

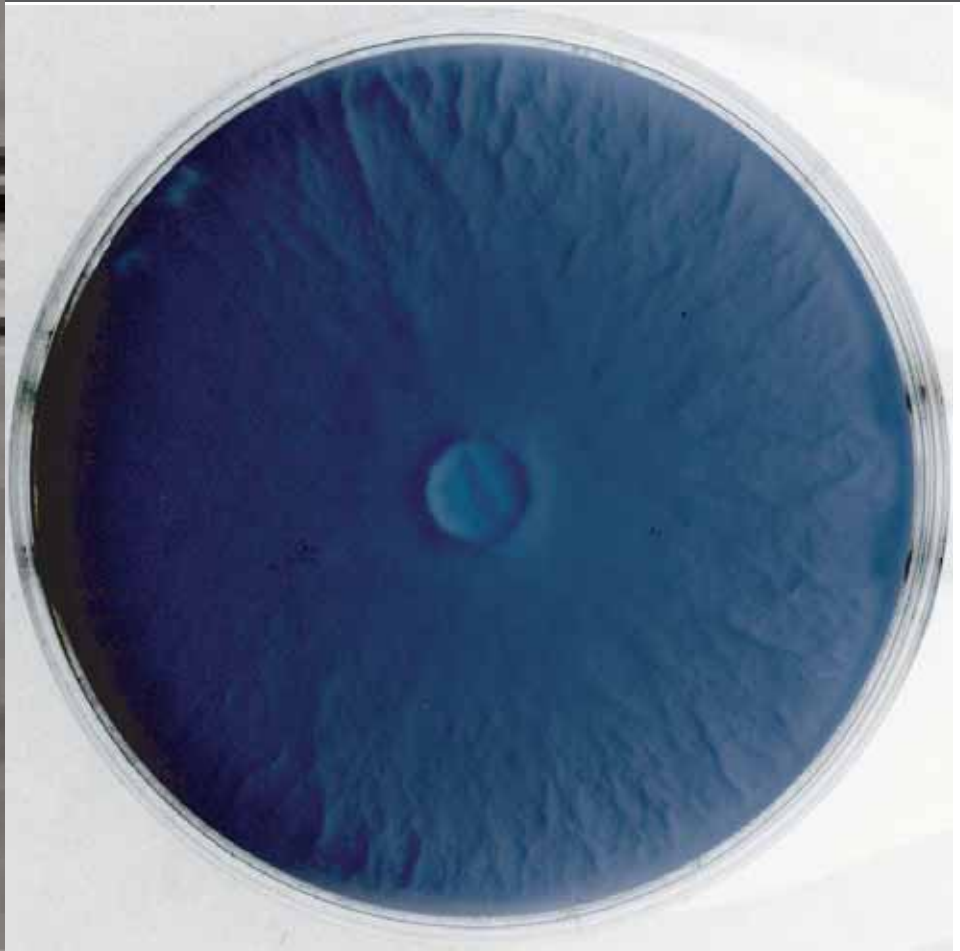
MYCOLOGY

CRITIQUE

Mycology Proficiency Testing Program
September 2004

Wadsworth Center

New York State Department of Health



Mycology PT Program

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Test Specimens and Grading Policy

Test Specimens

A minimum of two strains each of the proposed mold specimens was examined for inclusion in the proficiency test event of September 2004. 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 the appropriate test media. The single strain that best demonstrated the morphologic and physiologic characteristics was used in the test. Similarly, two or more strains of each of the proposed yeast pathogens 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. Additionally, 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 each of the proposed yeast pathogens was used in the test.

Grading Policy

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

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

Acceptable results for antifungal susceptibility testing are MICs within +/-2 dilutions of the reference result for a particular organism against a single drug. If a result falls outside of this range, the laboratory gets a score of zero for that particular test component or set. The current testing format is based on the two drugs amphotericin B and fluconazole. Five yeasts are to be tested against these two drugs. A test component/set involving one yeast against both drugs receives a maximum score of 20 (10 for first drug + 10 for second drug). The maximum total score is $5 \times 20 = 100$. However, a laboratory that routinely does not perform tests with either of the two drugs is scored with the maximum score for a single isolate against one drug. Again, for five yeasts isolates, the total will be $20 \times 5 = 100$.

For *Cryptococcus* antigen test, laboratories are evaluated on the basis of their responses and on overall performance for all the analytes tested in the Direct Detection category. Appropriate responses are determined by participant consensus requiring 80% agreement in the test. Qualitative/quantitative results are graded in relation to results given by participants for specific test kits. When the number of participants that used a specific test kit is less than 6, results are graded considering results given for the method used. Target values and acceptable ranges are mean value +/- 2 dilutions or positive or negative. 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.

Laboratories failure to attain an overall score of at least 80% is unsatisfactory performance. Laboratories failing two out of three consecutive proficiency test events for the permit category will fail the proficiency testing program for the category.

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

Answer Key

Mycology – General

Specimen Key	Validated Specimen	Other Acceptable Answers
M-1 <i>Conidiobolus coronatus</i>	<i>Conidiobolus coronatus</i>	
M-2 <i>Cunninghamella bertholletiae</i>	<i>Cunninghamella bertholletiae</i>	<i>Cunninghamella</i> sp.
M-3 <i>Syncephalastrum racemosum</i>	<i>Syncephalastrum racemosum</i>	<i>Syncephalastrum</i> sp.
M-4 <i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	<i>Rhizopus</i> sp.
M-5 <i>Mucor circinelloides</i>	<i>Mucor circinelloides</i>	<i>Mucor</i> sp. <i>Mucor racemosus</i>

Mycology – Yeast Only

Specimen Key	Validated Specimen	Other Acceptable Answers
Y-1 <i>Cryptococcus laurentii</i>	<i>Cryptococcus laurentii</i>	
Y-2 <i>Prototheca zopfii</i>	(Not validated)	
Y-3 <i>Sporobolomyces salmonicolor</i>	<i>Sporobolomyces salmonicolor</i>	
Y-4 <i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>	
Y-5 <i>Rhodotorula mucilaginosa</i>	<i>Rhodotorula mucilaginosa</i>	

Mycology – Antifungal Susceptibility Testing for Yeasts

Specimen Key	Reference range (µg/ml)		Validated range (µg/ml)	
	Amphotericin B	Fluconazole	Amphotericin B	Fluconazole
S-1 <i>Candida parapsilosis</i> ATCC 22019	0.25 – 1.0	2.0 – 8.0	0.12 – 2.0	1.0 – 16.0
S-2 <i>Candida krusei</i> ATCC 6258	0.25 – 2.0	16.0 – 64.0	0.12 – 4.0	8.0 – >64.0
S-3 <i>Candida albicans</i> ATCC 90028	0.5 – 2.0	0.25 – 1.0	0.25 – 4.0	0.12 – 2.0
S-4 <i>Candida albicans</i> ATCC 24433	0.25 – 1.0	0.25 – 1.0	0.12 – 2.0	0.12 – 2.0
S-5 <i>Candida tropicalis</i> ATCC 750	0.5 – 2.0	1.0 – 4.0	0.25 – 4.0	0.5 – 8.0

Mycology – Direct detection (Cryptococcus Antigen Test)

Specimen Key	Validated Specimen	Other Acceptable Titer Range
Cn-Ag-1 Positive (1:8)	Positive (1:8)	1:2 – 1:32
Cn-Ag-2 Negative	Negative	
Cn-Ag-3 Negative	Negative	
Cn-Ag-4 Negative	Negative	1:32 – 1:512
Cn-Ag-5 Positive (1:128)	Positive (1:128)	

Laboratory Results

Mycology - General	Correct Responses/ Total # Laboratories (%)	Referees (%)
M – 1 <i>Conidiobolus coronatus</i>	81/82 (99)	10/10 (100)
M – 2 <i>Cunninghamella bertholletiae</i>	82/82 (100)	10/10 (100)
M – 3 <i>Syncephalastrum racemosum</i>	82/82 (100)	10/10 (100)
M – 4 <i>Rhizopus oryzae</i>	78/82 (95)	10/10 (100)
M – 5 <i>Mucor circinelloides</i>	70/82 (85)	9/10 (90)

Mycology - Yeast Only	Correct Responses/ Total # Laboratories (%)	Referees (%)
Y – 1 <i>Cryptococcus laurentii</i>	59/59 (100)	10/10 (100)
Y – 2 <i>Prototheca zopfii</i> (Not validated)	47/60 (78)	8/10 (80)
Y – 3 <i>Sporobolomyces salmonicolor</i>	60/60 (100)	10/10 (100)
Y – 4 <i>Candida parapsilosis</i>	56/59 (95)	10/10 (100)
Y – 5 <i>Rhodotorula mucilaginosa</i>	56/59 (100)	10/10 (100)

Mycology - Antifungal Susceptibility Testing for Yeasts

	Correct Responses/Total # Laboratories (%)	
	Amphotericin B	Fluconazole
S- 1 <i>Candida tropicalis</i> ATCC 750	22/22 (100)	27/27 (100)
S- 2 <i>Candida krusei</i> ATCC 6258	22/22 (100)	27/27 (100)
S- 3 <i>Candida parapsilosis</i> ATCC 22019	22/22 (100)	27/27 (100)
S- 4 <i>Candida parapsilosis</i> ATCC 90018	22/22 (100)	24/27 (89)
S- 5 <i>Candida albicans</i> ATCC 90028	22/22 (100)	26/27 (96)

Mycology - Direct detection (*Cryptococcus* Antigen Test)

	Correct Responses/Total # Laboratories (%)	
	Qualitative	Quantitative
Cn-Ag-1 Positive (1:8)	84/85 (99)	82/85 (96)
Cn-Ag-2 Negative	85/85 (100)	NA
Cn-Ag-3 Negative	85/85 (100)	NA
Cn-Ag-4 Negative	85/85 (100)	NA
Cn-Ag-5 Positive (1:128)	85/85 (100)	80/85 (94)

	General	Yeast Only	Antifungal Susceptibility Testing for Yeasts	Direct Detection
Number of participating laboratories	82	61	27	85
Number of referee laboratories	10	10	3	85
Number of laboratories responding by deadline	82	61	27	85
Number of laboratories responding after deadline	0	0	0	0
Number of laboratories not responding	0	0	0	0
Number of laboratories successfully completing this test	79	60	27	85
Number of laboratories unsuccessfully completing this test	3	1	0	0

Number of Laboratories Using Commercial Yeast Identification System*

API 20C AUX	51
AMS Vitek system	26
Remel Uni-Yeast-Tek	7
IDS Rapid System	1
Microscan	1

Number of Laboratories Using Antifungal Susceptibility System/Method

YeastOne Colorimetric microdilution method	14
Broth microdilution method	9
Etest	3
Disk diffusion method	1

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

EIA method	3
Meridien Diagn.	3
Latex Agglutination method	74
Meridien Diagn.	37
Murex	23
Wampole	13
Immuno-Mycologics	1
Not specified	8

(*Includes multiple systems used by some laboratories)

Source: Skin

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	81
Labs with incorrect ID:	1
<i>(Basidiobolus ranarum)</i>	(1)

Clinical Significance: *Conidiobolus coronatus* causes infections most commonly as chronic sinusitis zygomycosis. Also, infections in pharynx and larynx, leading to dysphagia and obstruction of the larynx as well as extensive and chronic lymphedema, have been reported.

