

Mycology Proficiency Testing Program

January 2010 Test Event

Critique



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New York State Department of Health

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Schedule of 2010 Mycology PT Mailouts*[‡]

GENERAL

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

GENERAL POSTMARK DEADLINES

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

YEASTS ONLY

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

YEASTS ONLY POSTMARK DEADLINES

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

DIRECT DETECTION TESTING

January 27, 2010
September 29, 2010

DIRECT DETECTION TESTING POSTMARK DEADLINES

February 12, 2010
October 15, 2010

ANTIFUNGAL SUSCEPTIBILITY FOR YEASTS

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

ANTIFUNGAL SUSCEPTIBILITY FOR YEASTS POSTMARK DEADLINES

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

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

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

TEST SPECIMENS AND GRADING POLICY

Test Specimens*

At least two strains of each mold specimen were examined for inclusion in the proficiency test event of January 2009. The colony morphology of these strains was studied on Sabouraud dextrose agar. The microscopic morphologic features were examined by potato dextrose agar slide cultures. The physiological characteristics, such as cycloheximide sensitivity and growth at higher temperatures were investigated with appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics typical of the species was used as a test analyte. Similarly, two or more strains of yeast species were examined for inclusion in the proficiency test. The colony morphology of all yeast strains was studied on corn meal agar with Tween 80 plates inoculated by Dalmau or streak-cut method. Carbohydrate assimilation was studied with the API 20C AUX identification kit. The fermentations of carbohydrates, i.e., glucose, maltose, sucrose, lactose, trehalose, and cellobiose, were also investigated using classical approaches. Additional physiologic characteristics such as nitrate assimilation, urease activity, and cycloheximide sensitivity were investigated with the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics of the proposed test analyte was selected.

Grading Policy

A laboratory's response for each sample is compared with the response that reflects 80 percent agreement of 10 referee laboratories and/or 80 percent of all participating laboratories. The referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of New York State and must have a record of excellent performance during the preceding three years. The maximum score for each specimen is 20 based on the formula:

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

Acceptable results for antifungal susceptibility testing are based on consensus MIC values +/- 2 dilutions or interpretation per CLSI (NCCLS) guidelines or other publications. One yeast is to be tested against following drugs: amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole. The participating laboratories are allowed to select any number of antifungal drugs from the test panel based upon testing practices in their facilities. A maximum score of 100 will be equally divided among the drugs selected by the individual laboratory. If a result is incorrect, then laboratory gets a score of zero for that particular test component or set.

For *Cryptococcus neoformans* antigen test, laboratories are evaluated on the basis of their responses and on overall performance for all the analytes tested in the Direct Detection category. Appropriate responses are determined by 80% agreement in participant responses. Target values and acceptable ranges are mean value +/- 2 dilutions; positive or negative answers will be acceptable from laboratories that do not report titers. When both qualitative and quantitative results are reported, ten points will be deducted for each incorrect result. When only qualitative OR quantitative results are reported, twenty points will be deducted from each incorrect result.

A failure to attain an overall score of 80% is considered unsatisfactory performance. Laboratories receiving unsatisfactory scores in two out of three consecutive proficiency test events may be subject to 'cease testing' of clinical specimens.

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

ANSWER KEY AND LABORATORY PERFORMANCE

Mycology – General

	Specimen Key	Validated Specimen	Other Acceptable Answers	Correct Responses / Total # Laboratories (%)
M-1	<i>Cladosporium</i> sp.	<i>Cladosporium</i> sp.		71/73(97)
M-2	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i> species complex	70/73(96)
M-3	<i>Penicillium</i> sp.	<i>Penicillium</i> sp.	<i>Penicillium brevicompactum</i>	69/71 (97)
M-4	<i>Microsporium gypseum</i>	Not validated		19/73 (26)
M-5	<i>Rhizopus</i> sp.	<i>Rhizopus</i> sp.		71/73 (97)
M-Edu.	<i>Gliocladium</i> sp.			

Mycology – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers	Correct Responses / Total # Laboratories (%)
Y-1	<i>Candida guilliermondii</i>	<i>Candida guilliermondii</i>	<i>Pichia ohmeri</i>	52/53 (98)
Y-2	<i>Cryptococcus neoformans</i>	<i>Cryptococcus neoformans</i>		49/53 (92)
Y-3	<i>Hansenula anomala</i>	<i>Hansenula anomala</i>	<i>Candida pelliculosa</i> <i>Pichia anomala</i>	51/53 (96) 52/53 (98)
Y-4	<i>Prototheca wickerhamii</i>	<i>Prototheca wickerhamii</i>		53/53 (100)
Y-5	<i>Candida glabrata</i>	<i>Candida glabrata</i>	<i>Torulopsis glabrata</i>	52/53 (98)

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	0.25 – 1.0	Susceptible / No interpretation	26/26 (100)
Anidulafungin	0.125 – 0.5	Susceptible	17/17 (100)
Caspofungin	1.0 – 4.0	Susceptible	22/22 (100)
Flucytosine (5-FC)	0.06 – 1.0	Susceptible	25/25 (100)
Fluconazole	≥ 64	Resistant	32/32 (100)
Itraconazole	Not validated	Not validated	22/29 (76%)
Ketoconazole	0.5 – 4.0	No interpretation	7/7 (100)
Micafungin	0.06 – 0.25	Susceptible	17/17 (100)
Posaconazole	0.06 – 0.25	Susceptible	17/17 (100)
Voriconazole	1.0 – 4.0	Susceptible / Susceptible-dose dependent	25/25 (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	Positive (1:64)	Positive (1:64)	70/70 (100)	1:16 – 1:256	59/64 (92)
Cn-Ag-2	Positive (1:32)	Positive (1:32)	70/70 (100)	1:8 – 1:128	61/64 (95)
Cn-Ag-3	Negative	Negative	70/70 (100)		NA
Cn-Ag-4	Negative	Negative	70/70 (100)		NA
Cn-Ag-5	Negative	Negative	70/70 (100)		NA
Cn-Ag-Edu	Positive (1:64)			1:16 – 1:512	

TEST STATISTICS

	General	Yeast Only	Antifungal Susceptibility Testing for Yeasts	Direct Detection
Number of participating laboratories	73	53	33	70
Number of referee laboratories	10	10	33	70
Number of laboratories responding by deadline	73	53	32	70
Number of laboratories responding after deadline	0	0	0	0
Number of laboratories not responding	0	0	1	0
Number of laboratories successfully completing this test	72	52	32	70
Number of laboratories unsuccessfully completing this test	1	1	1	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	25
Etest	4
Disk diffusion method	0
Others [†]	5

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>	41
<i>Remel</i>	5
<i>Wampole</i>	17

(*Include multiple systems used by some laboratories)

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

MOLD DESCRIPTIONS

M-1 *Cladosporium* sp.

Source: Sputum

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	71
Laboratories with incorrect ID:	2
(<i>Phaeoannellomyces werneckii</i>)	(2)
Outcome:	Validated

Clinical Significance: *Cladosporium* sp. is a common airborne mold, but rarely causes human disease such as allergic fungal sinusitis. The fungus has also been implicated in cutaneous infection.

Ecology: *Cladosporium* sp. is often isolated from soil and plant litter. It is most frequently found in outdoor air in temperate climates.

Laboratory Diagnosis:

1. **Culture** – *Cladosporium* sp. grew rapidly on Sabouraud's dextrose agar; after 5 days at 25°C, *Cladosporium* sp. colony was grayish green, powdery or velvety on its surface. (Figure 1A). The reverse was greenish-black to brownish-black (Figure 1B).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed conidia often in long, branched chains with variable size. Conidia were unicellular and ellipsoidal to round at the tip. Prominent scars were visible at the points of attachment (Figure 2).
3. **Differentiation from other molds** – Generally, *Cladosporium* spp. have longer chains of conidia than *Fonsecaea* spp. and have small dark scars of attachment. On the country, the distal end of the conidiophore of *Fonsecaea* develops swollen denticles that bore primary single-celled ovoid conidia. *Xylohypha bantiana* is differentiated from *Cladosporium* by its lack of disjuncture scars on conidia.

4. **In vitro susceptibility testing** – *Cladosporium* species are generally susceptible to fluconazole.
5. **Molecular tests** – Restriction fragment length polymorphisms (RFLP) of the ribosomal small subunit gene and internal transcribed spacer (ITS) regions were used to distinguish *Cladosporium* species from other closely related molds such as *Fonsecaea*, *Phialophora*, and *Rhinocladiella* spp.

Comments: Two laboratories reported this specimen as *Phaeoannellomyces werneckii*, which is yeast-like moist slow growing mold.

Further Reading:

1. Caligiorne, R.B., De Resende, M.A., Dias-Nias-Neto, E., Oliveira, S.C., and Azevedo, V. 1999. Dematiaceous fungal pathogens: analysis of ribosomal DNA gene polymorphism by polymerase chain reaction-restriction fragment length polymorphism. *Mycoses* 42: 609-614.
2. Chiba, S., Okada, S., Suzuki, Y., Watanuki, Z., Mitsuishi, Y., Igusa, R., Sekii, T., and Uchiyama, B. 2009. *Cladosporium* species-related hypersensitivity pneumonitis in household environments. *Intern Med.* 48: 363-367.
3. Gugnani, H.C., Ramesh, V., Sood, N., Guarro, J., Moin-Ul-Haq, Paliwal-Joshi, A., Singh, B. 2006. Cutaneous phaeohyphomycosis caused by *Cladosporium oxysporum* and its treatment

with potassium iodide. *Med Mycol.* 44: 285-288.

4. Kantarcioglu, A.S., Yucel, A., and De Hoog, G.S. 2002. Case report. Isolation of *Cladosporium cladosporioides* from cerebrospinal fluid. *Mycoses* 45: 500-503.

