

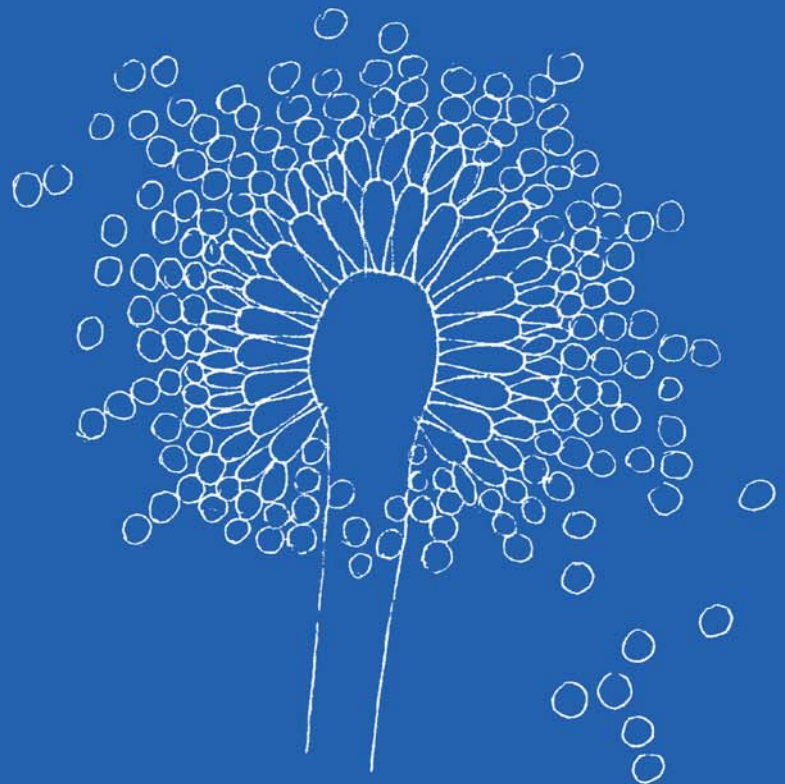


**MYCOLOGY** CRITIQUE

**Mycology Proficiency Testing Program  
October 2002**

**Wadsworth Center**

New York State Department of Health



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	Page
Contents	2
Test Specimens and Grading Policy	3
Answer Keys	4
Laboratory Results	5
Test Statistics	6
Mold Descriptions	7
<i>Cladosporium</i> sp.	
<i>Aspergillus versicolor</i>	
<i>Alternaria</i> sp.	
<i>Trichophyton mentagrophytes</i>	
<i>Chrysosporium</i> sp.	
<i>Mucor racemosus</i>	
Yeast Descriptions	16
<i>Trichosporon asahii</i>	
<i>Hansenula anomala</i>	
<i>Candida parapsilosis</i>	
<i>Candida stellatoidea</i>	
<i>Cryptococcus neoformans</i> var. <i>neoformans</i>	
<i>Cryptococcus neoformans</i> var. <i>grubii</i>	
Antifungal Susceptibility Testing for Yeasts	27
Figures	31
Bibliography	42
Acknowledgement	43

# *Test Specimens and Grading Policy*

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## Test Specimens

A minimum of two strains of each of the proposed mold specimens were examined for inclusion in the proficiency test event of October 2002. 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 of the proposed fungal pathogen 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 90 percent agreement of 10 referee laboratories or 90 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 grading formula used for each specimen is:

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

Participating laboratories must achieve a score of 80% or better on two (2) of three (3) consecutive test events to maintain acceptable proficiency levels.

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 lab 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 lab 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  $20 \times 5 = 100$ .

# Answer Key

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## Mycology – General

	Specimen Key	Validated Specimen	Other Acceptable Answers
M-1	<i>Cladosporium</i> sp.	<i>Cladosporium</i> sp.	
M-2	<i>Aspergillus versicolor</i>		
M-3	<i>Alternaria</i> sp.	<i>Alternaria</i> sp.	
M-4	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton mentagrophytes</i>	
M-5	<i>Chrysosporium</i> sp.	<i>Chrysosporium</i> sp.	
Ed.sp.	<i>Mucor racemosus</i>		

## Mycology – Yeast Only

	Specimen Key	Validated Specimen	Other Acceptable Answers
Y-1	<i>Trichosporon asabii</i>	<i>Trichosporon asabii</i>	<i>Trichosporon beigeli</i>
Y-2	<i>Hansenula anomala</i>	<i>Hansenula anomala</i>	<i>Candida pelliculosa</i> <i>Pichia anomala</i>
Y-3	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>	
Y-4	<i>Candida stellatoidea</i>	<i>Candida stellatoidea</i>	<i>Candida albicans</i>
Y-5	<i>Cryptococcus neoformans</i>	<i>Cryptococcus neoformans</i>	
Ed.sp.	<i>Cryptococcus neoformans</i> var. <i>grubii</i>		

## Mycology – Antifungal Susceptibility Testing for Yeasts

	Specimen Key
S-1	<i>Candida albicans</i> ATCC 90028
S-2	<i>Candida krusei</i> ATCC 6258
S-3	<i>Candida parapsilosis</i> ATCC 90018
S-4	<i>Candida parapsilosis</i> ATCC 22019
S-5	<i>Candida tropicalis</i> ATCC 750

# Laboratory Results

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## Mycology – General

	Correct Responses/ Total # Labs (%)	Referees (%)
M - 1 <i>Cladosporium</i> sp.	79/81 (98)	10/10 (100)
M - 2 <i>Aspergillus versicolor</i> (Not validated)	17/81 (21)	3/10 (30)
M - 3 <i>Alternaria</i> sp.	81/81 (100)	10/10 (100)
M - 4 <i>Trichophyton mentagrophytes</i>	77/81 (95)	9/10 (90)
M - 5 <i>Chrysosporium</i> sp.	72/81 (89)	9/10 (90)

## Mycology – Yeast Only

	Correct Responses/ Total # Labs (%)	Referees (%)
Y - 1 <i>Trichosporon asabii</i>	65/65 (100)	10/10 (100)
Y - 2 <i>Hansenula anomala</i>	65/65 (100)	10/10 (100)
Y - 3 <i>Candida parapsilosis</i>	65/65 (100)	10/10 (100)
Y - 4 <i>Candida stellatoidea</i>	64/65 (98)	9/10 (90)
Y - 5 <i>Cryptococcus neoformans</i>	62/65 (97)	10/10 (100)

## Mycology – Antifungal Susceptibility Testing for Yeasts

	Correct Responses/ Total # Labs (%)	Correct Responses/ Total # Labs (%)
	Amphotericin B	Fluconazole
S- 1 <i>Candida albicans</i> ATCC 90028	16/17 (94)	19/19 (100)
S- 2 <i>Candida krusei</i> ATCC 6258	17/17 (100)	19/19 (100)
S- 3 <i>Candida parapsilosis</i> ATCC 90018	16/17 (94)	19/19 (100)
S- 4 <i>Candida parapsilosis</i> ATCC 22019	17/17 (100)	18/19 (95)
S- 5 <i>Candida tropicalis</i> ATCC 750	15/17 (88)	18/19 (95)

## Mycology – General

Number of participating laboratories	81
Number of referee laboratories	10
Number of laboratories responding by deadline	81
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	78
Number of laboratories unsuccessfully completing this test	3

## Mycology – Yeast Only

Number of participating laboratories	65
Number of referee laboratories	10
Number of laboratories responding by deadline	65
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	65
Number of laboratories unsuccessfully completing this test	0

## Mycology – Antifungal Susceptibility Testing for Yeasts

Number of participating laboratories	19
Number of referee laboratories	3
Number of laboratories responding by deadline	19
Number of laboratories responding after deadline	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	19
Number of laboratories unsuccessfully completing this test	0

## Commercial Identification Systems Used\*

AMS Vitek system	25
API 20C AUX	39
Microscan	2
Remel Uni-Yeast-Tek	2
Other	3

(\*Includes multiple systems used by some labs)

## M-1 *Cladosporium* species

Source: Nasal polyps

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	79
Labs with incorrect ID:	2
( <i>Cladophialophora carrionii</i> )	(1)
( <i>Fonsecaea pedrosoi</i> )	(1)

**Clinical Significance:** *Cladosporium* sp. is a common airborne mold but rarely causes human disease. Cases of allergic fungal sinusitis caused by *Cladosporium* sp. are more commonly reported.