Ecology: *C. coronatus* is found worldwide in soils and plant detritus. It is more concentrated in warm, wet climates, particularly countries in West Africa.

Laboratory Diagnosis:

1. **Culture** – *C. coronatus* grew rapidly. On Sabouraud’s dextrose agar, at 25°C after 5 days, colony was white, waxy to powdery on the surface (Figure 1).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed unbranched conidiophores. The conidiophores (sporangiophores) produced single-cell (one-spored) large sporangiole with papilla (Figure 2).
3. **Differentiation from other Zygomycetes** – *C. coronatus* was distinguished from *Basidiobolus* by its conidia, which were round to pyriform with prominent papillae. Conidia of *C. coronatus* also might produce hair-like appendages called villae. In contrast to *Basidiobolus* and *C. incongruus*, *C. coronatus* is heterothallic and rarely produces zygospore.
4. **In vitro susceptibility testing** – *In vitro* susceptibility testing using amphotericin B revealed both sensitive and resistant strains. The combinations of sulfamethoxazole-trimethoprim and potassium iodide, ketoconazole and saturated potassium iodide, septrim and ketoconazole, and amphotericin B and terbinafine have reportedly been effective in treating *Conidiobolus* infections where single-drug therapy had failed.
5. **Molecular tests** – Real-time PCR was reported to be a promising tool for the diagnosis of invasive fungal infections in human tissue samples.

Comments: All the participating laboratories except one correctly reported this specimen. *Basidiobolus* is distinguished from *Conidiobolus* by its zygospores with conjugation beaks. *Conidiobolus* possesses sporangiophores with unswollen apices and sporangioles, which, once ejected, possess a papilla.

Further Reading:

1. Gugnani, H.C., Ezeanolue, B.C., Khalil, M., Amoah, C.D., Ajuiu, E.U., and Oyewo, E.A. 1995. Fluconazole in the therapy of tropical deep mycoses. *Mycoses*. 38: 485-488.
2. Imhof, A., Schaer, C., Schoedon, G., Schaer, D.J., Walter, R.B., Schaffner, A., and Schneemann, M. 2003. Rapid detection of pathogenic fungi from clinical specimens using LightCycler real-time fluorescence PCR. *Eur. J. Clin. Microbiol. Infect. Dis.* 22: 558-60.
3. Mukhopadhyay, D., Ghosh, L.M., Thammayya, A., and Sanyal, M. 1995. Entomophthoromycosis caused by *Conidiobolus coronatus*: clinicomycological study of a case. *Auris. Nasus. Larynx*. 22: 139-142.
4. Ribes, J.a., Vanover-Sams, C.L., and Baker, D.J. 2000. Zygomycetes in human disease. *Clin. Microbiol. Rev.* 13: 236-301.
5. Valle, A.C., Wanke, B., Lazera, M.S., Monteiro, P.C., and Viegas, M.L. 2001. Entomophthoramycosis by *Conidiobolus coronatus*. Report of a case successfully treated with the combination of itraconazole and fluconazole. *Rev. Inst. Med. Trop. Sao. Paulo.* 43: 233-236.

Source: Bronchial Walsh

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	82
Labs with incorrect ID:	0

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

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

Laboratory Diagnosis:

1. Culture – *C. bertholletiae* grew fast. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony showed white to gray color on the surface and cottony texture (Figure 3, left panel). Reverse appeared pale or buff (Figure 3, right panel).
2. Microscopic morphology – Lactophenol cotton blue mount showed broad, hyaline, and aseptate hyphae. Sporangioophores are branched with swollen vesicles at the end. Vesicles are covered with single-spored sporangioles supported by short denticles projecting from the vesicle (Figure 4).
3. Differentiation from other Zygomycetes – *C. bertholletiae* is distinct from other common Zygomycetes by their single-spored sporangia formed on denticles on the vesicle surface.
4. In vitro susceptibility testing – *C. bertholletiae* is susceptible to amphotericin B but resistant to ketoconazole and miconazole.
5. Molecular tests – No information available.

Comments: All the participating labs reported the acceptable answers for this specimen.

Further Reading:

1. Garey, K.W., Pendland, S.L., Huynh, V.T., Bunch, T.H., Jensen, G.M., and Pursell, K.J. 2001. *Cunninghamella bertholletiae* infection in a bone marrow transplant patient: amphotericin lung penetration, MIC determinations, and review of the literature. *Pharmacotherapy* 21: 855-860.
2. Lemmer, K., Losert, H., Rickerts, V., Just-Nubling, G., Sander, A., Kerkmann, M.L., Tintelnot, K. 2002. Molecular biological identification of *Cunninghamella* species. *Mycoses* 45 1:31-36.
3. Ribes, J.A., Vanover-Sams, C.L., and Baker, D.J. 2000. Zygomycetes in human disease. *Clin. Microbiol. Rev.* 13: 236-301.
4. Rickerts, V., Bohme, A., Viertel, A., Behrendt, G., Jacobi, V., Tintelnot, K., Just-Nubling, G. 2000. Cluster of pulmonary infections caused by *Cunninghamella bertholletiae* in immunocompromised patients. *Clin. Infect. Dis.* 31: 910-913.
5. Sato, M., Gemma, H., Sano, T., Ono, T., Atsumi, E., Ito, I., Chida, K., Nakamura, H. 2001. Pulmonary mucormycosis caused by *Cunninghamella bertholletiae* in a non-immunocompromised woman. *Nihon Kokyuki Gakkai Zasshi* 39: 758-762.
6. Tomsikova, A. 2002. Causative agents of nosocomial mycoses. *Folia Microbiol.* 47: 105-112.

Source: Lung Biopsy

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	82
Labs with incorrect ID:	0

Clinical Significance: *Syncephalastrum racemosum* is rarely known to be a pathogen in humans.

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

Laboratory Diagnosis:

1. **Culture** – *S. racemosum* grew rapidly. On Sabouraud’s dextrose agar, after 5 days at 25°C, the colony produced low-growing or tall erect mycelia that cover the whole plate. The surface were white to gray and black (Figure 5, left panel), and pale brown on reverse (Figure 5, right panel).
2. **Microscopic morphology** – Lactophenol cotton blue mount showed aseptate hyphae. Sporangiphores were branched and ended in round or oval terminal vesicles called ampullae. The vesicles were surrounded by rod- or club-shaped structure called merosporangia in which the multiple round to oval sporangiospores developed (Figure 6).
3. **Differentiation from other species** – It may be necessary to distinguish between the vesicle structures of *S. racemosum* and *Aspergillus* conidiphores.
4. **In vitro susceptibility testing** – No information available.
5. **Molecular tests** – The nuclear small-subunit (18S) ribosomal DNA and domains D1 and D2 of the nuclear large-subunit (28S) ribosomal DNA was used to investigate phylogenetic relationships among representative species of Zygomycetes.

Comments: All participating laboratories correctly identified this specimen.

Further Reading:

1. Kamalam, A. and Thambiah, A.S. 1980. Cutaneous infection by *Syncephalastrum*. *Sabouraudia* 18: 19-20.
2. Voigt, K., Cigelnik, E., and O’donnell, K. 1999. Phylogeny and PCR identification of clinically important Zygomycetes based on nuclear ribosomal-DNA sequence data. *J Clin Microbiol.* 37: 3957-3964.

Source: Sinus

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	83
Labs with incorrect ID:	4
(<i>Rhizomucor pusillus</i>)	(3)
(<i>Mucor sp.</i>)	(1)

Clinical Significance: *Rhizopus oryzae* is one of the most common organisms isolated from patients with zygomycetes. It causes angioinvasion, thrombosis, infarction, and necrosis of the involved tissues. The sites of infection most often involved are sinuses and rhinocerebral structures. Disseminated disease can involve virtually any organ in the body, with the skin, central nervous system, liver, spleen, and kidney being most common.

Ecology: *R. oryzae* has a worldwide distribution in soil, hay, decaying grass, and a variety of food stuffs.

Laboratory Diagnosis:

- Culture** – *R. oryzae* grew rapidly. On Sabouraud's dextrose agar, after 5 days at 25°C, floccose aerial mycelia covered whole plate, grayish in color on surface (Figure 7, left panel) and yellow to light brown on reverse (Figure 7, right panel).
- Microscopic morphology** – Lactophenol cotton blue mount showed broad, aseptate hyphae, either single or tufts of brown sporangiophores (conidiophores) arising from hyphae (stolons) opposite well-developed rhizoids (root like structures). Sporangiophores end in sporangia with a round columella (vesicle, enlarged at the apex), producing round to oval sporangiospores or sexual spores (Figure 8).
- Differentiation from other Zygomycetes** – *R. oryzae* was distinguished from other Zygomycetes by the presence of well-developed rhizoids situated opposite sporangiophores. Sporangiophores were unbranched and in tufts unlike in *Mucor*, *Rhizomucor*, *Absidia*. *Rhizopus* spp. produced striated or grooved sporangiospores, which was useful in differentiating *Rhizopus* spp. from *Absidia*, *Mucor*, and *Thamnidium* spp., all of which produce smooth sporangiospores.
- In vitro susceptibility testing** – Most clinical isolates were susceptible to amphotericin B.
- Molecular tests** – PCR assay for the rapid and accurate identification of the agents of mucormycosis has been reported.

Comments: Three laboratories reported this specimen as *Rhizomucor pusillus*, which has no apophysis or very tiny; sporangiophores are mostly unbranched in *Rhizopus oryzae* which they are branched in *Rhizomucor pusillus*. One laboratory reported this specimen as *Mucor sp.*, which can be differentiated from *Rhizopus* spp. by the absence of rhizoids and stolons.