5. Matsuwaki, Y., Nakajima, T., Iida, M., Nohara, O., Haruna, S., and Moriyama, H. 2001. A case report of allergic fungal sinusitis caused by *Penicillium* sp. and *Cladosporium* sp. *Nippon Jibiinkoka Gakkai Kaiho* 104: 1147-1150.

A.



B.

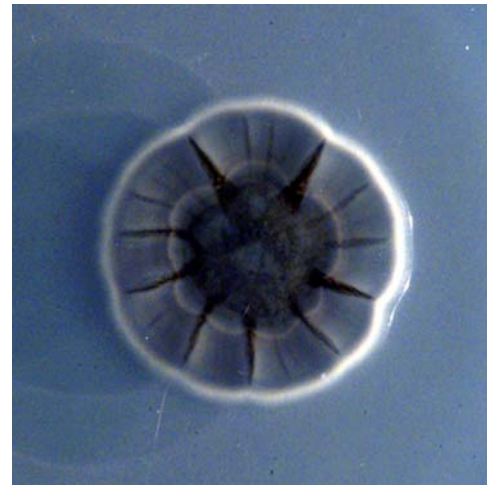
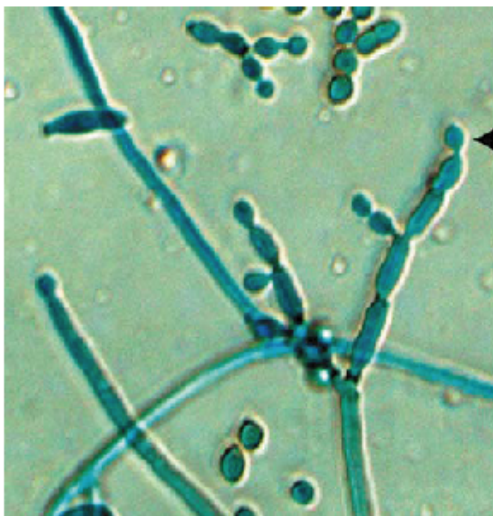


Figure 1. (A) Five-day-old, grayish green colony of *Cladosporium* sp. on Sabouraud's dextrose agar. (B) The reverse of five-day-old colony of *Cladosporium* sp. on Sabouraud's dextrose agar.

A.



B.

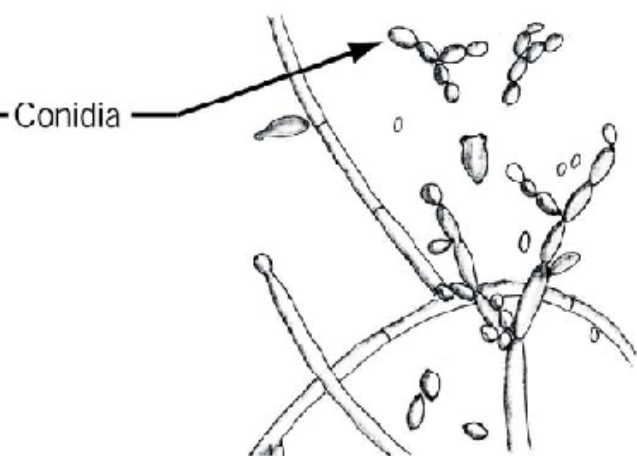


Figure 2. Microscopic morphology of *Cladosporium* sp. conidia occur in long, branched chains with variable size. The scars at the points of attachment of conidia are evident (A, 400× magnification; B, line drawing not to scale).

M-2 *Aspergillus niger*

Source: Bronchial wash

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	70
Laboratories with incorrect ID:	3
(<i>Aspergillus flavus</i>)	(1)
(<i>Aspergillus glaucus</i>)	(1)
(<i>Aspergillus nidulans</i>)	(1)
Outcome:	Validated

Clinical Significance: *Aspergillus niger* commonly causes ear infections. It is also implicated in allergic aspergillosis, pulmonary aspergilloma and rarely in primary cutaneous disease.

Ecology: *A. niger* is cosmopolitan in soil and on plants.

Laboratory Diagnosis:

1. **Culture** – *A. niger* was a fast grower on Sabouraud’s dextrose agar; after 5 days at 25°C, the initial growth was white, becoming black later on giving “salt and pepper appearance” (Figure 3A) and reverse turning pale yellow (Figure 3B). Good growth was seen at 37°C.
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate hyphae with smooth-walled, simple conidiophores measuring up to 1 mm in length. Conidiophores end in vesicle, which was globose and entirely covered (radiating) with two series of sterigmata (biseriate). Conidia produced from these sterigmata were brown to black, round, rough walled, and in chains measuring 4 – 5 µm in diameter (Figure 4).
3. **Differentiation from other molds** – *A. niger* is easily differentiated from other *Aspergillus* species by its rapid growth, black colonies, biseriate, radiating heads with black, round, rough conidia.
4. **In vitro susceptibility testing** – Most clinical isolates are susceptible to amphotericin B and variably susceptible to itraconazole and

resistant to fluconazole. Posaconazole, ravuconazole, and voriconazole exhibit promising activity against *A. niger*. *A. niger* is also susceptible to caspofungin.

5. **Molecular tests** – PCR method has been described to differentiate various species in the *A. niger* aggregate.

Comments: Few laboratories misidentified this specimen as other *Aspergillus* species.

Further Reading:

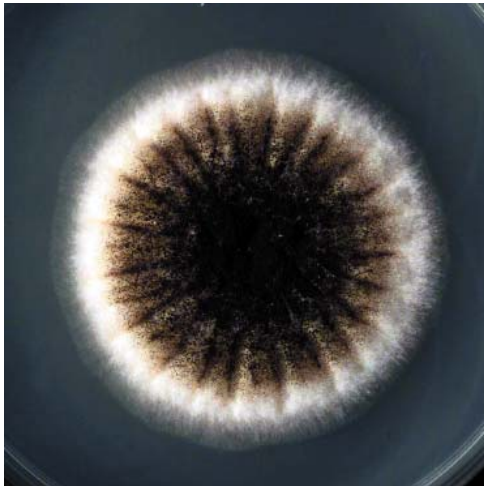
1. Fasunla, J., Ibekwe, T., and Onakoya, P. 2008. Otomycosis in western Nigeria. *Mycoses*. 51: 67-70.
2. Imhof, A., Balajee, S.A., Marr, K.A. 2003. New methods to assess susceptibilities of *Aspergillus* isolates to caspofungin. *J. Clin. Microbiol.* 41: 5683-5688.
3. McCracken, D., Barnes, R., Poynton, C., White, P.L., Isik, N., and Cook, D. 2003. Polymerase chain reaction aids in the diagnosis of an unusual case of *Aspergillus niger* endocarditis in a patient with acute myeloid leukaemia. *J. Infect.* 47: 344-347.
4. Mishra, G.S., Mehta, N., and Pal, M. 2004. Chronic bilateral otomycosis caused by *Aspergillus niger*. *Mycoses*. 47: 82-84.
5. Person AK, Chudgar SM, Norton BL, Tong BC, Stout JE. 2010. *Aspergillus niger*: an unusual cause of invasive pulmonary aspergillosis. *J Med Microbiol.* [Epub ahead of print]
6. Pfaller, M.A., Messer, S.A., Hollis, R.J., Jones, R.N., SENTRY Participants Group.

2002. Antifungal activities of posaconazole, ravuconazole, and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: report from SENTRY Antimicrobial Surveillance

Program, 2000. *Antimicrob. Agents Chemother.* 46: 1032-1037.

7. Shah, A., Maurya, V., Panjabi, C., and Khanna, P. 2004. Allergic bronchopulmonary aspergillosis without clinical asthma caused by *Aspergillus niger*. *Allergy.* 59: 236-237.

A.



B.

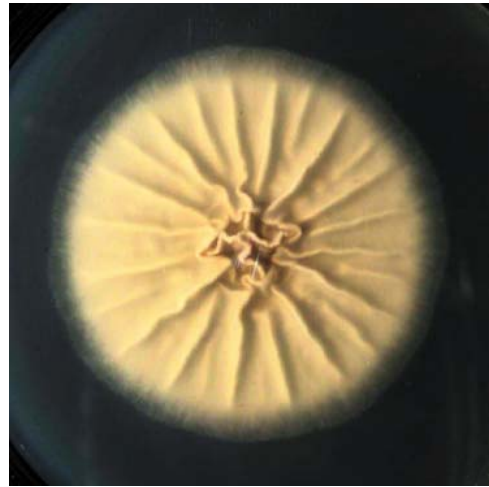
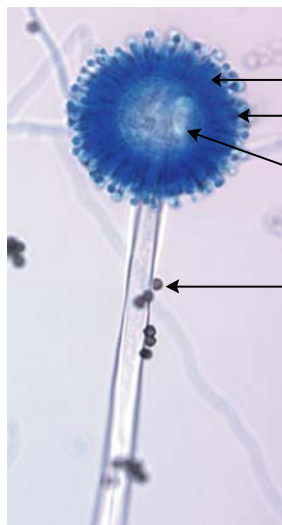


Figure 3. (A) Five-day-old, black colony of *Aspergillus niger* on Sabouraud's dextrose agar. The colony shows typical "salt and pepper appearance", which results from darkly pigmented conidia borne in large numbers on conidiophores. (B) The reverse of five-day-old colony of *Aspergillus niger*.

A.



B.