**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. growth is slow to rapid. *Cladosporium* sp. colony is grayish green, powdery or velvety on its surface, on Sabouraud's dextrose agar, at 25°C after 8 days (Figure 1L). The reverse is greenish-black to brownish-black (Figure 1R).
2. **Microscopic morphology** – Lactophenol cotton blue mount show conidia often in long, branched chains, and variations in size. Conidia are unicellular and ellipsoidal to round at the tip. Prominent scars are visible at the points of attachment (Figure 2).
3. **Differentiation from other molds** – Generally, the chains of conidia is longer in *Cladosporium*-type conidiation, and conidia have small dark scars of attachment. For *Fonsecaea*-type conidiation, the distal end of the conidiophore develop 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 studied to distinguish *Cladosporium* species from other closely related molds such as *Fonsecaea*, *Phialophora*, and *Rhinochadiella* spp.

**Comments:** One lab reported this specimen as *Cladosporium carrionii*, which usually has longer-chain conidia than seen in the specimen used in this test. The conidia of *C. carrionii* are also more pointed than the conidia of the test specimen. *Fonsecaea pedrosoi* does not form long-chain conidia, and its far-end conidiophore develops swollen denticles that bear primary conidia.

### Further Reading

1. Caligiorno, 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. Kantarcioglu, A.S., Yucel, A., and De Hoog, G.S. 2002. Case report. Isolation of *Cladosporium cladosporioides* from cerebrospinal fluid. *Mycoses* 45: 500-503.
3. 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 Kaibo* 104: 1147-1150.

## M-2 *Aspergillus versicolor*

Source: Auditory canal

Scoring:

No. Labs

Referee Labs with correct ID:

3

Labs with correct ID:

17

Labs with incorrect ID:

64

<i>(Aspergillus nidulans)</i>	(48)	<i>(Aspergillus fumigatus)</i>	(1)
<i>(Aspergillus sp.)</i>	(7)	<i>(Aspergillus glaucus</i> group)	(1)
<i>(Aspergillus flavus)</i>	(4)	<i>(Aspergillus unguis)</i>	(1)
<i>(Aspergillus terreus)</i>	(2)		

**Clinical Significance:** *Aspergillus versicolor* rarely causes deep infection in humans.

Occasionally, it is responsible for cases of onychomycosis. Infection of the external auditory canal are also reported.

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

### Laboratory Diagnosis:

1. **Culture** – *Aspergillus versicolor* grows moderately fast. On Sabouraud's dextrose agar, after 8 days at 25°C, *A. versicolor* colony is olive-green (Figure 3L) and sometimes shows a clear to wine-red exudate on the surface. The reverse is yellowish to light brown or orange (Figure 3R).
2. **Microscopic morphology** – Lactophenol cotton blue mount shows radiate conidial head. Phialides are biseriata. Conidia are round, smooth, or rough (Figure 4). Reduced conidiogenous structures are often seen. Hülle cells are sometimes present.
3. **Differentiation from other *Aspergillus* species** – *A. versicolor* usually produces round hülle cells similarly to *A. nidulans* but it has no cleistothecia, which distinguishes it from *A. nidulans* and *A. glaucus*. *A. versicolor* has biseriata conidial heads, differentiating it from *A. flavus*, which has both uniseriate and biseriata conidial heads. The colony surface of *A. versicolor* is pale-green, compared to brown for *A. terreus* and black for *A. niger*.
4. **In vitro susceptibility testing** – *In vitro*, *A. versicolor* is resistant to griseofulvin, fluconazole, and amphotericin B. MICs for itraconazole and ketoconazole are variable, but within a range of 0.50-4.0 µg/ml; in contrast, MICs for terbinafine are very low (<0.1 µg/ml).
5. **Molecular tests** – Nested PCR targeting of ribosomal DNA internal transcribed spacer regions was used for identification of *Aspergillus versicolor* and related *Aspergillus* species. Reverse-hybridization line probe assay (LiPA) combined with PCR amplification was reported to detect and identify clinically significant fungal pathogens, including *A. versicolor* and related species.

**Comments:** This specimen was not validated. Many labs reported this specimen as *A. nidulans*. The presence of cleistotheca and ridged ascospores in *A. nidulans* can be used to distinguish it from *A. versicolor*. *A. fumigatus* grows well at 45°C, which is a very important feature used for identification. *A. terreus* appears as a brown colony unlike *A. versicolor*, which is pale-green on the colony surface. *A. flavus* has both uniseriate and biseriata conidial heads, whereas *A. versicolor* has only biseriata conidial heads.

### Further Reading:

1. 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* sp. and other filamentous fungi: report from SENTRY antimicrobial surveillance program, 2000. *Antimicrob. Agents Chemother.* 46: 1032-1037.
2. Rotoli, M., Sascaro, G., and Cavalieri, S. 2001. *Aspergillus versicolor* infection of the external auditory canal successfully treated with terbinafine. *Dermatology* 202: 143.
3. Torres-Rodrigues, J.M., Madrenys-Brunet, N., Siddat, M., Lopez-Jodra, O., and Jimenez, T. 1998. *Aspergillus versicolor* as cause of onychomycosis: report of 12 cases and susceptibility testing to antifungal drugs. *J. Eur. Acad. Dermatol. Venereol.* 11: 25-31.

## M-3 *Alternaria species*

Source: Corneal scraping

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	81
Labs with incorrect ID:	0

**Clinical Significance:** *Alternaria* sp. causes onychomycosis, ulcerated cutaneous infection, and keratitis. It is also important as a causal agent of occupational respiratory allergy.

**Ecology:** *Alternaria* sp. is a common saprophyte found on plants, foodstuffs, textiles, and in soil worldwide.

### Laboratory Diagnosis:

1. **Culture** – *Alternaria* sp. is a rapid-growing fungus. On Sabouraud's dextrose agar, after 8 days at 25°C, *Alternaria* sp. colony is pale gray or dark olive-green, with white fringe and wooly surface (Figure 5L), brown to black on reverse (Figure 5R).
2. **Microscopic morphology** – Lactophenol cotton blue mount shows septate hyphae darkly pigmented. Conidiophores are brown, septate, simple or branched, and darkly pigmented. Conidia are large, smooth or rough, with both horizontal and transverse septa termed "muriform". They are club-shaped or elliptical and produced in chain described as "beaked" (Figure 6).
3. **Differentiation from other mold** – *Alternaria* sp. has dark brown or dark olive-green colony with a white fringe, and large club-shaped muriform conidia produced in chains. This makes it readily distinguishable from other molds.
4. **In vitro susceptibility testing** – In general, *Alternaria* species are susceptible to miconazole and ketoconazole. The activities of itraconazole, amphotericin B, and fluconazole are variable among different strains. All of the isolates are resistant to flucytosine.
5. **Molecular tests** – The major allergen Alt a 1 is well characterized and it has been produced as a recombinant protein to be used for standardization of *Alternaria alternata* allergy testing.