Further Reading:

- Dannaoui, E., Meletiadis, J., Mouton, J.W., Meis, J.F., Verweij, P.E. and Eurofung Network. 2003. *In vitro* susceptibilities of zygomycetes to conventional and new antifungals. *J. Antimicrob. Chemother.* 51: 45-52.
- Gomez-Lopez, A., Cuenca-Estrella, M., Mellado, E., and Rodriguez-Tudela, J.L. 2003. In vitro evaluation of combination of terbinafine with itraconazole or amphotericin B against Zygomycota. *Diagn. Microbiol. Infect. Dis.* 45: 199-202.
- Hilal, A.A., Taj-Aldeen, S.J., and Mirghani, A.H. 2004. Rhinoorbital mucormycosis secondary to *Rhizopus oryzae*: a case report and literature review. *Ear Nose Throat J.* 83: 556, 558-60, 562.
- Romano, C., Miracco, C., Massai, L., Piane, R., Alessandrini, C., Petrini, C., and Luzzi, P. 2002. Case report. Fatal rhinocerebral zygomycosis due to *Rhizopus oryzae*. *Mycoses.* 45: 45-9.
- Voigt, K., Cigelnik, E., and O'donnell, K. 1999. Phylogeny and PCR identification of clinically important zygomycetes based on nuclear ribosomal-DNA sequence data. *J. Clin. Microbiol.* 37: 3957-3964.

Source: Lung

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	70
Labs with incorrect ID:	12
(<i>Basidiobolus ranarum</i>)	(8)
(<i>Basidiobolus sp.</i>)	(2)
(<i>Chrysosporium sp.</i>)	(2)

Clinical Significance: *Mucor circinelloides* is rarely associated with human disease. It may cause superficial nail infection.

Ecology: *M. circinelloides* is saprobic and ubiquitous in nature. It is found in soil and environmental samples.

Laboratory Diagnosis:

1. Culture – *M. circinelloides* was rapid growing. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony was pale gray or yellowish on surface (Figure 9, left panel). Reverse appeared pale yellow (Figure 9, right panel).
2. Microscopic morphology – Lactophenol cotton blue mount showed hyaline hyphae. Sporangiohores were sympodially branched. Sporangiohores bear spherical sporangia that were variable in size. Columellae were somewhat spherical, but might vary in shape. Sporangiospores were smooth walled, ellipsoidal, and hyaline (Figure 10). Chlamydoconidia occasionally were produced in some cultures; they were spherical, cylindrical, or irregular in shape.
3. Differentiation from other Zygomycetes – *M. circinelloides* differs from *Rhizopus* and *Rhizomucor* by the absence of rhizoids, and from *Absidia* by absence of an apophysis. The maximum temperature for growth in *Mucor* is less than 37°C, but *Rhizomucor* can grow at about 54°C. *M. circinelloides* is positive for nitrate assimilation.
4. In vitro susceptibility testing – None of the triazoles were active against *Mucor* spp. (MIC₅₀ > 8 µg/ml).
5. Molecular tests – No information available.

Comments: This specimen was incorrectly identified it as *Basidiobolus ranarum* or *Basidiobolus sp.* *Basidiobolus* grows moderate to rapid at 30°C and less rapid at 37°C. Colonies are flat and furrowed, are yellowish to grayish on the surface with a pale reverse, and have a waxy texture. It produces two types of sporangiophores. Sporangiohores with inflated apices produce single-spored sporangioles (ballistospores or conidia). Conidia are ejected from the inflated, apical portion of the sporangiophore when the subconidial vesicle or swelling emits a stream of fluid that propels the conidium. This propulsion of conidia is a characteristic of the genus. Narrow sporangiophores with adhesive apices also produce single-spored sporangioles, but these sporangioles are released passively. Sporangiospores may undergo cleavage to produce meristospores. They may also produce secondary sporangia.

Further Reading:

1. Chandra, S. and Woodgyer, A. 2002. Primary cutaneous zygomycosis due to *Mucor circinelloides*. *Australas. J. Dermatol.* 43: 39-42.
2. Fingerroth, J.D, Roth, R.S., Talcott, J.A., and Rinaldi, M.G. 1994. Zygomycosis due to *Mucor circinelloides* in a neutropenic patient receiving chemotherapy for acute myelogenous leukemia. *Clin. Infect. Dis.* 19:135-137.
3. Stewart, N.J., Munday, B.L., and Hawkesford, T. 1999. Isolation of *Mucor circinelloides* from a case of ulcerative mycosis of platypus (*Ornithorhynchus anatinus*), and a comparison of the response of *Mucor circinelloides* and *Mucor amphibiorum* to different culture temperatures. *Med. Mycol.* 37: 201-206.

Source: CSF

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	59
Labs with incorrect ID:	0

Clinical Significance: *Cryptococcus laurentii* has been infrequently reported as an etiologic agent of infections in humans. In susceptible hosts such as diabetics, immunocompromised individuals, etc., several cases ranging from fungemia to eye infections, have been documented.

Ecology: *C. laurentii* is found in various sources 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 11).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *C. laurentii* showed round to oval cells (Figure 12). 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: All participating laboratories reported this specimen correctly.

* Size of the capsule determines the texture of the colony – strains with large capsule produce mucoid colonies while strains with tiny capsule produce dull, glassy texture colonies.

Further Reading:

1. Averbuch, D., Boekhout, 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.
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7. Sugita, T., Takashima, M., Ikeda, R., Nakase, T., and Shinoda, T. 2000. Intraspecies diversity of *Cryptococcus laurentii* as revealed by sequences of internal transcribed spacer regions and 28S rRNA gene and taxonomic position of *C. laurentii* clinical isolates. *J. Clin. Microbiol.* 38: 1468-1471.
8. Vlchikova-Lashkoska, M., Kamberova, S., Starova, A., Goleva-Mishevskva, L., Tsatsa-Biljanovska, N., Janevska, V., and Petrovska, M. 2004. Cutaneous *Cryptococcus laurentii* infection in a human immunodeficiency virus-negative subject. *J. Eur. Acad. Dermatol. Venereol.* 18: 99-100.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 regions of rDNA. The sequence is deposited in GenBank under the accession numbers AY591353.

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1
(AF410468) TCCGTAGGTG AACCTGCGGA AGGATCATT AAGATTGACC GAAAGGTCTT
ATCC18803 (AY591353) TCCGTAGGTG AACCTGCGGA AGGATCATT AAGATTGACC GAAAGGTCTT

51
ATCTCTATAT CCCTCACCTC TGTGAACTGT GGACCTCCGG GTCTGTCTTA
ATCTCTATAT CCCTCACCTC TGTGAACTGT GGACCTCCGG GTCTGTCTTA

101
ACAAACATCA GTGTAATGAA CGTATAAATC ATTAAACAAA ACAAACCTTT
ACAAACATCA GTGTAATGAA CGTATAAATC ATTAAACAAA ACAAACCTTT

151
CAACAACGGA TCTCTTGGCT CTCGCATCGA TGAAGAACGC AGCGAAATGC
CAACAACGGA TCTCTTGGCT CTCGCATCGA TGAAGAACGC AGCGAAATGC

201
GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG
GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG

251
CACCTTGCGC CTTTTGGTAT TCCGAAAGGC ATGCCTGTTT GAGTGTCTATG
CACCTTGCGC CTTTTGGTAT TCCGAAAGGC ATGCCTGTTT GAGTGTCTATG

301
AAATCTCAAT CCCCCGGGT TTATGATCTG GTCGGGACTT GGACATGGGC
AAATCTCAAT CCCCCGGGT TTATGATCTG GTCGGGACTT GGACATGGGC

351
GTCTGCCGGT CACACGGCTC GCCTCAAATG ACTTAGTGGA TCTCTCTGCA
GTCTGCCGGT CACACGGCTC GCCTCAAATG ACTTAGTGGA TCTCTCTGCA

401
TCCGTGACAG ACGTAATAAG TTTCGTCTTG TCCCTTGCGT ACGAGTCCGC
TCCGTGACAG ACGTAATAAG TTTCGTCTTG TCCCTTGCGT ACGAGTCCGC

451
TCATAACCTG CCATCGCGCA CTTTAGACTC TGACCTCAAA TCAGGTAGGA
TCATAACCTG CCATCGCGCA CTTTAGACTC TGACCTCAAA TCAGGTAGGA

510
CTACCCGCTG AACTTAAGCA TATCAATAAG CGGAGGA
CTACCCGCTG AACTTAAGCA TATCAATAAG CGGAGGA

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Alignment of primary sequences of the ITS1 and ITS2 regions of *Cryptococcus laurentii* and PT specimen *C. laurentii* ATCC18803. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Source: Sputum

Scoring:	No. Labs
Referee Labs with correct ID:	8
Labs with correct ID:	47
Labs with incorrect ID:	13
(<i>Prototheca wickerhamii</i>)	(7)
(<i>Prototheca sp.</i>)	(2)
(<i>Blastoschizomyces capitatus</i>)	(1)
(<i>Candida krusei</i>)	(1)
(<i>Geotrichum candidum</i>)	(1)
(<i>Trichosporon asabii</i>)	(1)

Clinical Significance: *Prototheca zopfii* is a causal agent of cutaneous lesions, peritonitis, olecranon bursitis and disseminated disease in immunosuppressed patients.

Ecology: *P. zopfii* is algae that lack chlorophyll, but are closely related to the green algae chlorella. It is ubiquitous in nature and has been isolated from a wide variety of sources including sewage, soil, and many water sources, including both fresh and salt water.

Laboratory Diagnosis:

- Culture – On Sabouraud’s dextrose agar after 5 days at 25°C, colony was dull white, and smooth (Figure 13).
- Microscopic morphology – On corn meal agar with Tween 80, round, immature sporangia of various sizes and mature sporangia filled with sporangiospores were seen (Figure 14). No blastoconidia or pseudo- and true hyphae were formed.
- Differentiation from other yeasts – *P. zopfii* did not grow on media containing cycloheximide, but grew well at 37°C. It was nitrate and urease negative. *P. zopfii* did not assimilate trehalose, which differentiated it from *P. wickerhamii*. It did not produce any zone of inhibition to 50 µg clotrimazole disk at 37°C, also could be used for differentiation *P. zopfii* from *P. wickerhamii*.
- In vitro susceptibility testing – Most of the clinical isolates are susceptible to amphotericin B, variably susceptible to itraconazole and ketoconazole, but resistance to flucytosine and fluconazole.
- Molecular tests – Sequence analysis of the imtrochondrial small subunit rRNA from *P. wickerhamii* showed higher homology with mitochondrial sequence from plants.

Comments: This specimen was not validated although it was sent as an educational specimen in January 2002 PT Event. Seven laboratories reported it as *P. wickerhamii*, which can be distinguished from *P. zopfii* by assimilation of trehalose. One laboratory each reported it as *Blastoschizomyces capitatus*, which has pseudohyphae and true hyphae, as *Geotrichum candidum*, which has true hyphae and does not grow on Sabouraud dextrose agar at 37°C, and as *Candida krusei* or *Trichosporon asabii*, which are urease positive.