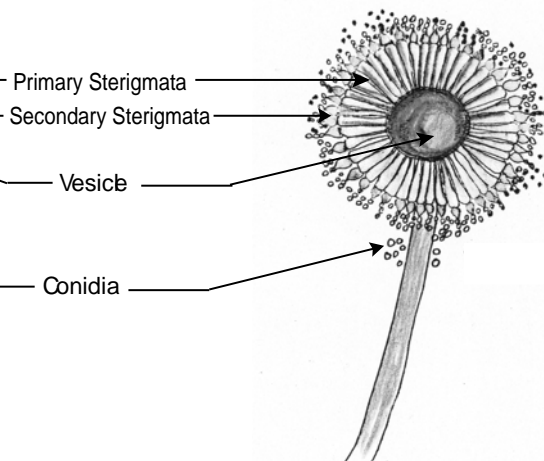


Figure 4. Microscopic morphology of *Aspergillus niger* showing globose vesicle with biserial, radiating head ; conidia are dark, round, and rough (A, 400× magnification; B, line drawing not to scale).

M-3 *Penicillium* sp.

Source: Hand

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	69
Laboratories with incorrect ID:	2
(<i>Aspergillus versicolor</i>)	(1)
(<i>Trichophyton mentagrophytes</i>)	(1)
Outcome:	Validated

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

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

Laboratory Diagnosis:

1. **Culture** – *Penicillium* sp. grew rapidly, velvety to powdery in texture. The colony was initially white and then becoming blue green, gray green, olive gray over time (Figure 5A). The reverse was pale to yellowish (Figure 5B).
2. **Microscopic morphology** – Lactophenol cotton blue or Calcofluor mounts showed septate hyaline hyphae, simple or branched conidiophores, and characteristic metulae, phialides. Metulae were secondary branches that form on conidiophores. The brush-like clusters of phialides, referred to as "penicilli". The unicellular conidia were round, and formed in chains at the tips of the phialides (Figure 6).
3. **Differentiation from other mold** – *Penicillium* sp. can be differentiated from *Paecilomyces* by flask-shaped phialides and globose to subglobose conidia; from

Gliocladium by chains of conidia; and from *Scopulariopsis* by phialides.

4. **In vitro susceptibility testing** – In general, *Penicillium* sp. is susceptible to amphotericin B, ketoconazole, itraconazole, and voriconazole.
5. **Molecular tests** – Internal transcribed spacer (ITS) regions can be used for *Penicillium* species identification.

Comments: This specimen is *Penicillium brevicompactum*, which was supplied by a vendor. *P. brevicompactum* is a ubiquitous fungal species that contaminates diverse substrates and commodities and produces an array of metabolites toxic to human and animals. Somehow this isolate could not grow at 30°C. Room temperature was suggested for growth. One laboratory reported this specimen as *Aspergillus versicolor*, which has reduced vesicles, could be confused with *Penicillium* sp., but regular sized vesicle conidial head should be able to be observed to distinguish it from *Penicillium* spp.

Further Reading:

1. Deshpande, S. D., and G. V. Koppikar. 1999. A study of mycotic keratitis in Mumbai. *Indian J Pathol Microbiol.* 42: 81-87.
2. Keceli, S., Yegenaga, I., Dagdelen, N., Mutlu, B., Uckardes, H., and Willke, A. 2005. Case report: peritonitis by *Penicillium* spp. in a patient undergoing continuous ambulatory peritoneal dialysis. *Int Urol Nephrol.* 37: 129-131.

3. Noritomi, D.T., Bub, G.L., Beer, I., da Silva, A.S., de Cleve, R., and Gama-Rodrigues, J.J. 2005. Multiple brain abscesses due to *Penicillium* spp infection. *Rev Inst Med Trop Sao Paulo*. 47: 167-170.

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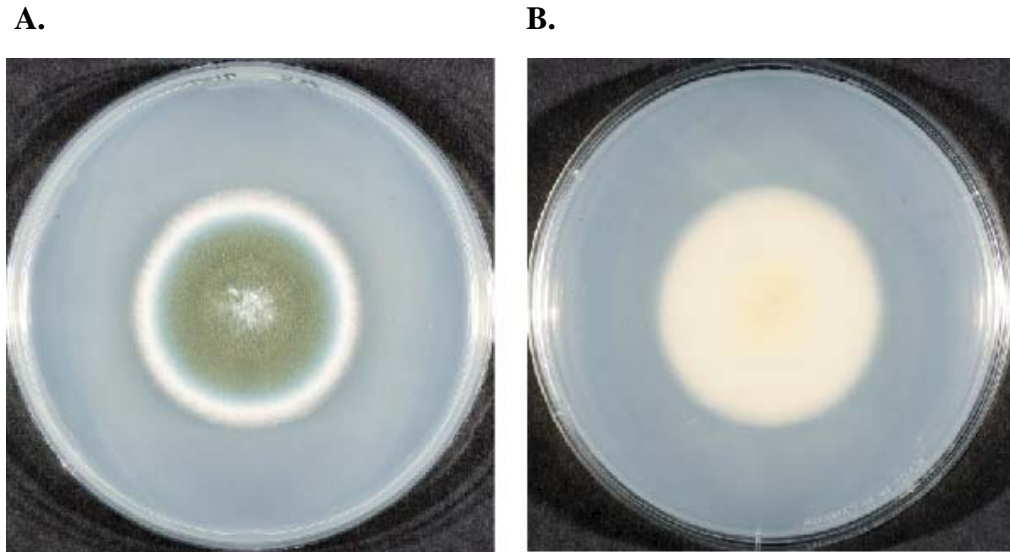


Figure 5. (A) Seven-day-old, white edge, blue green, to olive green colony of *Penicillium* sp. on Sabouraud's dextrose agar. (B) The reverse of seven-day-old *Penicillium* sp. colony.

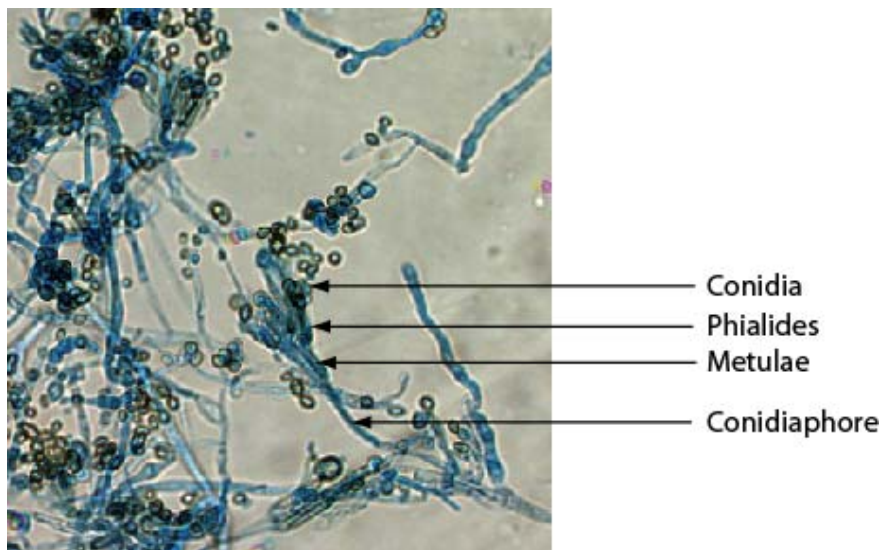


Figure 6. Microscopic morphology of *Penicillium* sp. showing broom-shaped phialides and round conidia (200× magnification).

M-4 *Microsporium gypseum*

Source: Toenail

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	2
Laboratories with correct ID:	19
Laboratories with incorrect ID:	54
(<i>Microsporium canis</i>)	(20)
(<i>Trichophyton mentagrophytes</i>)	(11)
(<i>Trichophyton rubrum</i>)	(8)
(<i>Microsporium persicolor</i>)	(7)
(<i>Trichophyton tonsurans</i>)	(5)
(<i>Chrysosporium</i> sp.)	(1)
(<i>Penicillium</i> sp.)	(1)
(<i>Trichophyton</i> sp.)	(1)
Outcome:	Non-validated

Clinical Significance: *Microsporium gypseum* is a well-known dermatophyte, which commonly infects hair and skin.

Ecology: *M. gypseum* is a geophilic dermatophyte and also isolated from fur of rodents.

Laboratory Diagnosis:

1. **Culture** – *M. gypseum* grew relatively rapidly. On Sabouraud's dextrose agar, after 5 days at 25°C, the texture of the colony was powdery to granular and the color was beige to cinnamon brown (Figure 7A). The reverse was yellow to brownish red (Figure 7B).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed septate hyphae, macroconidia and microconidia. Macroconidia were abundant, fusiform and symmetrical in shape with rounded ends. The walls of macroconidia were thin and rough and they contain 3-6 cells. Microconidia were moderately numerous in number, club-shaped and located along the hyphae. (Figure 8).
3. **Differentiation from other dermatophyte** – *Microsporium* differs from *Trichophyton* and *Epidermophyton* by its spindle-shaped macroconidia with echinulate to rough walls

4. ***In vitro* susceptibility testing** – Limited information is available. It was reported that *M. gypseum* was susceptible to terbinafine and itraconazole
5. **Molecular tests** – PCR and PCR-restriction fragment length polymorphism (RFLP) methods targeting the DNA topoisomerase II genes was reported for identification of dermatophytes.

Comments: This specimen was invalidated for current PT event. Majority of laboratories did not observe the characteristic of macroconidia from this lyophilized specimen.

Further Reading:

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2. Iwasawa M, Yorifuji K, Sano A, Takahashi Y, Nishimura K. 2009. Case of kerion celsi caused by *Microsporium gypseum* (*Arthroderma gypseum*) in a child. *Nippon Ishinkin Gakkai Zasshi*. 50: 155-160.
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II gene for rapid clinical diagnosis of the etiologic agent of dermatophytosis. *J Dermatol Sci.* 34: 35-48.

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due to *Microsporum gypseum* in a HIV-positive boy in Uganda, east Africa. *Mycoses.* 50: 153-155.

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A.



B.