### Further Reading:

1. Aden, E., Weber, B., Bossert, J., Teppke, M., Frank, E., Wahl, R., Fiebig, H., and Cromwell, O. 1999. Standardization of *Alternaria alternata*: extraction and quantification of alt a 1 by using an mAb-based 2-site binding assay. *J. Allergy Clin. Immunol.* 104: 128-135.
2. Benito, N., Moreno, A., Puig, J., and Rimola, A. 2001. Alternariosis after liver transplantation. *Transplantation* 72: 1840-1843.
3. De Moragas, J.M., Prats, G., and Verger, G. 1981. Cutaneous alternariosis treated with miconazole. *Arch. Dermatol.* 117: 292-294.
4. Downs, S.H., Mitakakis, T.Z., Marks, G.B., Car, N.G., Belousova, E.G., Leuppi, J.D., Xuan, W., Downie, S.R., Tobias, A., and Peat, J.K. 2001. Clinical importance of *Alternaria* exposure in children. *Am. J. Respir. Crit. Care Med.* 164: 455-459.
5. Ferrer, C., Munoz, G., Alio, J.L., Abad, J.L., and Colomm, F. 2002. Polymerase chain reaction diagnosis in fungal keratitis caused by *Alternaria alternata*. *Am. J. Ophthalmol.* 133: 398-399.
6. Pujol, I., Aguilar, C., Gene, J. and Guarro, J. 2000. In vitro antifungal susceptibility of *Alternaria* spp. and *Ulocladium* spp. *J. Antimicrobiol Chemotherapy* 46: 323-342.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 rDNA. The sequence is deposited in GenBank under the accession number AY228764.

```

1
STE-U4262 (AF397245) TCCGTAGGTG AACCTGCGGA GGGATCATT CACAAATATG AAGGCGGGCT
NYSDOH 736-02 (AY228764) TCCGTAGGTG AACCTGCGGA GGGATCATT CACAAATATG AAGGCGGGCT

51
GGAACCTCTC GGGGTTACAG CCTTGCTGAA TTATTCACCC TTGTCTTTTG
GGAACCTCTC GGGGTTACAG CCTTGCTGAA TTATTCACCC TTGTCTTTTG

101
CGTACTTCTT GTTTCCTTGG TGGGTTTCGCC CACCACTAGG ACAAACATAA
CGTACTTCTT GTTTCCTTGG TGGGTTTCGCC CACCACTAGG ACAAACATAA

151
ACCTTTTGTA ATTGCAATCA GCGTCAGTAA CAAATTAATA ATTACAACCTT
ACCTTTTGTA ATTGCAATCA GCGTCAGTAA CAAATTAATA ATTACAACCTT

201
TCAACAACGG ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG
TCAACAACGG ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGC

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Figure 7. Alignment of primary sequences of the ITS1 regions of *Alternaria alternata* STE-U4262 and PT specimen *Alternaria* sp. NYSDOH 736-02. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** All participating laboratories correctly identified this specimen.

Source: Toenail

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	77
Labs with incorrect ID:	4
( <i>Trichophyton tonsurans</i> )	(2)
( <i>Trichophyton rubrum</i> )	(1)
( <i>Trichophyton terrestre</i> )	(1)

**Clinical Significance:** *Trichophyton mentagrophytes* frequently causes chronic infection of the feet, nails, hair, and groin. Anthropophilic isolates, such as *T. mentagrophytes* var. *interdigitale*, cause chronic infection of nails, feet, and groin. Zoophilic isolates, such as *T. mentagrophytes* var. *mentagrophytes*, cause infection of skin, scalp, and beard.

**Ecology:** *T. mentagrophytes* is a cosmopolitan dermatophyte, either anthropophilic or zoophilic. Zoophilic isolates are mainly present in rodents, rabbits, hedgehogs, and other small animals.

**Laboratory Diagnosis:**

1. **Culture** – *Trichophyton mentagrophytes* grows rapidly. On Sabouraud's dextrose agar, after at 9 days 25°C, the colony shows white to cream color and powdery surface (Figure 8L). Reverse appears yellowish to tan (Figure 8R).
2. **Microscopic morphology** – Lactophenol cotton blue mount shows hyaline septate hyphae with both macro- and micro-conidia. Macroconidia are club-shaped with multiseptation and thin walls. Microconidia are round and clustered on conidiophores (Figure 9). Spiral hyphae are often seen.
3. **Differentiation from other dermatophytes** – Microscopically, *T. mentagrophytes* is differentiated from *T. rubrum* by its round conidia produced in clusters, and the presence of spiral hyphae. *T. mentagrophytes* is urease-positive, hair perforation-positive, and has no specific growth requirements. It is differentiated from *Microsporium persicolor* by alkaline reaction on BCP-milk-glucose agar. It is differentiated from *T. terrestre* by good growth at 37°C. It has round-shaped microconidia, which distinguishes it from *T. tonsurans* which has diverse shapes and sizes of microconidia.
4. **In vitro susceptibility testing** – Susceptibility testing using the NCCLS protocol (M38-P) indicates that common clinical isolates are susceptible to terbinafine and itraconazole.
5. **Molecular tests** - ITS1 sequences of clinical isolates are species-specific. Species-specific primers of chitin synthase 1 gene have been used to differentiate the *T. mentagrophytes* complex in specimens from humans and animals. The random amplified polymorphic DNA (RAPD) method has been used to study the genetic diversity of clinical isolates.

**Further Reading:**

1. Fernandez-Torres, B., Carrillo, A.J., Martin, E., Del Palacio, A., Moore, M.K., Valverde, A., Serrano, M., and Guarro, J. 2001. *In vitro* activities of 10 antifungal drugs against 508 dermatophyte strains. *Antimicrob. Agents Chemother.* 45: 2524-2528.
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3. Howell, S.A., Barnard, R.J., and Humphreys, E. 1999. Application of molecular typing methods to dermatophyte species that cause skin and nail infection. *J. Med. Microbiol.* 48: 33-40.
4. Kim, J.A., Takahashi, R., Tanaka, K., Fukushima, K., Nishimura, K., and Miyaji, M. 2001. Identification and subtyping of *Trichophyton mentagrophytes* by random amplified polymorphic DNA. *Mycoses* 44:157-165.
5. Makimura, K., Tamura, Y., Mochizuki, A., Hasegawa, A., Tajiri, Y., Hanazawa, R., Uchida, K., Saito, H., and Yamaguchi, H. 1999. Phylogenetic classification and species identification of dermatophyte strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. *J. Clin. Microbiol.* 37: 920-924.
6. Salim, A., and Young, E. 2002. Erythema multiforme associated with *Trichophyton mentagrophytes* infection. *J. Eur. Acad. Dermatol. Venereol.* 16:645-646.
7. Skorepova, M., Stork, J., and Hrabakova, J. 2002. Case reports. Tinea gladiatorum due to *Trichophyton mentagrophytes*. *Mycoses* 45:431-433.
8. Sommer, S., Barton, R.C., Wilkinson, S.M., Merchant, W.J., Evans, E.G., and Moores, M.K. 1999. Microbiological and molecular diagnosis of deep localized cutaneous infection with *Trichophyton mentagrophytes*. *Br. J. Dermatol.* 141: 323-325.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 rDNA. The sequence is deposited in GenBank under the accession number AY222457.