Further Reading:

- Casal, M.J. and Gutierrez Aroca, J. 1995. Simple new test for rapid differentiation of *Prototheca stagnora* from *P. wickerhamii* and *P. zopfii*. *Mycopathologia*. 130: 93-94.
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- Linares, M.J., Munoz, J.F., Solis, F., Rodriguez, E.C., Valero, A., and Casal, M. 1998. Study of the susceptibility of yeast isolates of clinical interest to five antifungal agents using the Etest. *Rev. Esp. Quimioter.* 11: 64-69.
- Van Bezooijen, B.P. and Newling, D.W. 2002. Protothecosis of the urinary tract. *J. Urol.* 167: 252.
- Zhao, J., Liu, W., Lv, G., Shen, Y., and Wu, S. 2004. Protothecosis successfully treated with amikacin combined with tetracyclines. *Mycoses.* 47: 156-158.

Source: Blood

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	60
Labs with incorrect ID:	0

Clinical Significance: *Sporobolomyces salmonicolor* is an infrequent casual agent of dermatitis. It has also been isolated from blood, sputum, and urine as a contaminant.

Ecology: *S. salmonicolor* is cosmopolitan, isolated from air and plants. It has also been isolated from skin of humans.

Laboratory Diagnosis:

1. Culture – On Sabouraud’s dextrose agar after 5 days at 25°C, colony was coral to salmon pink color, wrinkled, sometimes with satellite colonies (Figure 16).
2. Microscopic morphology – On corn meal agar with Tween 80, oval to elongated yeast like cells and kidney-shaped ballistoconidia were seen (Figure 17). True hyphae and pseudohyphae were rarely produced.
3. Differentiation from other yeasts – *S. salmonicolor* produced abundant satellite colonies from the ballistoconidia, differentiating it from pink-red *Rhodotorula* species.
4. In vitro susceptibility testing – Most isolates of *S. salmonicolor* are susceptible to amphotericin B and to commonly used azoles like fluconazole and itraconazole.
5. Molecular tests – Cytochrome b sequences were used for both species identification and the study of phylogenetic relationships among basidiomycetous yeast. The random amplified polymorphic DNA (RAPD) method was used to differentiate the members of *Sporobolomyces* from *Sterigmatomyces* and *Tilletiopsis*.

Comments: All the participating laboratories correctly identified this specimen.

Further Reading:

1. Biswas, S.K., Yokoyama, K., Nishimura, K., and Miyaji, M. 2001. Molecular phylogenetics of the genus *Rhodotorula* and related basidiomycetous yeasts inferred from the mitochondrial cytochrome b gene. *Int. J. Syst. Evol. Microbiol.* 51: 1191-1199.
2. Espinel-Ingroff A. 1998. In vitro activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. *J. Clin. Microbiol.* 36: 198-202.
3. Espinel-Ingroff, A., Pfaller, M., Messer, S.A., Knapp, C.C., Killian, S., Norris, H.A., and Ghannoum, M.A. 1999. Multicenter comparison of the sensititre YeastOne Colorimetric Antifungal Panel with the National Committee for Clinical Laboratory standards M27-A reference method for testing clinical isolates of common and emerging *Candida* spp., *Cryptococcus* spp., and other yeasts and yeast-like organisms. *J. Clin. Microbiol.* 37: 591-595.
4. Messner, R., Prillinger, H., Altmann, F., Lopandic, K., Wimmer, K., Molnar, O., and Weigang, F. 1994. Molecular characterization and application of random amplified polymorphic DNA analysis of *Mrakia* and *Sterigmatomyces* species. *Int. J. Syst. Bacteriol.* 44: 694-703.
5. Serena, C., Pastor, F.J., Ortoneda, M., Capilla, J., Nolard, N., and Guarro, J. 2004. In vitro antifungal susceptibilities of uncommon basidiomycetous yeasts. *Antimicrob. Agents Chemother.* 48: 2724-2726.
6. Seuri, M., Husman, K., Kinnunen, H., Reiman, M., Kreus, R., Kuronen, P., Lehtomaki, K., and Paananen, M. 2000. An outbreak of respiratory diseases among workers at a water-damaged building--a case report. *Indoor Air.* 10: 138-45.

Source: Rectal Swab

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	56
Labs with incorrect ID:	3
(<i>Candida albicans</i>)	(1)
(<i>Candida dubliniensis</i>)	(1)
(<i>Candida lambica</i>)	(1)

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

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

Laboratory Diagnosis:

- Culture** – On Sabouraud's dextrose agar after 5 days at 25°C, colony was white to cream, dull with smooth surface (Figure 17).
- Microscopic morphology** – On corn meal agar with Tween 80, long, multibranched pseudohyphae, together with small elongated blastoconidia clustered on them, were seen (Figure 18).
- Differentiation from other yeasts** – *C. parapsilosis* fermented glucose, but not maltose, sucrose, lactose, or trehalose. It did not grow on media containing cycloheximide, but it grew at 37°C. It assimilated glucose, maltose, and sucrose, but it was urease- and nitrate-negative. Biochemically, it was very similar to *C. lusitanae*, but microscopically, it formed long pseudohyphae that differentiated it from *C. lusitanae*.
- In vitro susceptibility testing** – *C. parapsilosis* is susceptible to amphotericin B, 5-flucytosine, and azoles such as fluconazole, ketoconazole, and itraconazole. A few clinical isolates are resistant to fluconazole.
- Molecular tests** – PCR assay of ITS regions of rDNA was used to identify *C. parapsilosis* in clinical specimens. Chromosome length polymorphism and RAPD procedures were used to characterize the genetic diversity of this organism.

Comments: One laboratory each reported this specimen as *C. albicans*, *C. dubliniensis*, and *C. lambica*. *C. parapsilosis* can not grow on the media containing cycloheximide, which differentiate it from *C. albicans* and *C. dubliniensis*. *C. parapsilosis* assimilate sucrose, which differentiate it from *C. lambica*.

Further Reading:

- Costa, S.F., Marinho, I., Araujo, E.A., Manrique, A.E., Medeiros, E.A., Levin, A.S. 2000. Nosocomial fungemia: a 2 – year prospective study. *J. Hospital Infect.* 45: 69-72.
- Da Silva, C.L., dos Santos, R.M., and Colombo, A.L. 2001. Cluster of *Candida parapsilosis* primary bloodstream infection in a neonatal intensive care unit. *Braz. J. Infect. Dis.* 5: 32-36.
- Dassanayake, R.S., and Samaranyake, L.P. 2000. Characterization of the genetic diversity in superficial and systemic human isolates of *Candida parapsilosis* by randomly amplified polymorphic DNA (RAPD). *APMIS.* 108: 153-160.
- Fujita S. and Hashimoto, T. 2000. DNA fingerprinting patterns of *Candida* species using *HinfI* endonuclease. *International J. Systematic & Evolutionary Microbiology.* 50: 1381-1389.
- Jones, J.M., Sarsam, M.A., Clarke, M.A., and Hedderwick, S.A. 2002. *Candida parapsilosis*: two cases of endocarditis in association with the Toronto stentless porcine valve. *J. Infect.* 44: 196-198.
- Segal, R., Kimchi, A., Kritzman, A., Inbar, R., and Segal, Z. 2000. The frequency of *Candida parapsilosis* in onychomycosis. An epidemiological survey in Israel. *Mycoses* 43: 349-353.
- Wong, P.N., Mak, S.K., Lo, K.Y., Tong, G.M., and Wong, A.K. 2000. A retrospective study of seven cases of *Candida parapsilosis* peritonitis in CAPD patients: the therapeutic implications. *Peritoneal Dialysis International* 20: 76-79.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 and ITS2 rDNA. The sequences are deposited in GenBank under the accession numbers AY217021 and AY217022, respectively.

```

1
wb176 (AF455530)      TTGGAAGTTA AAAGTCGTAA CAAGGTTTCC GTAGGTGAAC CTGCGGAAGG
ATCC 22019 (AY217021)      TCC GTAGGTGAAC CTGCGGAAGG

51
ATCATTACAG AATGAAAAGT GCTTAACTGC ATTTTTTCTT ACACATGTGT
ATCATTACAG AATGAAAAGT GCTTAACTGC ATTTTTTCTT ACACATGTGT

101
TTTTCTTTTT TTGAAAACCT TGCTTTGGTA GGCCTTCTAT ATGGGGCCTG
TTTTCTTTTT TTGAAAACCT TGCTTTGGTA GGCCTTCTAT ATGGGGCCTG

151
CCAGAGATTA AACTCAACCA AATTTTATTT AATGTCAACC GATTATTTAA
CCAGAGATTA AACTCAACCA AATTTTATTT AATGTCAACC GATTATTTAA

201
TAGTCAAAAC TTTCAACAAC GGATCTCTTG GTTCTCGCAT CGATGAAGAA
TAGTCAAAAC TTTCAACAAC GGATCTCTTG GTTCTCGCAT CGATGAAGAA

251
CGCAGCGAAA
CGCAGC

```

Alignment of primary sequences of the ITS1 regions of *C. paraposilosis* wb176 and PT specimen *C. paraposilosis* ATCC 22019. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
wb176 (AF455530)      CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATATGAATT GCAGATATTC
ATCC 22019 (AY217022) CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATATGAATT GCAGATATTC

51
GTGAATCATC GAATCTTTGA ACGCACATTG CGCCCTTTGG TATTCCAAAG
GTGAATCATC GAATCTTTGA ACGCACATTG CGCCCTTTGG TATTCCAAAG

101
GGCATGCCTG TTTGAGCGTC ATTTCTCCCT CAAACCCTCG GGTTTGGTGT
GGCATGCCTG TTTGAGCGTC ATTTCTCCCT CAAACCCTCG GGTTTGGTGT

151
TGAGCGATAC GCTGGGTTTG CTTGAAAGAA AGGCGGAGTA TAAACTAATG
TGAGCGATAC GCTGGGTTTG CTTGAAAGAA AGGCGGAGTA TAAACTAATG

201
GATAGGTTTT TTCCACTCAT TGGTACAAAC TCCAAAACCTT CTTCCAAATT
GATAGGTTTT TTCCACTCAT TGGTACAAAC TCCAAAACCTT CTTCCAAATT

251
CGACCTCAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG
CGACCTCAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG

301
CGGAGGA
CGGAGGAA

```

Alignment of primary sequences of the ITS2 regions of *C. paraposilosis* wb176 and PT specimen *C. paraposilosis* ATCC 22019. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

Source: Nail

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	56
Labs with incorrect ID:	3
(<i>Rhodotorula glutinis</i>)	(1)
(<i>Rhodotorula sp.</i>)	(1)

Clinical Significance: *Rhodotorula mucilaginosa* is a not very common casual agent of catheter-associated fungemia, dialysis-related peritonitis, and post surgery ventriculitis, endocarditis and meningitis.

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

Laboratory Diagnosis:

- Culture** – On Sabouraud’s dextrose agar after 7 days at 25°C, colony was smooth, moist, soft, pink to coral red (Figure 19).
- Microscopic morphology** – On corn meal agar with Tween 80, oval to round yeast cells, sometimes in short chains were seen (Figure 20). Rarely a faint capsule and rudimentary pseudohyphae were observed.
- Differentiation from other yeasts** – *R. mucilaginosa* did not ferment any carbohydrate, grew at 37°C, but did not grow on media containing cucloheximide. It formed pink pigment, thereby differentiating it from other yeast species. It did not produce ballistoconidia, thus differing from *Sporobolomyces* species. *R. mucilaginosa* did not assimilate nitrate or nitrite, which distinguished it from *R. glutinis*.
- In vitro susceptibility testing** – *R. mucilaginosa* is susceptible to amphotericin B and 5-fluorocytosine, variably susceptible to itraconazole, and resistant to fluconazole.
- Molecular tests** – Using species-specific oligonucleotide primers for PCR, identification of the basidiomycetous yeasts *Cryptococcus neoformans*, *Trichosporon cutaneum*, and *R. mucilaginosa* were done from single and mixed yeast populations. The cytochrome b sequences were used to identify various genera and species, phylogenetic relationship among basidiomycetous yeasts.

Comments: Two participating laboratories have reported this isolate as *R. glutinis*, which can be distinguished from *R. mucilaginosa* by nitrate assimilation test.

Further Reading:

- Biswas, S.K., Yokoyama, K., Nishimura, K., and Miyaji, M. 2001. Molecular phylogenetics of the genus *Rhodotorula* and related basidiomycetous yeasts inferred from the mitochondrial cytochrome b gene. *Int. J. Syst. Evol. Microbiol.* 51: 1191-1199.
- Gyaurgieva, O.H., Bogomolova, T.S., Gorshkova, G.I. 1996. Meningitis caused by *Rhodotorula rubra* in an HIV-infected patient. *J. Med. Vet. Mycol.* 34: 357-359.
- Huttova, M., Kralinsky, K., Horn, J., Marinova, I., Iligova, K., Fric, J., Spanik, S., Filka, J., Uher, J., Kurak, J., and Krcmery, V. Jr. 1998. Prospective study of nosocomial fungal meningitis in children--report of 10 cases.
- Kiraz, N., Gulbas, Z., and Akgun, Y. 2000. Case report. *Rhodotorula rubra* fungaemia due to use of indwelling venous catheters. *Mycoses.* 43: 209-210.
- Papadogeorgakis, H., Frangoulis, E., Papaefstathiou, C., and Katsambas, A. 1999. *Rhodotorula rubra* fungaemia in an immunosuppressed patient. *J. Eur. Acad. Dermatol. Venereol.* 12: 169-170.
- Posteraro, B., Romano, L., Sanguinetti, M., Masucci, L., Morace, G., and Fadda, G. 2000. Commercial systems for fluconazole susceptibility testing of yeasts: comparison with the broth microdilution method. *Diagn Microbiol Infect Dis.* 38: 29-36.

Introduction: Document M27-A2 published by the National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Antifungal Susceptibility Testing is the current standard reference guide for determining the antifungal susceptibility testing of pathogenic yeasts. It includes two methods, broth microdilution and broth macrodilution. Various commercial systems are also available on FDA approved devices for antifungal susceptibility testing of yeasts, such as Sensititre YeastOne Colorimetric Panel and Etest. The disk diffusion testing method approved by NCCLS (M44-A) is another good method for antifungal susceptibility testing of yeast, where the results could be read after 24 hr incubation rather than after 48 hr.

Materials & Methods: Twenty-five microbiology laboratories within the United States and one reference laboratory each from Canada and United Kingdom participated in this event. Two NCCLS quality control strains, *Candida parapsilosis* ATCC 22019 (S-1) and *Candida krusei* ATCC 6258 (S-2), three NCCLS reference strains, *Candida albicans* ATCC 90028 (S-3), ATCC 24433 (S-4), and *Candida tropicalis* ATCC 750 (S-5) were included in the September 29, 2004 antifungal proficiency testing event. These isolates have been well characterized, and their MIC ranges against amphotericin B and fluconazole have been published. MICs within ± 2 dilutions of the reference result (range of MICs for a particular yeast described in NCCLS, M27-A2) are the acceptable results in this event.