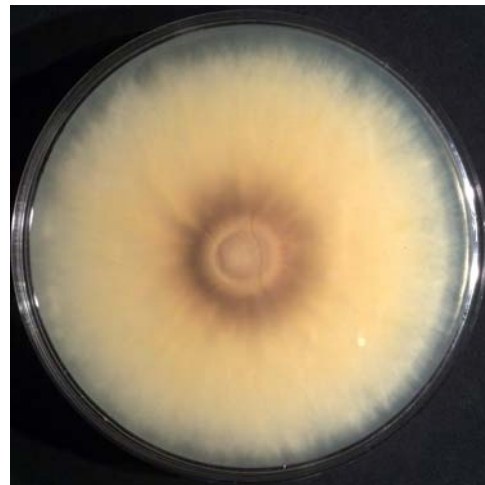


Figure 7. (A) Five-day old, powdery to granular, beige to cinnamon brown colony of *Microsporum gypseum* on Sabouraud's dextrose agar. (B) The reverse of the five-day-old colony of *M. gypseum*.

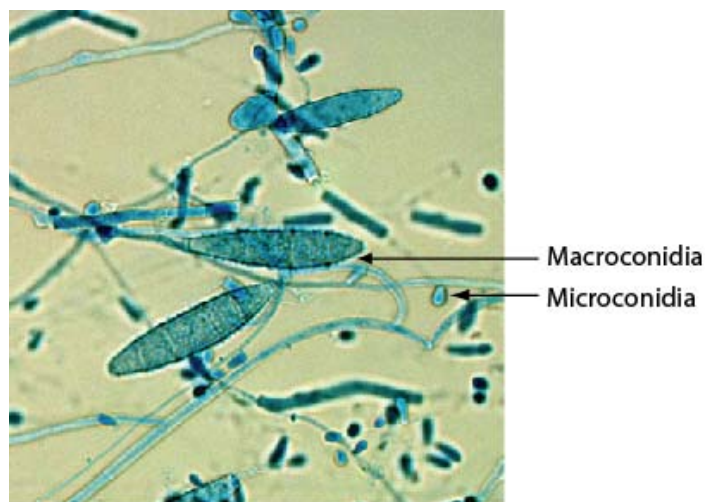


Figure 8. Microscopic morphology of *Microsporum gypseum* showing 3-6 celled macroconidia with thin and rough wall and club-shaped microconidia (200× magnification).

M-5 *Rhizopus* sp.

Source: Chest

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	71
Laboratories with incorrect ID:	2
(<i>Mucor</i> sp.)	(2)
Outcome:	Validated

Clinical Significance: *Rhizopus* sp. is the most common agent of zygomycosis. The predisposing factors are diabetic ketoacidosis, malnutrition, burns, and immunocompromising conditions such as hematologic malignancy, corticosteroid therapy, etc.

Ecology: *Rhizopus* sp. is cosmopolitan in distribution, mainly isolated from soil, decaying vegetables and bread.

Laboratory Diagnosis:

1. **Culture** – At 25°C, colonies on Sabouraud's dextrose agar, were wooly in texture, grayish brown, growing very rapidly, filling the culture plate in 24 – 48 h (Figure 9).
2. **Microscopic morphology** – Lactophenol cotton blue showed broad, aseptate hyphae, either single or tufts of brown sporangiophores (conidiophores) arising from hyphae (stolons) opposite well-developed rhizoids (root like structures). Sporangiophores had terminal sporangia with a round columella (vesicle, enlarged at the apex), containing round to oval sporangiospores or sexual spores (Figure 10).
3. **Differentiation from other zygomycetes** – *Rhizopus* species is distinguished from other members by the presence of well-developed rhizoids situated opposite sporangiophores. Sporangiophores are often unbranched and in tufts unlike in *Mucor*, *Rhizomucor*, *Absidia* (Table 1).
4. **In vitro susceptibility testing** – Most clinical isolates are susceptible to amphotericin B, itraconazole, and posaconazole, but resistant to voriconazole.

5. **Molecular tests** – PCR assay for the rapid and accurate identification of the agents of zygomycosis has been reported by Voigt et al.

Comments: This isolate is *Rhizopus stolonifer*. All the laboratories except two reported correct identification. Two laboratories reported it as *Mucor* sp., possibly because characteristic rhizoids were not observed.

Further reading:

1. Arikan S, Sancak B, Alp S, Hascelik G, McNicholas P. 2008. Comparative in vitro activities of posaconazole, voriconazole, itraconazole, and amphotericin B against *Aspergillus* and *Rhizopus*, and synergy testing for *Rhizopus*. *Med Mycol.* 46: 567-573.
2. Ciesla MC, Kammeyer PL, Yeldandi V, Petruzzelli GJ, Yong SL. 2000. Identification of the asexual state of *Rhizopus* species on histologic tissue sections in a patient with rhinocerebral mucormycosis. *Archives Pathology & Lab Medicine.* 124: 883 – 887.
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Table 1. Scheme for differentiation of various genera of zygomycetes pathogenic for humans and animals

Genus	Rhizoids	Conidiophores	Sporangia	Columella	Apophysis	Conidia
<i>Absidia</i>	Present	Branched	Pyriform	Hemi-spherical	Present	Globose, smooth
<i>Mucor</i>	Absent	Branched – single or Multiple	Globose	Various forms – globose, elongated	Absent	Globose - cylindrical
<i>Rhizopus</i>	Present	Single or group	Globose, gray – brown	Sub-globose	Present, but inconspicuous	Angular, striated
<i>Rhizomucor</i>	Present	Sympodial	Globose, gray	Sub-globose, brown	Absent	Sub-globose, small

A.

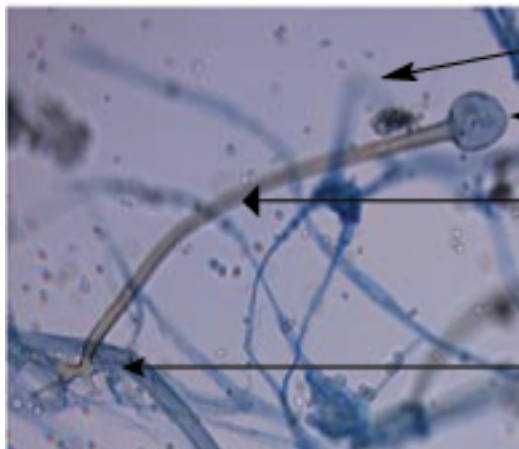


B.



Figure 9. (A) Three-day-old grayish brown colony of *Rhizopus* sp. on Sabouraud's dextrose. (B) The reverse of three-day-old colony of *Rhizopus* sp.

A.



B.

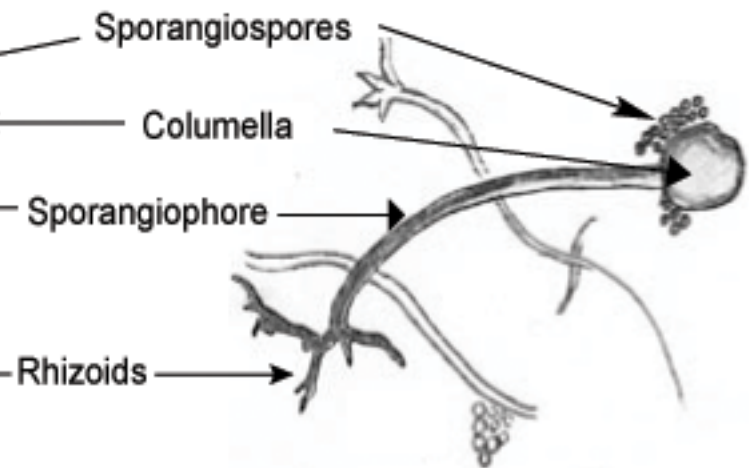


Figure 10. Microscopic morphology of *Rhizopus* sp. showing broad, aseptate hyphae, sporangiophores arising opposite rhizoids, sporangia with round columella, and oval sporangiospores (A, 100X magnification; B, line drawing not to drawn to scale).

M-Edu. *Gliocladium* sp.

Source: Skin

Laboratory Performance:	No. Laboratories
Referee Laboratories with correct ID:	10
Laboratories with correct ID:	72
Laboratories with incorrect ID: (<i>Paecilomyces</i> sp.)	1 (1)

Clinical Significance: *Gliocladium* sp. is considered a common laboratory contaminant as there are no reported clinical cases.

Ecology: *Gliocladium* species have a world-wide distribution and they are commonly isolated from a wide range of plant debris and soil.

Laboratory Diagnosis:

1. **Culture** – *Gliocladium* sp. was a rapid growing mold. At 25°C, initial growth was white, becoming olive green in the center, fluffy; reverse was pale yellow (Figure 11).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed hyaline, septate hyphae with *Penicillium*-like branched conidiophores. Conidia clumped together to form large balls, which were adjacent to phialides (Figure 12).

3. **Differentiation from other molds** – The microscopic morphology of *Gliocladium* species resembles that of *Penicillium* spp. However, the conidia of *Gliocladium* are not formed in chain like *Penicillium* spp.
4. **In vitro susceptibility testing** – No information available
5. **Molecular tests** – No information available

Comments: All the laboratories except one correctly identified this specimen.

Further reading:

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A.



B.