```

1
CBS558.66 (TMZ99001) AAGTAAAAGT CGTAACAAGG TTTCCGTAGG TGAAC-TGCG GAAGGATCAT
NFI 0105 (AY222457) TCCGTAGG TGAACCTGCG GAAGGATCAT

51
TAGCGCGCAG GCCGGAGGCT GGCCCCCCAC GATAGGGCCA AACGTCCGTC
TAGCGCGCAG GCCGGAGGCT GGCCCCCCAC GATAGGGCCA AACGTCCGTC

101
AGGGGTGAGC AGATGTGCGC CGGCCGTACC GCCCCATTCT TGTCTACATT
AGGGGTGAGC AGATGTGCGC CGGCCGTACC GCCCCATTCT TGTCTACATT

151
ACTCGGTTGC CTCGGCGGGC CGCGCTCTCC CAGGAGAGCC GTTCGGCGAG
ACTCGGTTGC CTCGGCGGGC CGCGCTCTCC CAGGAGAGCC GTTCGGCGAG

201
CCTCTCTTTA GTGGCTAAAC GCTGGACCGC GCCCGCCGGA GGACAGACGC
CCTCTCTTTA GTGGCTAAAC GCTGGACCGC GCCCGCCGGA GGACAGACGC

251
AAAAAAATTC TTTCAGAAGA GCTGTCAGTC TGAGCGTTAG CAAGCAAAAA
AAAAAAATTC TTTCAGAAGA GCTGTCAGTC TGAGCGTTAG CAAGCAAAAA

301
TCAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA TCGATGAAGA
TCAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA TCGATGAAGA

351
ACGCAGCGAA ATGCGATAAG TAATGTGAAT TGCAGAATTC CGTGAATCAT
ACGCAGC

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Figure 10. Alignment of primary sequences of the ITS1 regions of *T. mentagrophytes* CBS 558.66 and PT specimen *T. mentagrophytes* NFI 0105. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** Two labs reported this specimen as *T. tonsurans* based on tear-drop or club-shaped microconidia with balloon forms. One lab each identified it as *T. terrestre* based on the club-shaped microconidia and no growth at 37°C, and as *T. rubrum* based on the urease-negative result. However, these features are not typical of the *T. mentagrophytes* specimen sent in this testing.

## M-5 *Chryso sporium* species

Source: Tibial abscess

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	72
Labs with incorrect ID:	9
( <i>Scedosporium apiospermum</i> )	(4)
( <i>Microsporium nanum</i> )	(3)
( <i>Emmonsia parva</i> )	(2)

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

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

### Laboratory Diagnosis:

1. **Culture** – *Chryso sporium* sp. grows moderately fast. On Sabouraud's dextrose agar, after 9 days at 25°C, the colony shows white to cream color on the surface and powdery to granular texture (Figure 11L). Reverse appears yellow or buff (Figure 11R). The species of *Chryso sporium* sent in this testing is cycloheximide-resistant.
2. **Microscopic morphology** – Lactophenol cotton blue mount shows hyaline septate hyphae. Ovoid or club-shaped conidia with broad truncated bases are seen either singly or in short chains borne directly on hyphae, or in short conidiophores (Figure 12).
3. **Differentiation from other mold** – *Chryso sporium* sp. is distinct from *Emmonsia* sp. in not developing adiaconidia at 37°C. It does not display thermal dimorphism and is negative with specific nucleic acid probe, which serves to differentiate it from *Blastomyces dermatitidis*. *Chryso sporium* sp. grows on the media with cycloheximide and is urease-positive, which distinguishes it from *Sporotrichum* sp.
4. **In vitro susceptibility testing** – No information available.
5. **Molecular tests** – No information available.

### Further Reading:

1. Carmichael, J.W. 1962. *Chryso sporium* and some other aleuriosporic hyphomycetes. *Canadian J. Botany* 40: 1137-1173.
2. Chabasse, D. 1988. Taxonomic study of keratinophilic fungi isolated from soil and mammals in France. *Mycopathologia* 101: 133-140.
3. Gaur, P.K., and Lichtwardt, R.W. 1980. Comparative study of a new *Chryso sporium* species with *Histoplasma capsulatum*. *Sabouraudia* 18:105-114.
4. Levy, F.E., Larson, J.T., George, E., and Maisel, R.H. 1991. Invasive *Chryso sporium* infection of the nose and paranasal sinuses in an immunocompromised host. *Otolaryngol. Head Neck Surg.* 104: 384-388.
5. Sigler, L., Guarro, J., and Punsola, L. 1986. New kera tinophilic species of *Chryso sporium*. *Canadian J. Botany* 64: 1212-1215.
6. Van Oorschot, C.A.N. 1980. A revision of *Chryso sporium* and allied genera. *Stud. Mycol.* 20: 1-89.

The identity of the test isolate was confirmed in the Mycology PTP program by sequencing of its ITS1 rDNA. The sequence is deposited in GenBank with the accession number AY222456.

```

1
UAMH 4320 (AJ007841) GGAAGTAAAA GTCGTAACAA GGTTCGCGTA GGTGAACCTG CGGAAGGATC
HYSDOH 467-98 (AY222456) TCCGTA GGTGAACCTG CGGAAGGAAC

51
ATTAAAGTGT TTCGGAGCCT GGTATGGGCA TCTCAACTCG AGGTGTCGGT
ACTAAAGTGT TTCGGAGCCT GGTATGGGCA TCTCGACTCG AGGTGTCGGT

101
GCCAGCGCCC CCACACGTGT TTACTIONACT TGGTTGCCTT GGTGAGCCTG
GCCAGCGCCC CCACACGTGT TTACTIONACT TGGTTGCCTT GGTGAGCCTG

151
CCCTTGTGGC TGCTGGGGAT GCCTCACGGT GTCCCGGGCT CGTGCTCGCC
CCCTTGTGGC TGCCGGGGAT GCCTCACGGT GTCTCGGGCT CGTGTTCAAC

201
AGTGGAACAT TTGAACTCTT ATGTGAAAAT AGTCAGTCTG AGCATTATGC
AGTGGAACAT TTGAACTCTT ACCTGAAAAT AGTCAGTCTG AGCCTTATGC

251
AAATTAAATA AAACCTTCAA CAACGGATCT CTTGGTCCG GCATCGATGA
AAATTAAATA AAACCTTCAA CAACGGATCT CTTGGTCCG GCATCGATGA

301
AGAACGCAGC GAAATGCGAT
AGAACGCAGC

```

Figure 13. Alignment of primary sequences of the ITS1 regions of *Chryso sporium articulatum* UAMH 4320 and PT *Chryso sporium* sp. NYSDOH 467-98. Unmatched nucleotide bases are shaded. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** Conidia of *Chryso sporium* sp. are pyriform with truncated bases, which are similar to those of *Scedosporium apiospermum*. However, *S. apiospermum* has large, dark, rounded cleistothecia with thick walls while *Chryso sporium* sp. does not. *Microsporium nanum* produces two-celled macroconidia, which are different from the unicellular conidia of *Chryso sporium* sp. *Emmonsia parva* develops adiaconidia at 37°C but *Chryso sporium* sp. does not.