Results: A total of 27 laboratories participated in this antifungal susceptibility testing event. The performances of all 27 laboratories were satisfactory. Of the 27 participating laboratories, 9 laboratories used the broth microdilution method, 14 laboratories used YeastOne Colorimetric microdilution method, and 3 laboratories used Etest and 1 laboratory used disk diffusion method. The supplementary information on antifungal susceptibility testing procedures is summarized in Table 1. The MIC results submitted by the 27 participants are illustrated in Figure 25. Good performance was noted for all specimens irrespective of the methodology used by the laboratories for both amphotericin B and fluconazole. Overall, agreement with the NCCLS reference ranges was 91% against amphotericin B and 88% against fluconazole for all isolates.

Further Reading:

1. Arthington-Skaggs, B.A., Lee-Yang, W., Ciblak, M.A., Frade, J.P., Brandt, M.E., Hajjeh, R.A., Harrison, L.H., Sofair, A.N., Warnock, D.W.; and Candidemia Active Surveillance Group. 2002. Comparison of visual and spectrophotometric methods of broth microdilution MIC end point determination and evaluation of a sterol quantitation method for in vitro susceptibility testing of fluconazole and itraconazole against trailing and nontrailing *Candida* isolates. *Antimicrob. Agents Chemother.* 46: 2477-2481.
2. 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.
3. Espinel-Ingroff, A., Pfaller, M., Messer, S.A., Knapp, C.C., Killian, S., Norris, H.A., and Ghannoum, M.A. 1999. Multicenter comparison of the sensititre YeastOne Colorimetric Antifungal Panel with the National Committee for Clinical Laboratory standards M27-A reference method for testing clinical isolates of common and emerging *Candida* spp., *Cryptococcus* spp., and other yeasts and yeast-like organisms. *J. Clin. Microbiol.* 37: 591-595.
4. Martin-Mazuelos, E., Gutierrez, M.J., Aller, A.I., Bernal, S., Martinez, M.A., Montero, O., and Quindos, G. 1999. A comparative evaluation of Etest and broth microdilution methods for fluconazole and itraconazole susceptibility testing of *Candida* spp. *J. Antimicrob. Chemother.* 43: 477-481.

*A*ntifungal susceptibility testing

S-1 *Candida parapsilosis* ATCC 22019

Summary	NCCLS Reference Range	Expanded Range
Amphotericin B	0.25-1.0 µg/ml	0.12-2.0 µg/ml
Fluconazole	2.0-8.0 µg/ml	1.0-16.0 µg/ml

Amphotericin B values were reported within NCCLS reference range by all laboratories. Fluconazole values were reported within NCCLS reference range by 23 participating laboratories, and within the expanded values by 5 laboratories.

S-2 *Candida krusei* ATCC 6258

Summary	NCCLS Reference range	Expanded range
Amphotericin B	0.25-2.0 µg/ml	0.12-4.0 µg/ml
Fluconazole	16-64 µg/ml	8->64 µg/ml

Amphotericin B values were reported within NCCLS reference range by all participating laboratories. Fluconazole values were reported within NCCLS reference range by 25 laboratories, and within the expanded values by 2 laboratories.

S-3 *Candida albicans* ATCC 90028

Summary	NCCLS Reference range	Expanded Range
Amphotericin B	0.5-2.0 µg/ml	0.25-4.0 µg/ml
Fluconazole	0.25-1.0 µg/ml	0.12-2.0 µg/ml

Amphotericin B values were reported within NCCLS reference range by 18 participating laboratories, and within the expanded values by 4 laboratories. Fluconazole values were reported within NCCLS reference range by 26 laboratories, and within the expanded values by 1 laboratory.

S-4 *Candida albicans* ATCC 24433

Summary	NCCLS Reference range	Expanded Range
Amphotericin B	0.25-1.0 µg/ml	0.12-2.0 µg/ml
Fluconazole	0.25-1.0 µg/ml	0.12-2.0 µg/ml

Amphotericin B values were reported within NCCLS reference range by 20 participating laboratories, and within the expanded values by 2 laboratories. Fluconazole values were reported within NCCLS reference range by 19 laboratories, and within the expanded values by 5 laboratories. Fluconazole MIC values higher than the expanded range were reported by 3 laboratories.

S-5 *Candida tropicalis* ATCC 750

Summary	NCCLS Reference range	Expanded range
Amphotericin B	0.5-2.0 µg/ml	0.25-4.0 µg/ml
Fluconazole	1.0-4.0 µg/ml	0.5-8.08 µg/ml

Amphotericin B values were reported within NCCLS reference range by 18 participating laboratories, and within the expanded values by 4 laboratories. Fluconazole values were reported within NCCLS reference range by 26 laboratories. The MIC value lower than the expanded range was reported by 1 laboratory.

Further Reading: (cont'd)

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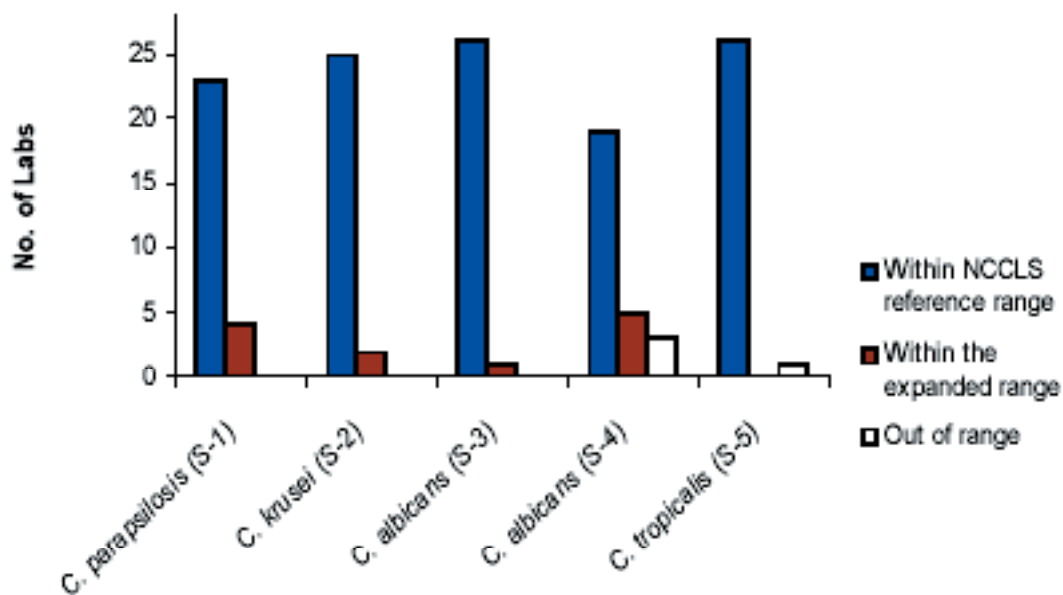
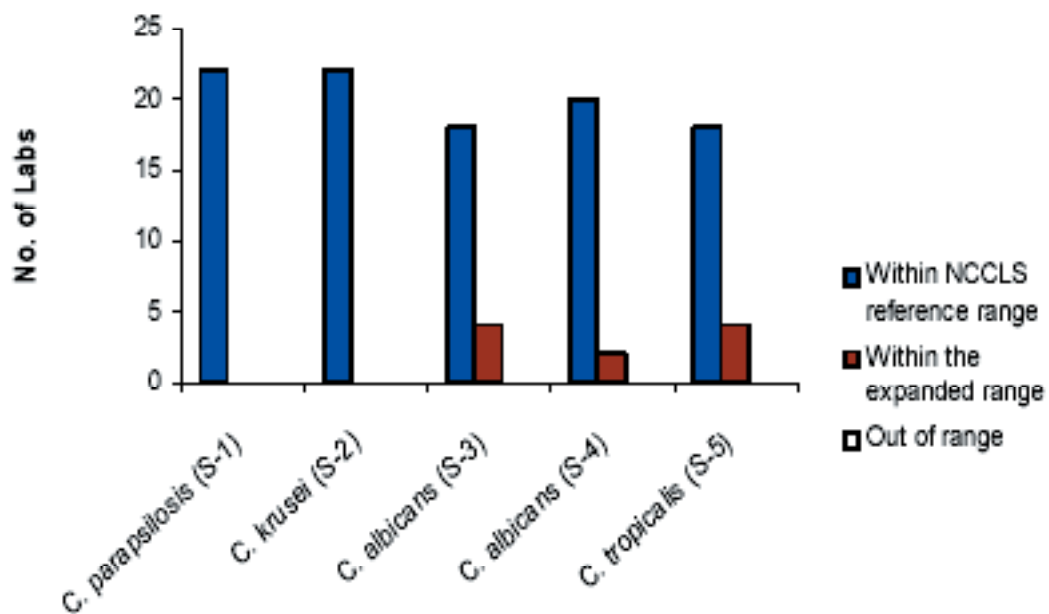
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*A*ntifungal susceptibility testing

Test Method*	No. Participant Laboratories
NCCLS broth microdilution	9
NCCLS broth macrodilution	0
Sensititre YeastOne Colorimetric	14
Etest	3
Disk diffusion	1
Medium employed*	
RPMI 1640	14
RPMI 1640 w / alamar blue	2
Antibiotic medium 3	1
Sabouraud dextrose	4
YeastOne broth	7
Mueller-Hinton Agar + 2% glucose + 0.5 g/ml Methylene Blue	1
Inoculum preparation*	
Spectrophotometric	9
MacFarland	19
Inoculum size (CFU/ml)	
0.5-2.5 × 10 ³	9
1.5-8 × 10 ³	12
0.5-1.0 × 10 ⁴	3
2.5 × 10 ⁶	1
MacFarland 0.5	2
Incubation temperature*	
35°C	24
37°C	3
Incubation duration*	
24 hr	18
48 hr	11
Endpoint reading*	
Visual	16
Colorimetric	12
Spectrophotometric	1
Biomlic	1
Scoring endpoint¹*	
100% inhibition	12
95% inhibition	1
80% inhibition	5
50% inhibition	4
Color change	11
Prominent decrease	1
QC organism	
NCCLS recommended strains	27
Unknown	0