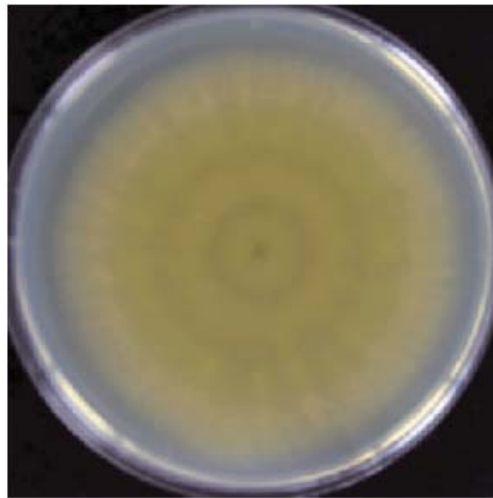
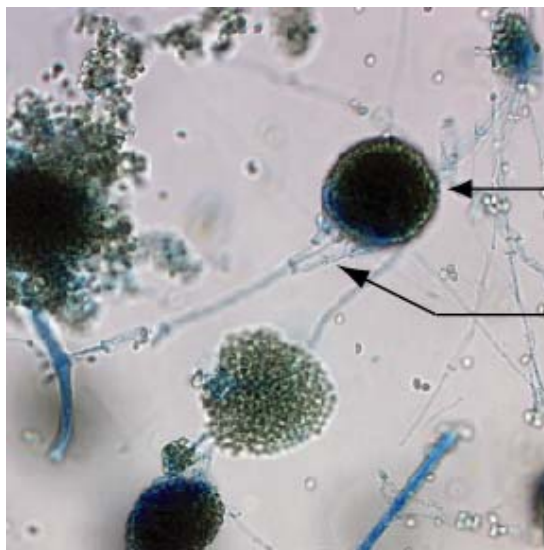


Figure 11. (A) Five-day-old, olive green colony of *Gliocladium* sp. on Sabouraud's dextrose agar. (B) The reverse of five-day-old *Gliocladium* sp. colony.



Conida clump together to form a ball

Phialides

Figure 12. Microscopic morphology of *Gliocladium* species with septate hyaline hyphae, brush like clusters of phialides and conidia clump together to form balls (400X magnification).

YEAST DESCRIPTIONS

Y-1 *Candida guilliermondii*

Source: Blood / Nail / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Candida famata*)

Outcome:

No. Laboratories

10

52

1

(1)

Validated

Clinical Significance: *Candida guilliermondii* is a frequent causal agent of nosocomial fungemia in immunosuppressed patient. It is also infrequent casual agent of urinary tract infections, brain abscess, and ocular infections.

Ecology: *C. guilliermondii* is widely distributed in nature (routinely isolated from insects, soil, plants, atmosphere, seawater, the exudates of various trees, and processed foods) and is a common constituent of the normal human microflora.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony was flat, smooth, cream-yellow (Figure 13).
2. **Microscopic morphology** – On corn meal agar with Tween 80, few short pseudohyphae with clusters of blastoconidia were seen (Figure 14).
3. **Differentiation from other yeasts** – *C. guilliermondii* is the anamorph (asexual form) of *Pichia guilliermondii/Kodamaea ohmeri*. It ferments glucose, sucrose, and trehalose, grows at 37°C, and on media containing cycloheximide. It does not form pink pigment thereby differentiating it from *Rhodotorula* species. It does not produce true hyphae, which differentiates it from *Candida ciferrii* and *Trichosporon beigeli*. Unlike *Candida lusitanae*, it is unable to grow at 45°C.
4. **In vitro susceptibility testing** – Most clinical isolates are susceptible to amphotericin B, 5-

flucytosine, and azoles such as fluconazole, ketocoazole, itraconazole and caspofungin. A few isolates are reported to have high MIC to azoles.

5. **Molecular tests** – Primers for large ribosomal subunit DNA sequences were used in PCR to differentiate *C. guilliermondii* from *C. famata/Debaryomyces hansenii* complex. Isolates of *C. guilliermondii* were identified using PCR to amplify ribosomal DNA, followed by restriction digestion of the PCR product.

Comments: One participating laboratory reported this isolate as *C. famata*, probably because in the API 20C AUX yeast identification system, *C. guilliermondii* and *C. famata* are assigned the same biocode. Supplementary test based upon melibiose assimilation is recommended for further differentiation of *C. guilliermondii* and *C. famata*. *C. guilliermondii* assimilates melezitose and raffinose frequently (90%), while *C. famata* assimilates the two carbohydrates infrequently (60%).

Further Reading:

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2. Kabbara, N., Lacroix, C., de Latour, R.P., Socié, G., Ghannoum, M., and Ribaud, P. 2008. Breakthrough *C. parapsilosis* and *C. guilliermondii* blood stream infections in allogeneic hematopoietic stem cell transplant

- recipients receiving long-term caspofungin therapy. *Haematologica*. 93: 639-640.
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 8. Pfaller, M.A., Boyken, L., Hollis, R.J., Messer, S.A., Tendolkar, S., and Diekema, D.J. 2006. *In Vitro* Susceptibilities of *Candida* spp. to Caspofungin: Four Years of Global Surveillance. *J. Clin. Microbiol*. 44: 760-763.
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Sequences alignment:

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

```

Query 1      CGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTTTGGCAGCGCTTAACTGCGCGG 60
          |||
Sbjct 2      CGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTTTGGCAGCGCTTAACTGCGCGG 61

Query 61     CGAAAAACCTTACACACAGTGTCTTTTGGATACAGAACTCTTGCTTTGGTTTGGCCTAGA 120
          |||
Sbjct 62     CGAAAAACCTTACACACAGTGTCTTTTGGATACAGAACTCTTGCTTTGGTTTGGCCTAGA 121

Query 121    GATAGGTTGGGCCAGAGGTTTAAACAAAAACACAATTTAATTATTTTACAGTTAGTCAAAT 180
          |||
Sbjct 122    GATAGGTTGGGCCAGAGGTTTAAACAAAAACACAATTTAATTATTTTACAGTTAGTCAAAT 181

Query 181    TTTGAATTAATCTTCAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAA 240
          |||
Sbjct 182    TTTGAATTAATCTTCAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAA 241

Query 241    CGCAG 245
          |||
Sbjct 242    CGCAG 246

```

Alignment of primary sequences of the ITS1 regions of *C. guilliermondii* strain SMB (Sbjct) and PT specimen *C. guilliermondii* M2167 (Query).



Figure 13. Seven-day-old, flat, smooth, cream-yellow colony of *Candida guilliermondii* on Sabouraud's dextrose agar.

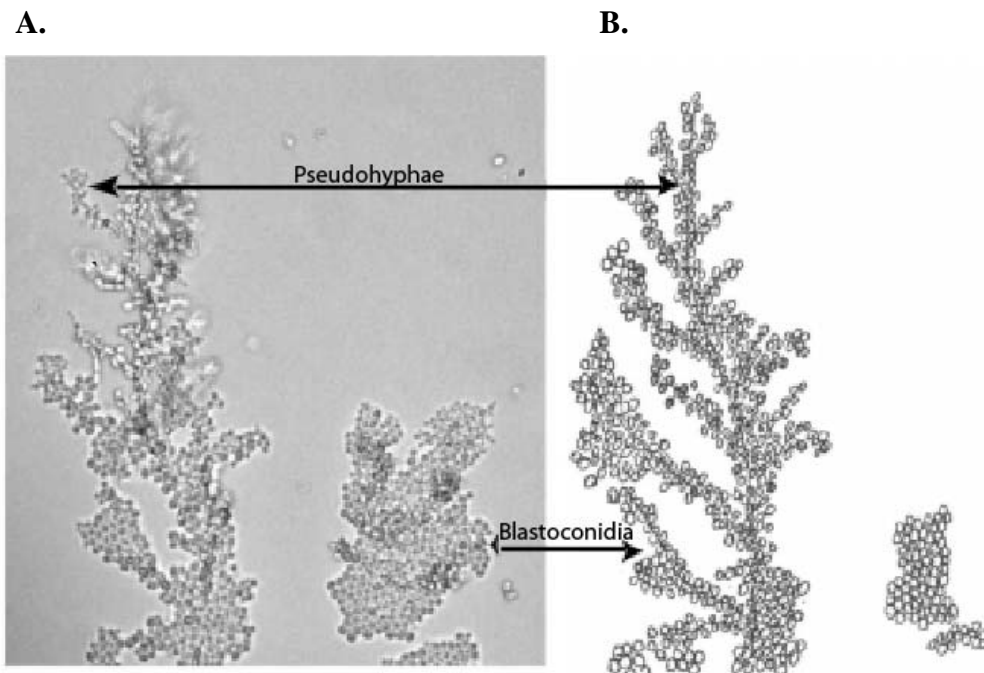


Figure 14. Microscopic morphology of *Candida guilliermondii*. On corn meal agar with Tween 80 culture, short pseudohyphae with clusters of blastoconidia are seen (A, 200× magnification; B, line drawing not to scale).

Y-2 *Cryptococcus neoformans*

Source: CSF / Sputum / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Cryptococcus laurentii*)

(*Cryptococcus terreus*)

(*Pichia ohmeri*)

Outcome:

No. Laboratories

10

49

4

(2)

(1)

(1)

Validated

Clinical Significance: The incidence of cryptococcosis due to *Cryptococcus neoformans* infection increased with the spread of AIDS and other immunosuppressive conditions. *Cr. neoformans* var. *grubii* and var. *neoformans* mainly cause meningoencephalitis in patients with AIDS or other underlying immune dysfunctions. *Cr. neoformans* var. *neoformans* infections are more likely to have cutaneous involvement, and to infect older patients, than are infections caused by *Cr. grubii*. *Cr. gattii* causes pulmonary cryptococcosis and systemic cryptococcosis in normal and immunocompromised hosts.

Ecology: *Cryptococcus neoformans* var. *neoformans* and var. *grubii* are commonly found in avian (pigeon) droppings. Both varieties have world-wide distributions. *Cr. gattii* is commonly found on *Eucalyptus* and other trees and mainly distributed in Australia, Southeast Asia, Southern California, Pacific Northwest, Vancouver Island, British Columbia, Canada, and South America.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony was cream to tan in color, smooth, moist, and soft (Figure 15).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *Cr. neoformans* cells were large and round, with no pseudohyphae or true hyphae. In India-ink preparation, encapsulated yeasts were seen (Figure 16).