Source: Skin

Scoring:

No. Labs

Referee Labs with correct ID:

3

Labs with correct ID:

20

Labs with incorrect ID:

61

( <i>Mucor</i> sp.)	(25)	( <i>Absidia corymbifera</i> )	(1)
( <i>Basidiobolus ranarum</i> )	(16)	( <i>Arthrographis</i> sp.)	(1)
( <i>Basidiobolus</i> sp.)	(4)	( <i>Chrysosporium</i> sp.)	(1)
( <i>Conidiobolus coronatus</i> )	(4)	( <i>Mucor plumbeus</i> )	(1)
( <i>Malbranchea</i> sp.)	(2)	( <i>Rhizomucor</i> sp.)	(1)
( <i>Mortierella</i> sp.)	(2)	( <i>Phycomyces</i> sp.)	(1)
( <i>Mucor circinelloides</i> )	(2)		

**Clinical Significance:** *Mucor racemosus* is an uncommon agent of zygomycosis in the severely debilitated patient.

**Ecology:** *M. racemosus* has a worldwide distribution. It is isolated from soil and decaying organic materials.

#### Laboratory Diagnosis:

1. **Culture** – *Mucor racemosus* grows rapidly. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony is grayish on surface, very wooly, and covers the whole petri dish (Figure 14L). Reverse appears pale yellow (Figure 14R).
2. **Microscopic morphology** – Lactophenol cotton blue mount shows *M. racemosus* has hyaline hyphae, which are broad and predominantly aseptate. The long and straight sporangiophores arise irregularly from the hyphae and are branched or unbranched. Sporangia with columellas lacks apophyses. Rhizoids and stolons are absent (Figure 15).
3. **Differentiation from other zygomycetes** – *Mucor* differs from *Rhizopus* and *Rhizomucor* by absence of rhizoids, and from *Absidia* by absence of an apophysis beneath the sporangium. The maximum temperature for growth in *Mucor* is less than 37°C, but *Rhizomucor* can grow at about 54°C. *M. racemosus* is different from other *Mucor* species such as *M. circinelloides*, *M. ramosissimus*, *M. indicus*, and *M. amphibiorum* in its ability to assimilate sucrose.
4. **In vitro susceptibility testing** – None of the triazoles were active against *Mucor* spp. (MIC<sub>50</sub> > 8 µg/ml)
5. **Molecular tests** – No information available.

**Comments:** More than half of the labs were able to identify this isolate to genus level according to its microscopic characteristics. Maximum temperature of growth needs to be determined, and certain biochemical tests need to be done, in order to further identify it the species level.

#### Further Reading:

1. Latte, K.P., and Kolodziej, H. 2000. Antifungal effects of hydrolysable tannins and related compounds on dermatophytes, mould fungi and yeasts. *Z Naturforsch.* 55:467-472.
2. Ribes, J.A., Vanover-Sams, C.L., and Baker, D.J. 2000. Zygomycetes in human disease. *Clin. Microbiol. Rev.* 13: 236-301.

# Y-1 *Trichosporon asabii*

Source: Toenail

Scoring:

Referee Labs with correct ID:

Labs with correct ID:

Labs with incorrect ID:

No. Labs

10

65

0

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

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

## Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar, after 7 days at 25°C, *T. asabii* colony is white to yellowish. The surface is wrinkled, velvety (Figure 16).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *T. asabii* has true and pseudohyphae with blastoconidia singly or in short chains. Rectangular-to-oval arthroconidia are prominent and are fragmented from both the main and side branches of hyphae (Figure 17).
3. **Differentiation from other yeasts** – *T. asabii* is nonfermentative, urease-positive, nitrate-negative, cycloheximide resistant, and metabolically active for assimilation of a wide range of carbohydrates. It can be distinguished from *Geotrichum candidum* by its wooly colony and production of urease.
4. **In vitro susceptibility testing** – *T. asabii* is susceptible to amphotericin B, but reduced-susceptibility isolates are often recovered from patients who do not respond to therapy with this drug. The susceptibilities to flucytosine and azoles are variable.
5. **Molecular tests** – Sequence analysis of the ribosomal DNA intergenic spacer regions provide a powerful method to distinguish between phylogenetically closely related species and clinical isolates.

## Further Reading:

1. Chakrabarti, A., Marhawa, R.K., Mondal, R., Trehan, A., Gupta, S., Rao-Raman, D.S., Sethi, S., and Padhyet, A.A. 2002. Generalized lymphadenopathy caused by *Trichosporon asabii* in a patient with Job's syndrome. *Med. Mycol.* 40: 83-86.
2. Gueho, E., Improvisi, L., De Hoog, G.S., and Dupont B. 1994. *Trichosporon* on humnas: a practical account. *Mycoses* 37:3-10.
3. Gueho, E., Smith, M.T., De Hoog, G.S., Billon-Grand, G., Christen, R., and Batenburg-van der Vegte, M.H. 1992. Contributions to a revision of the genus *Trichosporon*. *Antonie van Leeuwenboek.* 61: 289-316.
4. Meyer, M.H., Letscher-Bru, V., Waller, J., Lutz, P., Marcellin, L., and Herbrecht, R. 2002. Chronic disseminated *Trichosporon asabii* infection in a leukemic child. *Clin. Infect. Dis.* 35: e22-25.
5. Nagai, H., Yamakami, Y., Hashimoto, A., Tokimatsu, I., and Nasu, M. 1999. PCR detection of DNA specific for *Trichosporon* species in serum of patients with disseminated trichosporosis. *J. Clin. Microbiol.* 37: 694-699.
6. Panagopoulou, P., Evdoridou, J., Bibashi, E., Filioti, J., Sofianou, D., Kremenopoulos, G., and Roilides, E. 2002. *Trichosporon asabii*: an unusual cause of invasive infection in neonates. *Pediatr. Infect. Dis. J.* 21: 169-170.
7. Sugita, T., Nakajima, M., Ikeda, R., Matsushima, T., and Shinoda, T. Sequence analysis of the ribosomal DNA intergenic spacer I regions of *Trichosporon* species. *J. Clin. Microbiol.* 40: 1826-1830.
8. Wolf, D.G., Falk, R., Hacham, M., Theelen, B., Boekhout, T., Scorzetti, G., Shapiro, M., Block, C., Salkin, I.F., and Polacheck, I. 2001. Multidrug-resistant *Trichosporon asabii* infection of nongranulocytopenic patients in three intensive care units. *J. Clin. Microbiol.* 39: 4420-4425.

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 AY217017 and AY217018, respectively.

Figure 18. Alignment of primary sequences of the ITS1 regions of *T. asabii* CBS 7137 and PT specimen *T. asabii* NYSDOH 651-02. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
CBS7137 (AF444466) TCCGTAGGTG AACCTGCGGA AGGATCATTG GTGATTGCCT TTATAGGCTT 50
NYSDOH 651-02 (AY217017) TCCGTAGGTG AACCTGCGGA AGGATCATTG GTGATTGCCT TTATAGGCTT

51
ATAACTATAT CCACTTACAC CTGTGAACTG TTCTACTACT TGACGCAAGT 100
ATAACTATAT CCACTTACAC CTGTGAACTG TTCTACTACT TGACGCAAGT

101
CGAGTATTTT TACAAACAAT GTGTAATGAA CGTCGTTTTA TTATAACAAA 150
CGAGTATTTT TACAAACAAT GTGTAATGAA CGTCGTTTTA TTATAACAAA

151
ATAAAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA TGAAGAACGC 200
ATAAAACTTT CAACAACGGA TCTCTTGGCT CTCGCATCGA TGAAGAACGC