¹Most laboratories used 100% inhibition for amphotericin B and either 50% or 80% inhibition for fluconazole.

* More than one value reported by individual laboratories



Introduction: This is a simple, sensitive latex test capable of detecting the capsular polysaccharide of *C. neoformans* in CSF and serum. It was described and proven to be superior in sensitivity to the India ink mount (1, 2). Clinical studies established the prognostic value of the test (3, 5, 6 and 7), and showed it to be a valuable aid in establishing a diagnosis when culture was negative (4). Paired serum and CSF specimens allowed detection of antigen in confirmed cases (7). Parallel serologic studies for both antigen and antibody are recommended to ensure detection of extrameningeal cryptococcosis. Newly emerging disease states and therapies have been shown to increase the opportunity for nonspecific interference in some serum specimens. Pretreatment of serum specimens with pronase prior to utilization of the latex agglutination test reduces nonspecific interference, and enhances the detection of capsular polysaccharide antigens of *Cryptococcus neoformans*.

Materials & Methods: Eighty-five laboratories participated in this event. Two positive serums for Cryptococcal antigen were included in the November 3, 2004 direct detection antigen testing event. One of these serums was of low titer (1:8) and the other was of high titer (1:128). Titers within ± 2 dilutions of the reference result are the acceptable results in this event.

Results: The performances of all 85 laboratories were satisfactory. Of the 85 participating laboratories, 74 laboratories used latex agglutination method, 3 laboratories used EIA method, and 8 laboratories did not specify the test method they use. The supplementary information on qualitative and quantitative assays on *Cryptococcus neoformans* antigen test are summarized in Table 2 and 3. Good performance was noted for all specimens irrespective of the methodology used by the laboratories.

Further Reading: (cont'd)

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Table 2. Summary of qualitative assay

Method (<i>Manufacture</i>)	Sample Total Number of labs	Cn-Ag-1 N/P*	Cn-Ag-2 N/P	Cn-Ag-3 N/P	Cn-Ag-4 N/P	Cn-Ag-5 N/P
EIA (<i>Meridien Diagn.</i>)	3	0/3	3/0	3/0	3/0	0/3
Latex Agglutination (<i>Meridien Diagn.</i>)	74	1/73	74/0	74/0	74/0	0/74
(<i>Murex</i>)	37	1/36	37/0	37/0	37/0	0/37
(<i>Wampole</i>)	23	0/23	23/0	23/0	23/0	0/23
(Immuno-Mycologics)	13	0/13	13/0	13/0	13/0	0/13
	1	0/1	1/0	1/0	1/0	0/1
Not specified	8	0/8	8/0	8/0	8/0	0/8

* N/P: number of laboratories reported Negative vs. number of laboratories reported Positive

Table 3. Summary of quantitative assay

The number of laboratories that reported titers is listed for positive test samples Cn-Ag-1 and Cn-Ag-5.

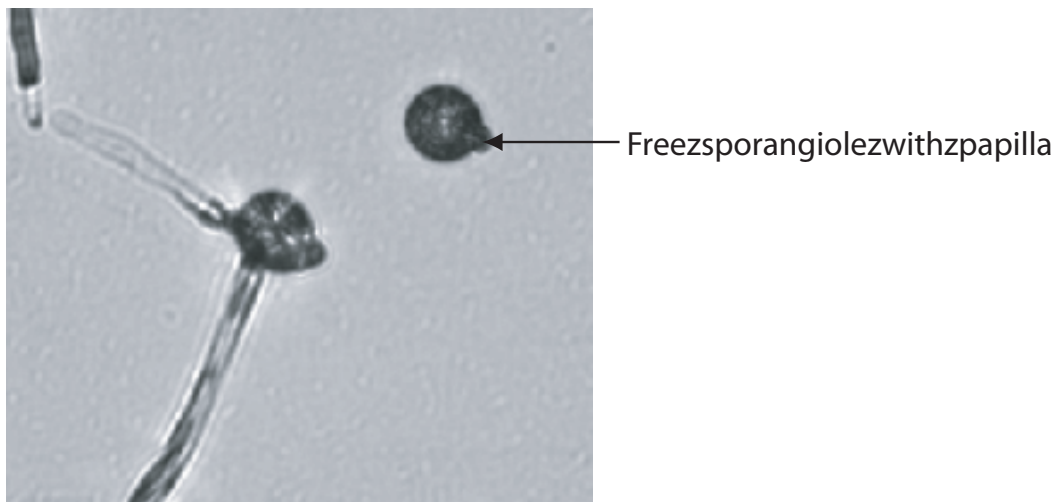
Method (<i>Manufacture</i>)	Sample Total # of labs	Cn-Ag-1 Titers										Cn-Ag-2 Titers						
		2	4	8	16	32	64	128	256	Other	8	16	32	64	128	256	512	Other
EIA (<i>Meridien Diagn.</i>)	3			2						1				1				2
Latex Agglutination (<i>Meridien Diagn.</i>)	32		5	16	7	1	1			1				9	14	6	1	2
(<i>Murex</i>)	19		4	6	7	1	1					1	1	6	4	7		
(<i>Wampole</i>)	11			1	3	4	2	1						1	2	5	3	
(Immuno-Mycologics)	1					1									1			
Not specified	8	2	1	3	1				1		1	2	2	1	1			1

Figure 1



Five-day-old, white, waxy to powdery texture colony of *Conidiobolous coronatus* on Sabouraud's dextrose agar

Figure 2



Microscopic morphology of *Conidiobolous coronatus* showing sporangiole with papilla (400× magnification).

Figure 3

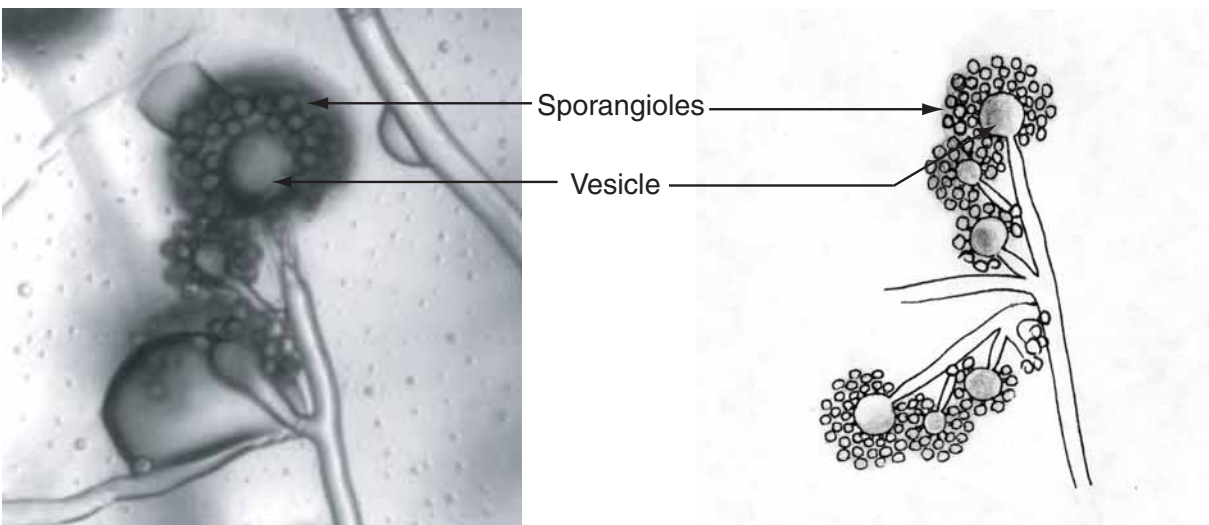


Five-day-old, white to gray cottony texture colony of *Cunninghamella bertholletiae* on Sabouraud's dextrose agar.



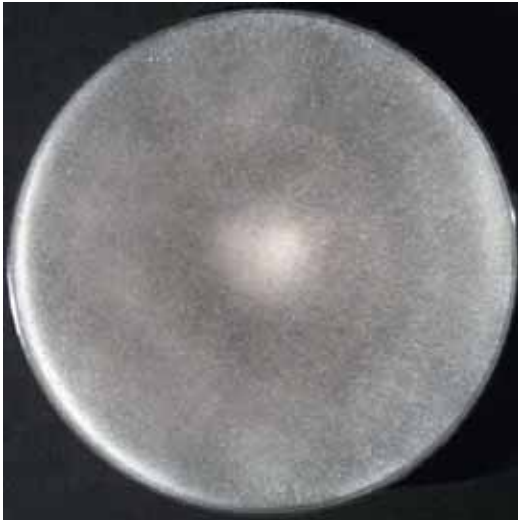
The reverse of the colony shows pale or buff.

Figure 4

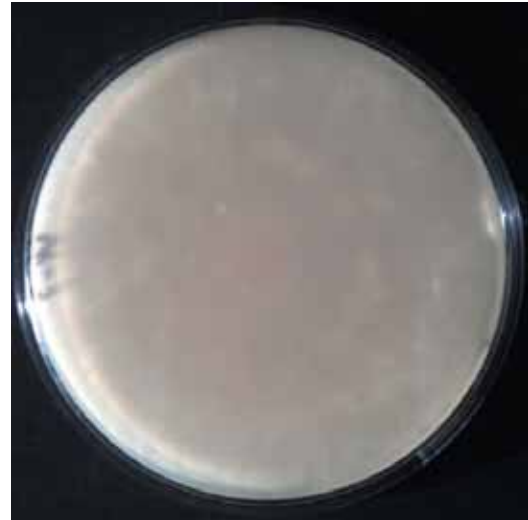


Microscopic morphology of *Cunninghamella bertholletiae* showing broad, hyaline, and aseptate hyphae. Sporangiophores branched and end with a swollen vesicle covered with single-spored sporangioles (left; 200× magnification, right; line drawing not to scale).

Figure 5

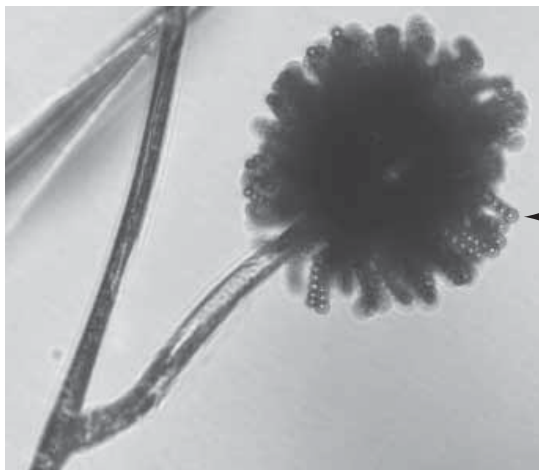


Five-day-old, white to gray and black woolly texture colony of *Syncephalastrum racemosum* on Sabouraud's dextrose agar.



The reverse side of the colony appears pale brown.

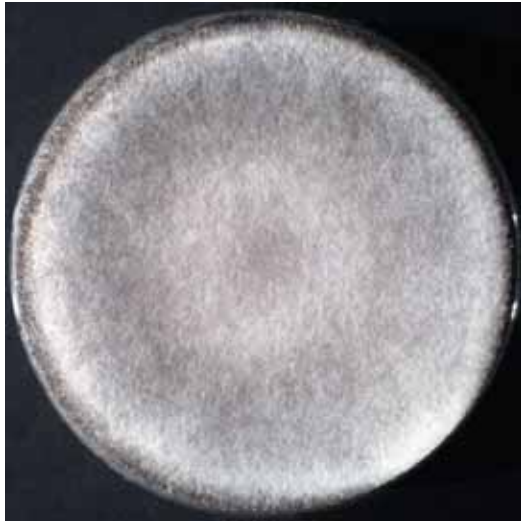
Figure 6



← Merosporangia and sporangiospores

Microscopic morphology of *Syncephalastrum racemosum* showing merosporangia arranged around the vesicle at the apex of sporangiophore and round sporangiospores formed in a linear series in the interior of the merosporangia (left; 400× magnification, right; line drawing not to scale).

Figure 7

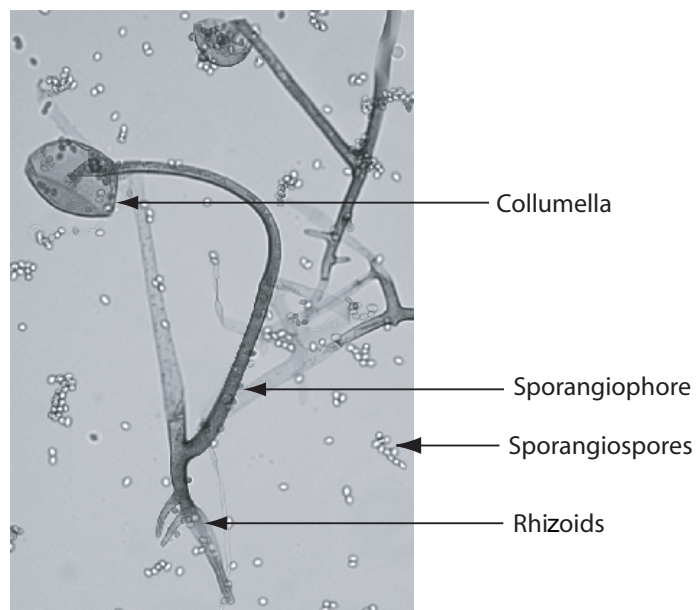


Five-day-old, grayish color colony of *Rhizopus oryzae* on Sabouraud's dextrose agar.



The reverse of the colony appears yellow to light brown.

Figure 8

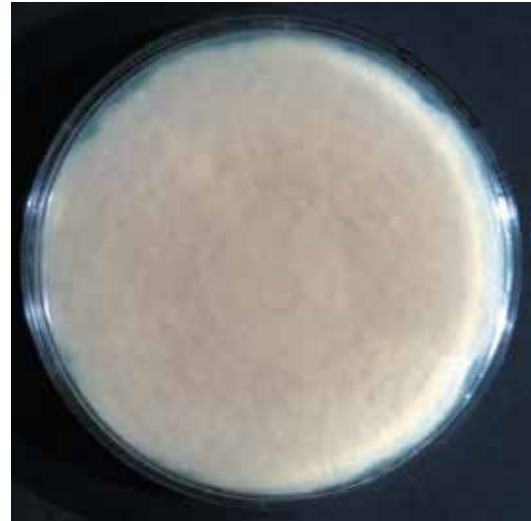


Microscopic morphology of *Rhizopus oryzae* showing collumella and rhizoids present and ovoid sporangiospores (200× magnification).

Figure 9

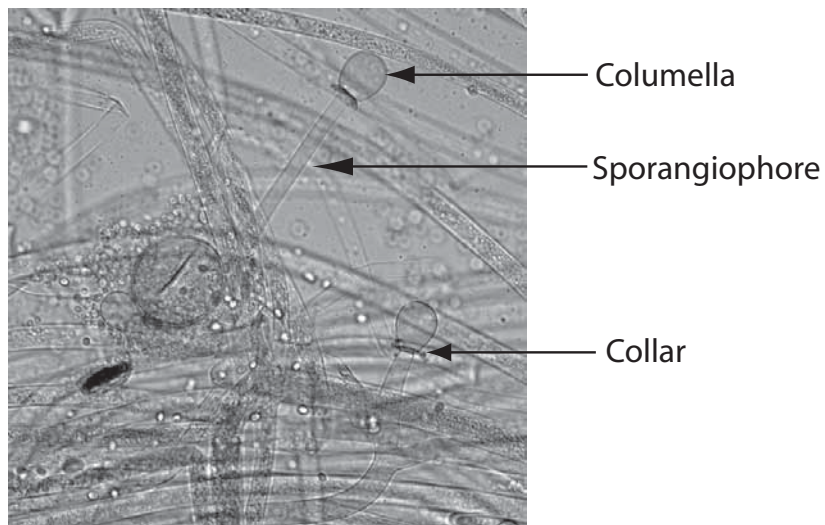


Five-day-old, pale gray to yellowish colony of *Mucor circinelloides* on Sabouraud's dextrose agar.