3. **Differentiation from other yeasts** – *Cr. neoformans* does not ferment any carbohydrates and does not grow on media containing cycloheximide, but it grows at 37°C. *Cr. neoformans* produces dark brown colonies on niger seed agar. It produces urease enzyme and it is negative on nitrate reaction. *Cr. neoformans* and *Cr. gattii* are differentiated by 1) growth and color change: *Cr. gattii* on canavanine-glycine-bromthymol blue (CGB) medium becomes blue-green after 2 – 5 days at 25°C; 2) PCR technique: *Cr. gattii* can be differentiated from the other two varieties using a number of primers; 3) serotyping: *Cr. neoformans* var. *grubii* is serotype A, *Cr. neoformans* var. *neoformans* is serotype D, *Cr. gattii* is serotype B and C.
4. **In vitro susceptibility testing** – Most isolates are susceptible to amphotericin B, 5-flucytocine, and to azoles like fluconazole, itraconazole, and posaconazole. A few isolates with high MIC to fluconazole have been isolated from AIDS patients.
5. **Molecular tests** – *Cr. neoformans* is one of the most intensely studied pathogenic fungi. The molecular biology of this organism has revealed various virulence factors and unique genotypes among clinical strains.

Comments: Originally, *Cryptococcus neoformans* was described as comprising of two varieties: *Cr. neoformans* var. *neoformans* (serotypes A & D) and *Cr. neoformans* var. *gattii* (serotype B & C). Recently, *Cr. neoformans* was further subdivided into two varieties: *Cr.*

neoformans var. *grubii* (serotype A) and *Cr. neoformans* var. *neoformans* (serotype D). *Cr. neoformans* var. *gattii* was re-named as *Cr. gattii*.

Further Reading:

1. Aller, A.I., Martin-Mazuelos, E., Lozano, F., Gomez-Mateos, J., Steele-Moore, L., Holloway, W.J., Gutierrez, M.J., Recio, F.J., and Espinel-Ingroff, A. 2000. Correlation of fluconazole MICs with clinical outcome in cryptococcal infection. *Antimicrob. Agents Chemother.* 44: 1544-1548.
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9. Kwon-Chung, K.J., Polacheck, I., and Bennett, J.E. 1982. Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotype A and D) and *Cryptococcus neoformans* var. *gattii* (serotype B and C). *J. Clin. Microbiol.* 15: 535-537.
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11. Singh N, Lortholary O, Dromer F, Alexander BD, Gupta KL, John GT, del Busto R, Klintmalm GB, Somani J, Lyon GM, Pursell K, Stosor V, Munoz 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; Cryptococcal Collaborative Transplant Study Group. 2008. Central nervous system cryptococcosis in solid organ transplant recipients: clinical relevance of abnormal neuroimaging findings. *Transplantation.* 86: 647-651.
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Figure 15. Seven-day-old, cream to tan colored, smooth, moist, and soft colony of *Cryptococcus neoformans* on Sabouraud's dextrose agar.

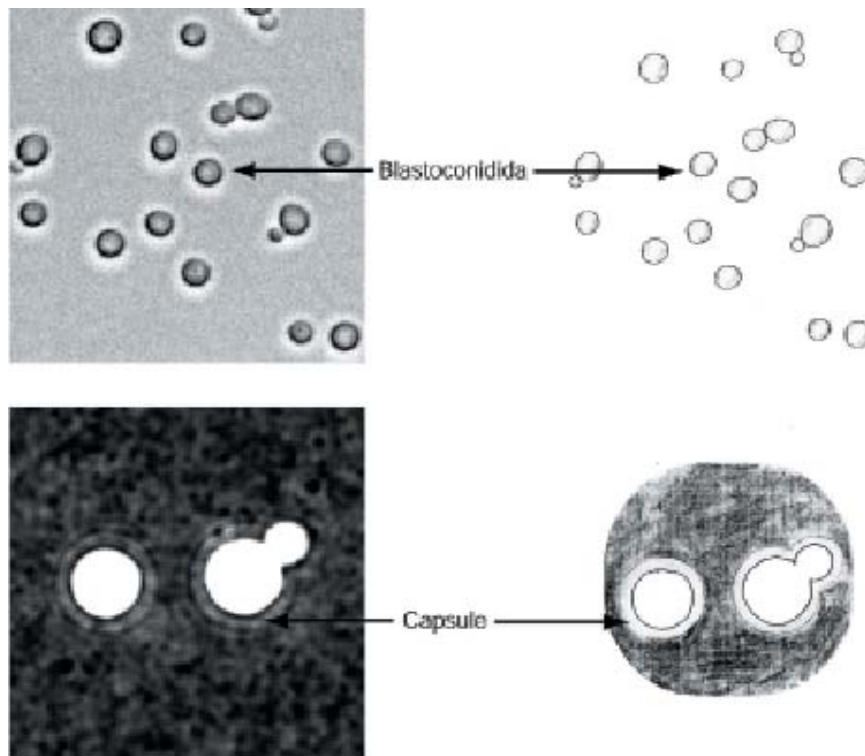


Figure 16. Microscopic morphology of *Cryptococcus neoformans* on corn meal agar with Tween 80. (Upper panel) Round, large blastoconidia. (Right, 400× magnification; Left, line drawing not to scale) (Lower panel) India-ink preparation revealing capsules (right, 1000× magnification; Left, line drawing not to scale).

Y-3 *Hansenula anomala*

Source: Chest / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Candida parapsilosis*)

(*Saccharomyces cerevisiae*)

Outcome:

No. Laboratories

10

51

2

(1)

(1)

Validated

Clinical Significance: *Hansenula anomala* is an infrequently encountered agent causing nosocomial infections. Several cases of fungemia in neonates, and endocarditis in immunosuppressed patients, are reported in the literature.

Ecology: *H. anomala* is found in soil and on various fruits and vegetables. It is also found on skin of humans and lower animals.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colonies appeared smooth, creamy, and soft (Figure 17).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *H. anomala* showed blastoconidia with ascospores, but no pseudohyphae (Figure 18).
3. **Differentiation from other yeasts** – *Hansenula anomala* is one of the synonyms of *Pichia anomala*. *Candida pelliculosa* is the anamorph (asexual form) of *Pichia anomala*. *H. anomala* does not grow on media containing cycloheximide, or at 42°C. It assimilates nitrate but is urease-negative.
4. **In vitro susceptibility testing** – *H. anomala* is susceptible to amphotericin B, 5-flucytosine, and azoles such as fluconazole, clotrimazole, and itraconazole.
5. **Molecular tests** – PCR amplification of a specific fragment of 18S rDNA and heteroduplex mobility assays were performed to detect and distinguish *H. anomala* from other clinically important yeasts. Phylogenetic analysis of domain sequences

placed four new species in the *H. anomala* clade.

Comments: One laboratory each reported this specimen as *Candida parapsilosis* and *Saccharomyces cerevisiae* respectively. *H. anomala* is nitrate positive, which differentiates it from *C. parapsilosis* and *S. cerevisiae*.

Further Reading:

1. Bakir, M., Cerikcioglu, N., Tirtir, A., Berrak, S., Ozek, E., and Canpolat, C. 2004. *Pichia anomala* fungaemia in immunocompromised children. *Mycoses*. 47: 231-235. 2007.
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Sequences alignment:

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

```

Query 1 GAACGCAGCGAAATGCGATACGTATTGTGAATTGCAGATTTTCGTGAATCATCGAATCTT 60
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Sbjct 246 GAACGCAGCGAAATGCGATACGTATTGTGAATTGCAGATTTTCGTGAATCATCGAATCTT 305

Query 61 TGAACGCACATTGCACCCTCTGGTATTCCAGAGGGTATGCCTGTTTGAGCGTCATTTCTC 120
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Sbjct 306 TGAACGCACATTGCACCCTCTGGTATTCCAGAGGGTATGCCTGTTTGAGCGTCATTTCTC 365

Query 121 TCTCAAACCTTCGGGTTTGGTATTGAGTGATACTCTGTCAAGGGTTAACTTGAAATATTG 180
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Sbjct 366 TCTCAAACCTTCGGGTTTGGTATTGAGTGATACTCTGTCAAGGGTTAACTTGAAATATTG 425

Query 181 ACTTAGCAAGAGTGTAATAAGCAGTCTTTCTGAAATAATGTATTAGGTTCTTCCAAC 240
      |||
Sbjct 426 ACTTAGCAAGAGTGTAATAAGCAGTCTTTCTGAAATAATGTATTAGGTTCTTCCAAC 485

Query 241 TCGTTATATCAGCTAGGCAGGTTTLAGAAGTATTTTAGGCTCGGCTTAACAACAATAAACT 300
      |||
Sbjct 486 TCGTTATATCAGCTAGGCAGGTTTLAGAAGTATTTTAGGCTCGGCTTAACAACAATAAACT 545

Query 301 AAAAGTTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA 352
      |||
Sbjct 546 AAAAGTTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA 597

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Alignment of primary sequences of the ITS2 regions of *H. anomala* M10 (Sbjct) and PT specimen *H. anomala* M2169 (Query).



Figure 17. Seven-day-old, smooth, creamy, soft colony of *Hansenula anomala* on Sabouraud's dextrose agar.

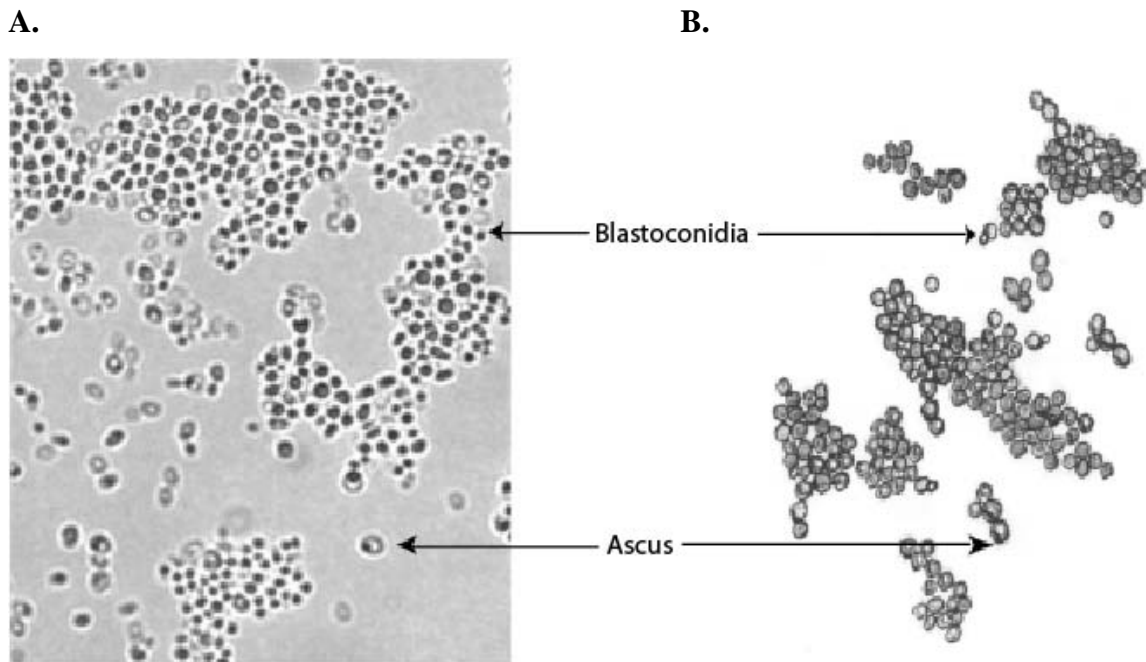


Figure 18. Microscopic morphology of *Hansenula anomala*. On corn meal agar with Tween 80 culture, blastoconidia with ascus are seen (A, 400 × magnification; B, line diagram not to scale).

Y-4 *Prototheca wickerhamii*

Source: Lung / Blood / Urine

Laboratory Performance:

Referee Laboratories with correct ID:

Laboratories with correct ID:

Laboratories with incorrect ID:

(*Malassezia pachydermatis*)

Outcome:

No. Laboratories

10

52

1

(1)

Validated

Clinical Significance: *Prototheca wickerhamii* causes protothecosis in humans. Most commonly, these yeast-like algae cause cutaneous and subcutaneous lesions termed bursitis. Rarely, *P. wickerhamii* causes systemic infections. The infection is acquired through traumatic implantation of algae in subcutaneous tissue.

Ecology: *P. wickerhamii* has been isolated from various environmental sources like sewage, slime, and stream sediment.

Laboratory Diagnosis:

1. **Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, the colony was moist, cream-colored, yeast-like (Figure 19).
2. **Microscopic morphology** – On corn meal agar with Tween 80, sporangia of various sizes, some filled with sporangiospores (endospores), were seen (Figure 20). There was no budding, no hyphae.
3. **Differentiation from related organism** – *P. wickerhamii* requires thiamine for growth, does not grow on media containing cycloheximide, grows well at 25°C and 37°C. The cells of *P. wickerhamii* are smaller than those of *P. zopfii*. On the API 20C AUX, a specific assimilation biocode differentiates it from other *Prototheca* species. The isolates of *P. zopfii* are resistant to 50-µg clotrimazole disk at 37°C while *P. wickerhamii* isolates produces a zone of inhibition.
4. **In vitro susceptibility testing** – Almost all isolates are susceptible to amphotericin B and voriconazole, but resistant to fluconazole

and 5 FC, variably susceptible to itraconazole and ketoconazole.

5. **Molecular tests** – Sequence analysis of the mitochondrial small subunit rRNA from *P. wickerhamii* showed higher homology with mitochondrial sequence from plants.

Comments: All the participating laboratories except one correctly identified this specimen. *P. wickerhamii* is distinguishable from *Malassezia pachydermatis* by no growth on the media containing cycloheximide.

Further Reading:

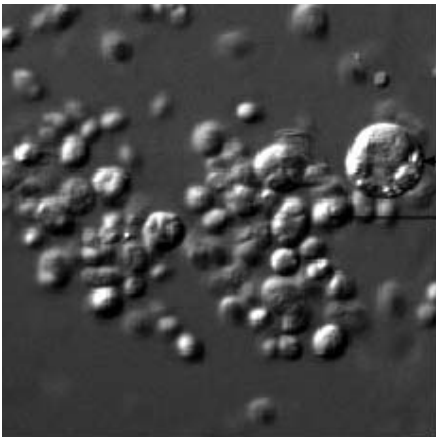
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Figure 19. Seven-day-old, moist, cream-colored colony of *Prototheca wickerhamii* on Sabouraud's dextrose agar.

A.



B.

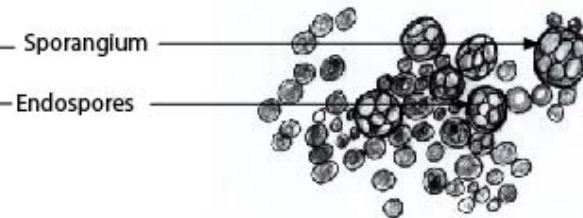


Figure 20. Microscopic morphology of *Prototheca wickerhamii* on corn meal agar showing sporangia of various sizes, some filled with sporangiospores (endospores) (A, 400× magnification; B, line drawing not to scale).

Y-5 *Candida glabrata*

Source: Tissue / 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 glabrata* commonly causes urinary tract infections and vaginitis. Incidence of candidiasis caused by *C. glabrata* has increased in immunosuppressed patients due to more intensive anticancer chemotherapy, bone marrow, and organ transplantation.

Ecology: Humans, lower mammals, and birds are the carriers of *C. glabrata*.

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar at 25°C for 3 to 5 days, colony was white to cream, smooth and shiny (Figure 21).
2. Microscopic morphology – On cornmeal agar with Tween 80, *C. glabrata* blastoconidia were tiny, round or elliptical in shape (Figure 22).
3. Differentiation from other yeasts – *C. glabrata* grows at 42°C but does not grow on media containing cycloheximide. It ferments glucose and trehalose. *C. glabrata* forms only blastoconidia and no pseudohyphae or true hyphae.
4. In vitro susceptibility testing – *C. glabrata* is susceptible to amphotericin B, caspofungin, and 5-FC but resistant to azoles like fluconazole and itraconazole.
5. Molecular tests – PCR amplification of a mitochondrial rRNA gene fragment, which is species specific, was developed to identify *C. glabrata*. Diversity of karyotype by pulse-field gel electrophoresis was used to confirm *C. glabrata* infection. Comparative sequence analysis of cytochrome oxidase gene has been reported for typing of *C. glabrata*.

Comments: All participating laboratories correctly identified this specimen.

Further Reading:

1. Becker, K., Badehorn, D., Keller, B., Schulte, M., Bohm, K.H., Peters, G., and Fegeler, W. 2001. Isolation and characterization of a species specific DNA fragment for identification of *Candida (Torulopsis) glabrata* by PCR. *J. Clin. Microbiol.* 39: 3356-3359.
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Antimicrob Agents Chemother. [Epub ahead of print]

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10. Thompson GR 3rd, Wiederhold NP, Vallor AC, Villareal NC, Lewis JS 2nd, Patterson TF. 2008. Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to *Candida glabrata* infection. *Antimicrob Agents Chemother.* 52: 3783-3785.

Sequences alignment:

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

```

Query 1 GCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCGGTGAAT 60
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Sbjct 3592 GCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCGGTGAAT 3651

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Sbjct 3652 CATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGGGGGCATGCCTGTTTGAG 3711

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Sbjct 3772 CTTGAAATTTGTAGGCCATATCAGTATGTGGGACACGAGCGCAAGCTTCTCTATTAATCTG 3831

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Sbjct 3832 CTGCTCGTTTGC GCGAGCGGCGGGGTTAATACTGTATTAGGTTTTACCAACTCGGTGTT 3891

Query 301 GATCTAGGGAGGGATAAGTGAGTGTTTTGTGCGTGCTGGGCAGACAGACGTCTTTAAGTT 360
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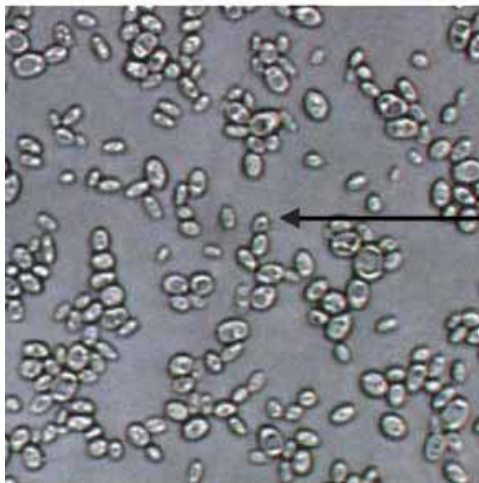
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Alignment of primary sequences of the ITS2 region of *C. glabrata* CBS 138 (Sbjct) and PT specimen *C. glabrata* M2171 (Query).



Figure 21. Four-day-old, white and shiny colony of *Candida glabrata* on Sabouraud's dextrose agar.

A.



B.