201
AGC 250
AGCGAATTGC GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA

```

Figure 19. Alignment of primary sequences of the ITS2 regions of *T. asabii* CBS 7137 and PT specimen *T. asabii* NYSDOH 651-02. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
CBS7137 (AF444466) CAACAACGGA TCTCTTGGCT CTCGCATCGA TGAAGAACGC AGCGAATTGC 50
NYSDOH 651-02 (AY217018) CAACAACGGA TCTCTTGGCT CTCGCATCGA TGAAGAACGC AGCGAATTGC

51
GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG 100
GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG

101
CAGCTTGCGC TCTCTGGTAT TCCGGAGAGC ATGCCTGTTT CAGTGTCTATG 150
CAGCTTGCGC TCTCTGGTAT TCCGGAGAGC ATGCCTGTTT CAGTGTCTATG

151
AAATCTCAAC CACTAGGGTT TCCTAATGGA TTGGATTTGG GCGTCTGCGA 200
AAATCTCAAC CACTAGGGTT TCCTAATGGA TTGGATTTGG GCGTCTGCGA

201
TTTCTGATCG CTCGCCTTAA AAGAGTTAGC AAGTTTGACA TTAATGTCTG 250
TTTCTGATCG CTCGCCTTAA AAGAGTTAGC AAGTTTGACA TTAATGTCTG

251
GTGTAATAAG TTTCACTGGG TCCATTGTGT TGAAGCGTGC TTCTAATCGT 300
GTGTAATAAG TTTCACTGGG TCCATTGTGT TGAAGCGTGC TTCTAATCGT

301
CCGCAAGGAC AATTACTTTG ACTCTGGCCT GAAATCAGGT AGGACTACCC 350
CCGCAAGGAC AATTACTTTG ACTCTGGCCT GAAATCAGGT AGGACTACCC

351
GCTGAACTTA AGCATATCAA TAAGCGGAGG A
GCTGAACTTA AGCATATCAA TAAGCGGAGG A

```

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

## Y-2 *Hansenula anomala*

Source: Urine

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	65
Labs with incorrect ID:	0

**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 isolated from soil and is found 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 20).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *H. anomala* showed blastoconidia with ascospores, but no pseudohyphae (Figure 21).
3. **Differentiation from other yeasts** – *Candida pelliculosa* is the anamorph (asexual form) of this yeast. It did not grow on media containing cycloheximide, or at 42°C. It assimilated nitrate but was 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.

### Further Reading:

1. Chakrabarti, A., Singh, K., Narang, A., Singhi, S., Batra, R., Rao, L., Ray, P., Gopalan, S., Das, S., Gupta, V., Gupta, K., Bose, S.M., and McNeil, M.M. 2001. Outbreak of *Pichia anomala* infection in the pediatric service of a tertiary-care center in Northern India. *J. Clin. Microbiol.* 39: 1702-1706.
2. Kalenic, S., Jandrrlic, M., Vegar, V., Zuech, N., Sekulic, A., and Mlinaric-Missoni, E. 2001. *Hansenula anomala* outbreak at a surgical intensive care unit: a search for risk factors. *Eur. J. Epidemiol.* 17: 491-496.
3. Kurtzman, C.P. 2000. Four new yeasts in the *Pichia anomala* clade. *Int. J. Syst. Evol. Microbiol.* 50 Pt 1: 395-404.
4. Ma, J.S., Chen, P.Y., Chen, C.H., and Chi, C.S. 2000. Neonatal fungemia caused by *Hansenula anomala*: a case report. *J. Microbiol. Immunol. Infect.* 33: 267-270.
5. Olicio, R., Almeida, C.A., and Seuanez, H.N. 1999. A rapid method for detecting and distinguishing clinically important yeasts by heteroduplex mobility assays (HMAs). *Mol. Cell Probes* 13: 251-255.
6. Wong, A.R., Ibrahim, H., Van Rostenberghe, H., Ishak, Z., and Radzi, M.J. 2000. *Hansenula anomala* infection in a neonate. *J. Paediatr. Child Health* 36: 609-610.
7. Yamada, Y., Maeda, K., and Mikata, K. 1994. The phylogenetic relationships of the hat-shaped ascospore-forming, nitrate-assimilating *Pichia* species, formerly classified in the genus *Hansenula* Sydow et Sydow, based on the partial sequences of 18S and 26S ribosomal RNAs (Saccharomycetaceae): the proposals of three new genera, *Ogataea*, *Kuraishia*, and *Nakazawaea*. *Biosci. Biotechnol. Biochem.* 58: 1245-1257.

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 AY217019 and AY217020, respectively.

```

1                                     50
CBS 605 (AF335926)   TCCGTAGGTG AACCTGCGGA AGGATCATTAGTAGTATTCTA TTGCCAGCGC
NYSDOH 835-90 (AY217019) TCCGTAGGTG AACCTGCGGA AGGATCATTAGTAGTATTCTA TTGCCAGCGC

51                                     100
TTAATTGCGC GCGGATAAAC CTTACACACA TTGTCTAGTT TTTTTGAACT
TTAATTGCGC GCGGATAAAC CTTACACACA TTGTCTAGTT TTTTTGAACT

101                                    150
TTGCTTTGGG TGGTGAGCCT GGCTTACTGC CCAAAGGTCT AAACACATTT
TTGCTTTGGG TGGTGAGCCT GGCTTACTGC CCAAAGGTCT AAACACATTT

151                                    200
TTTTAATGTT AAAACCTTTA ACCAATAGTC ATGAAAATTT TTAACAAAAA
TTTTAATGTT AAAACCTTTA ACCAATAGTC ATGAAAATTT TTAACAAAAA

201                                    250
TTAAAATCTT CAAAACCTTC AACAACGGAT CTCTTGGTTC TCGCATCGAT
TTAAAATCTT CAAAACCTTC AACAACGGAT CTCTTGGTTC TCGCATCGAT

251
GAAGAACGCA GC
GAAGAACGCA GC

```

Figure 22. Alignment of primary sequences of the ITS1 regions of *H. anomala* CBS 605 and PT specimen *H. anomala* NYSDOH 835-90. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1                                     50
CBS 605T (AF218991) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTATTGTG AATTGCAGAT
NYSDOH 835-90 (AY217020) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTATTGTG AATTGCAGAT

51                                     100
TTTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCACCCT CTGGTATTCC
TTTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCACCCT CTGGTATTCC

101                                    150
AGAGGGTATG CCTGTTTGAG CGTCATTTCT CTCTCAAACC TTCGGGTTTG
AGAGGGTATG CCTGTTTGAG CGTCATTTCT CTCTCAAACC TTCGGGTTTG

151                                    200
GTATTGAGTG ATACTCTGTC AAGGGTTAAC TTGAAATATT GACTTAGCAA
GTATTGAGTG ATACTCTGTC AAGGGTTAAC TTGAAATATT GACTTAGCAA

201                                    250
GAGTGTAATA ATAAGCAGTC TTTCTGAAAT AATGTATTAG GTTCTTCCAA
GAGTGTAATA ATAAGCAGTC TTTCTGAAAT AATGTATTAG GTTCTTCCAA

251                                    300
CTCGTTATAT CAGCTAGGCA GGTTTAGAAG TATTTTAGGC TCGGCTTAAC
CTCGTTATAT CAGCTAGGCA GGTTTAGAAG TATTTTAGGC TCGGCTTAAC

301                                    350
AACAATAAAC TAAAAGTTTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA
AACAATAAAC TAAAAGTTTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA

351
CTTAAGCATA TCAATAAGCG GAGGA
CTTAAGCATA TCAATAAGCG GAGGA

```

Figure 23. Alignment of primary sequences of the ITS2 regions of *H. anomala* CBS 605 and PT specimen *H. anomala* NYSDOH 835-90. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** All participating laboratories reported the correct identification of this isolate, which was sent as an educational specimen in the October 2001 PTP event.

## Y-3 *Candida parapsilosis*

Source: Blood

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	65
Labs with incorrect ID:	0

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

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

### Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony is white to cream, dull with smooth surface (Figure 24).
2. **Microscopic morphology** – On corn meal agar with Tween 80, long, multibranched pseudohyphae together with small elongated blastoconidia clustered on them, are seen (Figure 25).
3. **Differentiation from other yeasts** – *C. parapsilosis* only ferments glucose but not maltose, sucrose, lactose, or trehalose. It does not grow on media containing cycloheximide, but it does grow at 37°C. It assimilates glucose, maltose, and sucrose but it is urease- and nitrate-negative. Biochemically, it is very similar to *C. lusitanae* but microscopically it forms long pseudohyphae that differentiates it from *C. lusitanae*.
4. **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.
5. **Molecular tests** – PCR assay of ITS regions of rDNA was used to identify *C. parapsilosis* in clinical specimens. Chromosome length polymorphism and RAPD procedures were used to characterize the genetic diversity of this organism.

### Further Reading:

1. 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.
2. Da Silva, C.L., dos Santos, R.M., and Colombo, A.L. 2001. Cluster of *Candida parapsilosis* primary bloodstream infection in a neonatal intensive care unit. *Braz. J. Infect. Dis.* 5: 32-36.
3. 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.
4. Fujita S. and Hashimoto, T. 2000. DNA fingerprinting patterns of *Candida* species using *HinfI* endonuclease. *International J. Systematic & Evolutionary Microbiology.* 50: 1381-1389.
5. 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.
6. 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.
7. 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                                     50
wb176 (AF455530)   TTGGAAGTTA AAAGTCGTAA CAAGGTTTCC GTAGGTGAAC CTGCGGAAGG
ATCC 22019 (AY217021)                               TCC GTAGGTGAAC CTGCGGAAGG

51                                     100
ATCATTACAG AATGAAAAGT GCTTAACTGC ATTTTTTCTT ACACATGTGT
ATCATTACAG AATGAAAAGT GCTTAACTGC ATTTTTTCTT ACACATGTGT

101                                    150
TTTTCTTTTT TTGAAAACCT TGCTTTGGTA GGCCTTCTAT ATGGGGCCTG
TTTTCTTTTT TTGAAAACCT TGCTTTGGTA GGCCTTCTAT ATGGGGCCTG

151                                    200
CCAGAGATTA AACTCAACCA AATTTTATTT AATGTCAACC GATTATTTAA
CCAGAGATTA AACTCAACCA AATTTTATTT AATGTCAACC GATTATTTAA

201                                    250
TAGTCAAAAC TTTCAACAAC GGATCTCTTG GTTCTCGCAT CGATGAAGAA
TAGTCAAAAC TTTCAACAAC GGATCTCTTG GTTCTCGCAT CGATGAAGAA

251
CGCAGCGAAA
CGCAGC

```

Figure 26. 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                                     50
wb176 (AF455530)   CGATGAAGAA CGCAGCGAAA TGCATAAGT AATATGAATT GCAGATATTC
ATCC 22019 (AY217022) CGATGAAGAA CGCAGCGAAA TGCATAAGT AATATGAATT GCAGATATTC

51                                     100
GTGAATCATC GAATCTTTGA ACGCACATTG CGCCCTTTGG TATTCCAAAG
GTGAATCATC GAATCTTTGA ACGCACATTG CGCCCTTTGG TATTCCAAAG

101                                    150
GGCATGCCTG TTTGAGCGTC ATTTCTCCCT CAAACCCTCG GGTTTGGTGT
GGCATGCCTG TTTGAGCGTC ATTTCTCCCT CAAACCCTCG GGTTTGGTGT

151                                    200
TGAGCGATAC GCTGGGTTTG CTTGAAAGAA AGGCGGAGTA TAAACTAATG
TGAGCGATAC GCTGGGTTTG CTTGAAAGAA AGGCGGAGTA TAAACTAATG

201                                    250
GATAGGTTTT TTCCACTCAT TGGTACAAAC TCCAAAACCT CTTCCAAATT
GATAGGTTTT TTCCACTCAT TGGTACAAAC TCCAAAACCT CTTCCAAATT

251                                    300
CGACCTCAAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG
CGACCTCAAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG

301
CGGAGGA
CGGAGGAA

```

Figure 27. 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.

**Comments:** This important clinical specimen was correctly identified by all of the participating labs.

## Y-4 *Candida stellatoidea*

Source: Sputum

Scoring:	No. Labs
Referee Labs with correct ID:	9
Labs with correct ID:	64
Labs with incorrect ID:	1
( <i>Candida dubliniensis</i> )	(1)

**Clinical Significance:** *Candida stellatoidea* can cause fungemia in patients with lymphoma associated with shock.

**Ecology:** *C. stellatoidea* is found as a commensal pathogen on humans and a number of other mammals. Also found on leaves and flowers, and in water and soil.

### Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony is white to cream, glossy, smooth, and soft (Figure 28).
2. **Microscopic morphology** – On corn meal agar with Tween 80, round blastoconidia bunched together with pseudohyphae are easily seen. Thick-walled, mostly terminal chlamydospores are prominent (Figure 29).
3. **Differentiation from other yeasts** – On the basis of morphology, *C. stellatoidea* is difficult to distinguish from *C. dubliniensis* and *C. albicans*. *C. stellatoidea* is not able to assimilate sucrose but *C. albicans* and *C. dubliniensis* can.
4. **In vitro susceptibility testing** – *C. stellatoidea* is sensitive to amphotericin B. Some isolates from AIDS patients are resistant to fluconazole.
5. **Molecular tests** – Many molecular tests are available for identification of *C. stellatoidea* such as PCR fingerprinting, RAPD, RFLPs. A combination of RFLPs generated by different restriction digestions of the PCR products of the V3 region of the 25S rDNA gene (rDNA) or of ITS were reported to be able to differentiate *Candida albicans* subgroups, *C. dubliniensis* and *C. stellatoidea*.

### Further Reading:

1. McCullough, M.J., Clemons, K.V., and Stevens, D.A. 1999. Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea*. *J. Clin. Microbiol.* 37: 417-421.
2. Newman, S.L., Flanigan, T.P., Fisher, A., Rinaldi, M.G., Stein, M., and Vigilante, K. 1994. Clinically significant mucosal candidiasis resistant to fluconazole treatment in patients with AIDS. *Clin. Infect. Dis.* 19: 684-686.
3. Sufliarsky, J., Sorkovska, D., Kunova, A., Helpianska, L., and Krcmery, V. Jr. 1995. Breakthrough fungemia caused by *Candida stellatoidea* in a patient with lymphoma associated with shock, successfully treated with amphotericin B lipid complex. *Infection* 23: 246-247.

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 AY217023 and AY217024, respectively.