The reverse of the colony appears pale yellow.

Figure 10



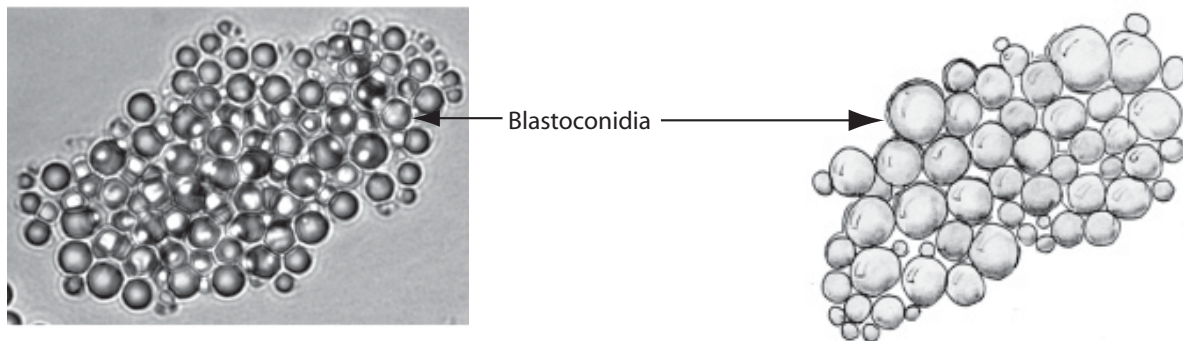
Microscopic morphology of *Mucor circinelloides* showing branching sporangiophores with a columella and sporangiospores (200× magnification).

Figure 11



Five-day-old, white creamy colony of *Cryptococcus laurentii* on Sabouraud's dextrose agar.

Figure 12



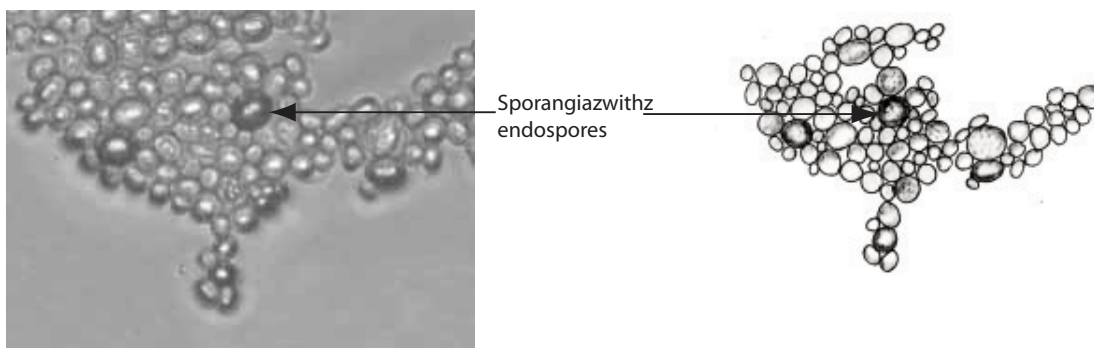
Microscopic morphology of *Cryptococcus laurentii* on corn meal agar showing blastoconidia (left; 400× magnification, right; line drawing not to scale).

Figure 13



Five-day-old, soft, smooth, and dull white colony of *Prototheca zopfii* on Sabouraud's dextrose agar.

Figure 14



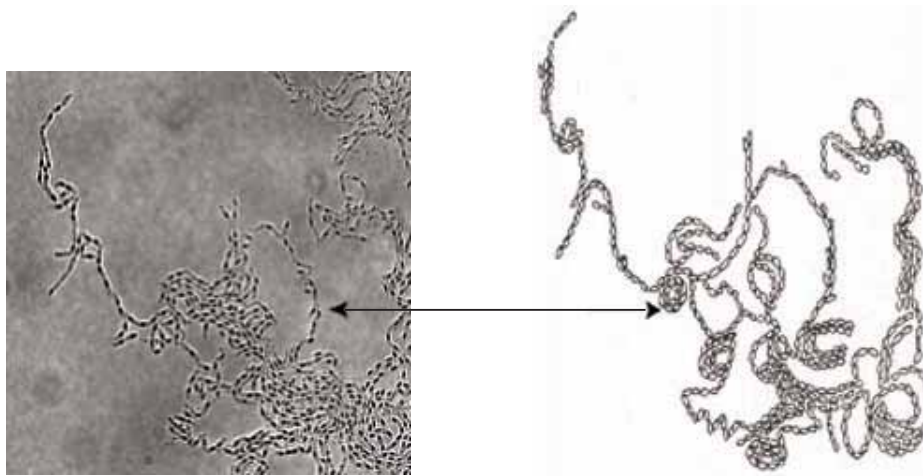
Microscopic morphology of *Prototheca zopfii* on corn meal agar with Tween 80 culture, round immature sporangia and mature sporangia with endospores are seen (left; 400× magnification, right; line drawing not to scale).

Figure 15



Five-day-old, pink, wrinkled colony of *Sporobolomyces salmonicolor* on Sabouraud's dextrose agar.

Figure 16



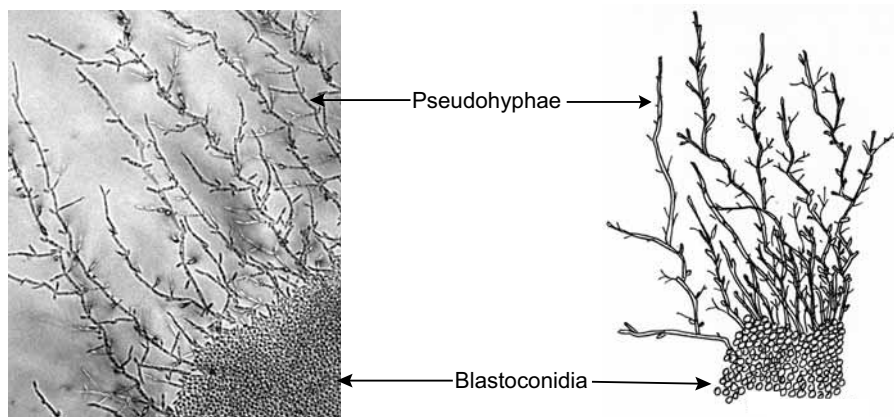
Microscopic morphology of *Sporobolomyces salmonicolor* on corn meal agar with Tween 80 culture, oval to elongated yeastlike blastoconidia are seen (left; 400× magnification, right; line drawing not to scale).

Figure 17



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

Figure 18



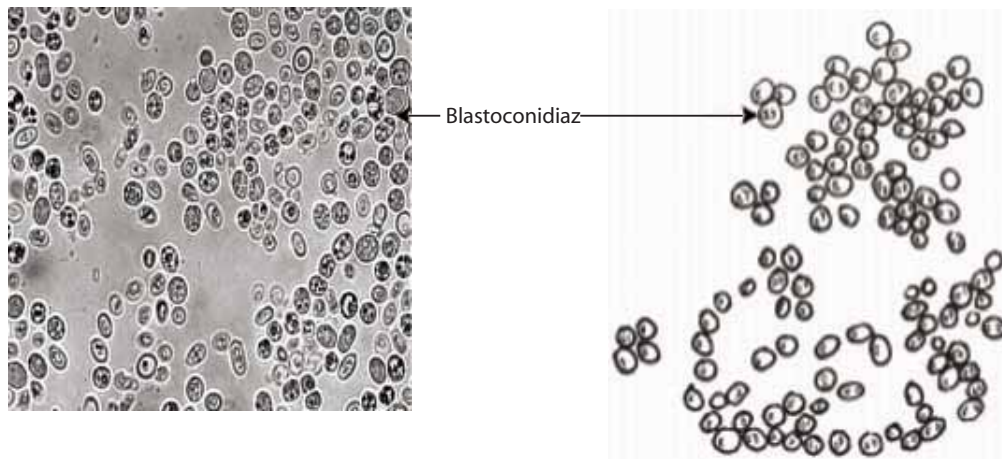
Microscopic morphology of *Candida parapsilosis*, on corn meal agar with Tween 80 showing long, multibranched pseudohyphae together with small cluster of elongated blastoconidia (left; 400× magnification, right; line drawing not to scale).

Figure 19



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

Figure 20



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

Total 145 copies of survey sheet were sent out and 138 labs responded.

Would your lab personnel be interested in attending

Yes	106
No	16
Possibly	2
No response	14

Preferred time:

Fall (September-October-November)	33
Spring (April-May)	72

Preferred length:

1 day	60
2 days	24
3 days	14

Preferred style:

Lectures	13
Hands-on	6
Both	97

Subjects would be of the greatest interest

Mold identification	69
Yeast identification	81
Antifungal susceptibility testing	36
Molecular techniques	27
Molecular identification	27
Molecular typing methods	22
Emerging pathogens	86
Epidemiology of fungal infections	50
Other	3

Summary of Zygomycetes Survey

Total 145 copies of survey sheet were sent out and 138 labs responded.

Numbers of organisms were isolated from all responded laboratories

Organisms	Year 2003	Year 2004 (till now)
<i>Rhizopus</i> species	433	380
<i>Rhizomucor</i> species	99	68
<i>Mucor</i> species	656	606
<i>Absidia</i> species	54	25
<i>Cunninghamella bertholletiae</i>	59	52
<i>Cokeromyces recurvatus</i>	0	1

Entomophthorales

<i>Conidiobolus coronatus</i>	1	0
<i>Basidiobolus</i> species	1	1

Sources for those zygomycetes isolates:

Nail	23	Feet	2
Sputum	13	Leg	2
Respiratory	9	Body fluids	1
Skin	8	Chest	1
Bronchial wash	7	Environmental	1
Sinus	7	Ethmoid	1
Scalp	6	Exudate	1
Tissue	6	Hair	1
Wound	5	Liver	1
Nasal	3	Lung	1
Stool	3	Throat	1
Blood	2	Urine	1
Ear	2	Misc.	1

Does your laboratory:

Send cultures to reference laboratories for confirmation

Yes	47
No	70
No Answer	21

Keep the cultures for reference or destroy them

Yes	71
No	62

Receive requests for susceptibility testing on these cultures

Yes	11
No	82

Total 145 copies of survey sheet were sent out and 138 labs responded.

Numbers of organisms were isolated from all responded laboratories

Organisms	Year 2003	Year 2004 (till now)
<i>Histoplasma capsulatum</i>	186	202
<i>Blastomyces dermatitidis</i>	124	112
<i>Coccidioides immitis</i> & <i>C. posadasii</i>	176	240

Does your laboratory:

Use Gen- {robe or similar kits for identification

Yes	13
No	109
No Answer	16

Send cultures to laboratories for confirmation

Yes	86
No	37

Keep the cultures for reference or destroy them

Yes	63
No	56

Receive requests for susceptibility testing on these cultures

Yes	6
No	110

*S*ummary of *Antifungal Testing Survey*

Do you send susceptibility testing specimens to reference laboratories?

State lab., Albany	42
Other Reference labs in New York State	23
Outside State labs	44

Does your facility currently perform antifungal susceptibility testing?

Yes	23
No	114

Antifungal agents used for susceptibility testing:

5-fluorocytosine	22
amphotericin B	22
fluconazole	25
voriconazole	15
caspofungin	5
itraconazole	22
ketoconazole	17
miconazole	2
Other	1

Test method employed:

NCCLS broth macrodilution using RPMI 1640	1
NCCLS broth microdilution	5
Etest	4
Agar dilution	0
Sensititre YeastOne	17

Does your facility perform to determine the levels of antifungal agents in patient's sera/body fluids? If yes, please specify antifungal agents and methods used.

Yes	2
No	115

Antifungal agents:

5-fluorocytosine
amphotericin B
fluconazole
voriconazole
itraconazole
ketoconazole

Methods used:

Bioassay
HPLC

Do you anticipate that your facility will initiate antifungal susceptibility testing or serum/body fluid level determinations within the next year?

Yes	8
No	115

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