Blastoconidia

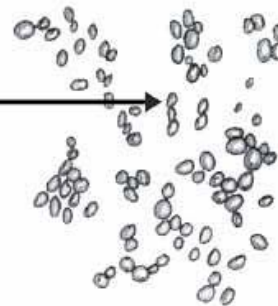


Figure 22. Microscopic morphology of *Candida glabrata* on corn meal agar with Tween 80 shows elliptical shaped blastoconidia (A, 400× magnification; B, line diagram not to scale).

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 based upon usual test practices in their facilities.

Materials & Results: *Candida albicans* M955 (S-1) was the analyte in the January 27, 2010 antifungal proficiency testing event. Thirty-one 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 draft document M27-S3 (December 2007)

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

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

³ For Ketoconazole, there is no assigned interpretative breakpoint.

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

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

Summary:

Table 3. Summary of Laboratory Performance, Antifungal Susceptibility Testing for Yeast Only, January 2010 PT Event

Acceptable Responses/Total # Laboratories (%)	
S- 1: <i>Candida albicans</i> M955	
Amphotericin B	26/26 (100)
Anidulafungin	17/17 (100)
Caspofungin	22/22 (100)
Flucytosine (5-FC)	25/25 (100)
Fluconazole	32/32 (100)
Itraconazole	22/29 (76%) Not validated
Ketoconazole	7/7 (100)
Micafungin	17/17 (100)
Posaconazole	17/17 (100)
Voriconazole	25/25 (100)

Table 4. Distribution of Antifungal MIC values (µg/ml) Reported by Participating Laboratories

S-1: *Candida albicans* M955

Drugs (µg/ml)	Total # of labs	≤0.03	0.06	0.12	0.19	0.25	0.38	0.5	0.75	1	2	3	4	8	16	32	≥64	≥128	≥256
Amphotericin B	26		1				1	7	1	16									
Anidulafungin	17			5		11		1											
Caspofungin	22									6	14	1	1						
Flucytosine (5-FC)	24		1	9		12				2									
Fluconazole	32															1	3	6	21
Itraconazole	29			2		3		17		4	1			1	1				
Ketoconazole	7							3		2	1		1						
Micafungin	17		1	2		14													
Posaconazole	17	1		1	1	14													
Voriconazole	25									9	12		4						

Table 5. Distribution of Antifungal Susceptibility Interpretations Reported by Participating Laboratories

S-1: *Candida albicans* M955

Antifungal Agent	Total # of labs	Susceptible	Susceptible -dose dependent	Intermediate	Resistant	Non-susceptible	No interpretation
Amphotericin B	26	13					13
Anidulafungin	17	17					
Caspofungin	22	19		1	1	1	
Flucytosine (5-FC)	25	25					
Fluconazole	32		1		31		
Itraconazole	29	2	18	2	7		
Ketoconazole	7	2					5
Micafungin	17	17					
Posaconazole	17	13					4
Voriconazole	25	9	10	1	5		

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR MOLDS (EDUCATIONAL)

Introduction: Eight laboratories participated in this educational test event. The document of M38-A2 published by Clinical Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards, NCCLS), is the current standard reference guide for antifungal susceptibility testing of pathogenic molds. The following 10 drugs were included in the antifungal susceptibility testing panel of NYSDOH Mycology Proficiency Test Program - amphotericin B, anidulafungin, caspofungin, flucytosine (5-FC), fluconazole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole.

Materials & Results: *Aspergillus fumigatus* M2039 was used. Laboratories were free to choose any number of drugs and preferred test method. Eight out of thirteen laboratories used CLSI Microdilution method, four laboratories used YeastOne Colorimetric method, and three laboratory used Etest. The acceptable range of MIC_{80/100} (µg/ml) values are listed in the Table 6. The MIC values and interpretations based upon yeast breakpoints criteria were reported by all the participating laboratories (Table 7 and Table 8).

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

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

Table 6. Acceptable range of MIC values for Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2039.

Acceptable Ranges of MIC (µg/ml) values	
Educational specimen: <i>Aspergillus fumigatus</i> M2039	
Amphotericin B	0.25 – 1.0
Anidulafungin	Not Available
Caspofungin	Not Available
Flucytosine (5-FC)	32 – 256
Fluconazole	≥ 64
Itraconazole	4 - 128
Ketoconazole	≥ 16
Micafungin	Not Available
Posaconazole	0.5 – 2.0
Voriconazole	0.5 – 2.0

Table 7. MIC ($\mu\text{g/ml}$) Values of Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2039

Drugs ($\mu\text{g/ml}$)	Total # of labs	0.015	0.03	0.06	0.12	0.25	0.5	0.75	1.0	2.0	3.0	4.0	≥ 8	≥ 16	≥ 32	≥ 64	≥ 128	≥ 256	≥ 512
Amphotericin B	12					1	5		5		1								
Anidulafungin	7	1	1	1	1								3						
Caspofungin	9		1	1	1	3							2	1					
Flucytosine (5-FC)	11											1			3	5	1	1	
Fluconazole	11															4	2	5	1
Itraconazole	11											1	2	5	1	1	1		
Ketoconazole	4													2	1	1			
Micafungin	6	2			1	1							3						
Posaconazole	9						1		6	1		1							
Voriconazole	11						2	1	3	4		1							

Colors represent the testing method used:

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

Table 8. Distribution of Interpretation Reported by Participating Laboratories for Mold Antifungal Susceptibility Educational Sample: *Aspergillus fumigatus* M2039*

Antifungal Agent	Total # of labs	Susceptible	Intermediate	Resistant	No interpretation
Amphotericin B	12	3			9
Anidulafungin	7	1		1	5
Caspofungin	9	2		1	6
Flucytosine (5-FC)	11			3	8
Fluconazole	12			3	9
Itraconazole	11			4	7
Ketoconazole	4				4
Micafungin	6	1		1	5
Posaconazole	9	3	1		5
Voriconazole	11	2	1	1	7

*Based upon yeast interpretation.

Further Reading:

1. Barry, A.L., Pfaller, M.A., Rennie, R.P., Fuchs, P.C., and Brown, S.D. 2002. Precision and accuracy of fluconazole susceptibility testing by broth microdilution, Etest, and disk diffusion methods. *Antimicrob. Agents Chemother.* 46: 1781-1784.
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DIRECT DETECTION (*CRYPTOCOCCUS NEOFORMANS* ANTIGEN TEST)

Introduction: A simple, sensitive latex test capable of detecting the capsular polysaccharide of *C. neoformans* in CSF and serum was described, and proven to be superior in sensitivity to the India ink mount (1, 2). Clinical studies established the prognostic value of the test (4, 6, 7 and 8), and showed it to be a valuable aid in establishing a diagnosis when culture was negative (5). Paired serum and CSF specimens allowed detection of antigen in confirmed cases (8). Parallel serologic studies for both antigen and antibody are recommended to ensure detection of extrameningeal cryptococcosis. Newly emerging disease states and therapies have been shown to increase the opportunity for nonspecific interference in some serum specimens. Pretreatment of serum specimens with pronase prior to utilization of the latex agglutination test reduces nonspecific interference, and enhances the detection of capsular polysaccharide antigens of *Cryptococcus neoformans*.

Materials & Methods: Seventy laboratories participated in the January 27, 2010 direct detection antigen testing event. Two positive serum samples for cryptococcal antigen were included. The titers were 1:64 and 1:32 for Cn-Ag-1 and Cn-Ag-2 respectively. One positive synthetic CSF sample for cryptococcal antigen was distributed as an educational specimen labeled as Cn-Ag-Edu. The titer was 1:64. Titers within ± 2 dilutions of the reference and/or consensus results were the acceptable results for this event.

Results: The performance of 70 laboratories was satisfactory in this test event. One laboratory reported the titer of specimen Cn-Ag-1 lower than the acceptable titer range and four laboratories reported the titer higher than the acceptable titer range. One laboratory reported the titer of specimen Cn-Ag-2 lower than the acceptable titer range and two laboratories

reported the titer higher than the acceptable titer range. The synthetic CSF specimen was the first time introduced in the proficiency test. All the participating laboratories were reported it qualitatively. The majority laboratories reported the titer as 1:64 or 1:128. Two laboratories reported the titer of this specimen lower than the acceptable range and four laboratories reported the titer higher than the acceptable range. The supplementary information on quantitative assays on *Cryptococcus neoformans* antigen test is summarized in Table 9.

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Table 9. Summary of quantitative assay

The number of laboratories that reported titers is listed for positive test samples Cn-Ag-1, Cn-Ag-2, and Cn-Ag-Edu.

Method	Sample	Cn-Ag-1 Titers									
		Total # of laboratories	4	16	32	40	64	80	128	256	512
EIA											
	(Meridien Diagnostic) 2			1				1			
Latex Agglutination											
	(Immuno-Mycologics) 3			2				1			
	(Meridien Diagnostic) 39			3	1	21	1	9	1	1	2
	(Remel) 4		1	1		1		1			
	(Wampole) 16	1		4		5		4	1	1	

Method	Sample	Cn-Ag-2 Titers								
		Total # of laboratories	4	8	16	20	32	40	64	128
EIA										
	(Meridien Diagnostic) 2			1				1		
Latex Agglutination										
	(Immuno-Mycologics) 3			1				2		
	(Meridien Diagnostic) 39		1	6	1	25	1	3	1	1
	(Remel) 4	1				1		2		
	(Wampole) 16			5		6		3	1	1

Method	Sample	Cn-Ag-Edu Titers											
		Total # of laboratories	4	8	32	64	80	100	128	256	512	1024	2048
EIA													
	(Meridien Diagnostic) 2			1				1					
Latex Agglutination													
	(Immuno-Mycologics) 3							1	1		1		
	(Meridien Diagnostic) 39		1	4	12	1	1	14	2	2		1	1
	(Remel) 4	1			2			1					
	(Wampole) 16			2	7			3	3		1		

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