```

1
ATCC 11006 (AF336831) TCCGTAGGTG AACCTGCGGA AGGATCATT A CTGATTTGCT TAATTGCACC 50
ATCC 36232 (AY217023) TCCGTAGGTG AACCTGCGGA AGGATCATT A CTGATTTGCT TAATTGCACC

51
ACATGTGTTT TTCTTTGAAA CAAACTTGCT TTGGCGGTGG GCCCAGCCTG 100
ACATGTGTTT TTCTTTGAAA CAAACTTGCT TTGGCGGTGG GCCCAGCCTG

101
CCGCCAGAGG TCTAAACTTA CAACCAATTT TTTATCAACT TGTCACACCA 150
CCGCCAGAGG TCTAAACTTA CAACCAATTT TTTATCAACT TGTCACACCA

151
GATTATTACT TAATAGTCAA AACTTTCAAC AACGGATCTC TTGGTTCTCG 200
GATTATTACT TAATAGTCAA AACTTTCAAC AACGGATCTC TTGGTTCTCG

201
CATCGATGAA GAACGCAGC
CATCGATGAA GAACGCAGC

```

Figure 30. Alignment of primary sequences of the ITS1 regions of *C. albicans* ATCC 11006 and PT specimen *C. stellatoidea* ATCC 36232. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
ATCC 11006 (AF335963) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATATG AATTGCAGAT 50
ATCC 36232 (AY217024) GCATCGATGA AGAACGCAGC GAAATGCGAT ACGTAATATG AATTGCAGAT

51
ATTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCT CTGGTATTCC 100
ATTCGTGAAT CATCGAATCT TTGAACGCAC ATTGCGCCCT CTGGTATTCC

101
GGAGGGCATG CCTGTTTGAG CGTCGTTTCT CCCTCAAACC GCTGGGTTTG 150
GGAGGGCATG CCTGTTTGAG CGTCGTTTCT CCCTCAAACC GCTGGGTTTG

151
GTGTTGAGCA ATACGACTTG GGTTCGCTTG AAAGACGGTA GTGGTAAGGC 200
GTGTTGAGCA ATACGACTTG GGTTCGCTTG AAAGACGGTA GTGGTAAGGC

201
GGGATCGCTT TGACAATGGC TTAGGTCTAA CCAAAAACAT TGCTTGCGGC 250
GGGATCGCTT TGACAATGGC TTAGGTCTAA CCAAAAACAT TGCTTGCGGC

251
GGTAACGTCT ACCACGTATA TCTTCAAAC TTAGACCTCAA ATCAGGTAGG 300
GGTAACGTCT ACCACGTATA TCTTCAAAC TTAGACCTCAA ATCAGGTAGG

301
ACTACCCGCT GAACTTAAGC ATATCAATAA GCGGAGGA
ACTACCCGCT GAACTTAAGC ATATCAATAA GCGGAGGA

```

Figure 31. Alignment of primary sequences of the ITS2 regions of *C. albicans* ATCC 11006 and PT specimen *C. stellatoidea* ATCC 36232. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** The code book for the API 20C lists *C. albicans* 1 and *C. albicans* 2. Actually, *C. albicans* 2 is the sucrose-negative strain, which is also named as *C. stellatoidea*. In this test event, we also consider *C. albicans* as a correct answer for *C. stellatoidea*.

## Y-5 *Cryptococcus neoformans* var. *neoformans*

Source: CSF

Scoring:	No. Labs
Referee Labs with correct ID:	10
Labs with correct ID:	62
Labs with incorrect ID:	3
( <i>Cryptococcus laurentii</i> )	(1)
( <i>Cryptococcus uniguttulatus</i> )	(2)

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

**Ecology:** *Cryptococcus neoformans* var. *neoformans* and var. *grubii* are commonly found in avian (pigeon) droppings. Both varieties have world-wide distributions. However, *Cr. neoformans* var. *gattii* is commonly found on *Eucalyptus* tree and mainly distributed in Australia, Southeast Asia, Southern California, and South America.

### Laboratory Diagnosis:

1. **Culture** – On Sabouraud's dextrose agar after 7 days at 25°C, colony is cream to tan in color, smooth, moist, and soft (Figure 32).
2. **Microscopic morphology** – On corn meal agar with Tween 80, *Cr. neoformans* cells are large and round, with no pseudohyphae or true hyphae (Figure 33U). In India-ink preparation, encapsulated yeasts are seen (Figure 33L).
3. **Differentiation from other yeasts** – *Cr. neoformans* does not ferment any carbohydrates and does not grow on media containing cycloheximide, but it does grow at 37°C. *Cr. neoformans* produced dark brown colonies on niger seed agar. It produces urease enzyme and it is negative on nitrate reaction. The three varieties are differentiated by 1) growth and color change: *Cr. neoformans* var. *gattii* on canavanine-glycine-bromthymol blue (CGB) medium becomes blue-green after 2 – 5 days at 25°C; 2) PCR technique: *Cr. neoformans* var. *gattii* can be differentiated from the other two varieties.
4. **In vitro susceptibility testing** – Most isolates are susceptible to amphotericin B, 5FC, and to azoles like fluconazole and itraconazole. 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. Recently, a direct PCR technique has been developed to differentiate the three varieties of this pathogen.

### 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 Espnel-Ingroff, A. 2000. Correlation of fluconazole MICs with clinical outcome in cryptococcal infection. *Antimicrob. Agents Chemother.* 44: 1544-1548.
2. Chaturvedi, S. Rodeghier, B., Fan, J., McClelland, C.M., Wickes, B.L., and Chaturvedi, V. 2000. Direct PCR of *Cryptococcus neoformans* MAT $\alpha$  and MAT $\alpha$  pheromones to determine mating type, ploidy, and variety: a tool for epidemiological and molecular pathogenesis studies. *J. Clin. Microbiol.* 38: 2007-2009.
3. De Baere, T., Claeys, G., Swinne, D., Verschraegen, G., Muylaert, A., Massonet C., and Vanechoutte, M. 2002. Identification of cultured isolates of clinically important yeast species using fluorescent fragment length analysis of the amplified internally transcribed rRNA spacer 2 region (ITS2). *BMC Microbiol.* 2: 21.
4. Hunger, R.E., Paredes, B.E., Quattroppani, C., Krahenbuhl, S., and Braathen, L.R. 2000. Primary cutaneous cryptococcosis in a patient with systemic immunosuppression after liver transplantation. *Dermatology* 200: 352-355.
5. 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.
6. Nunez, M., Peacock, J.E., and Chin, R. Jr. 2000. Pulmonary cryptococcosis in the immunocompetent host. Therapy with oral fluconazole: a report of four cases and a review of the literature. *Chest* 118: 527-534.
7. Sorrell, T.C., Brownlee, A.G., Ruma, P., Malik, R., Pfeiffer, T.J., and Ellis, D.H. 1996. Natural environmental sources of *Cryptococcus neoformans* var. *gattii*. *J. Clin. Microbiol.* 34: 1261-1263.
8. Steenberg, J.N., and Casadevall. 2000. Prevalence of *Cryptococcus neoformans* var. *neoformans* (serotype D) and *Cryptococcus neoformans* var. *grubii* (serotype A) isolates in New York City. *J. Clin. Microbiol.* 38:1974-1976.

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 AY217025 and AY217026, respectively.

```

1
ATCC 32045 (AF335936) TCCGTAGGTG AACCTGCGGA AGGATCAGTA GAGAATATTG GACTTTGGTC
JEC21 ITS1 (AY217025) TCCGTAGGTG AACCTGCGGA AGGATCAGTA GAGAATATTG GACTTTGGTC

51
CATTTATCTA CCCATCTACA CCTGTGAACT GTTTATGTGC TTCGGCACGT
CATTTATCTA CCCATCTACA CCTGTGAACT GTTTATGTGC TTCGGCACGT

101
TTTACACAAA CTTCTAAATG TAATGAATGT AATCATATTA TAACAATAAT
TTTACACAAA CTTCTAAATG TAATGAATGT AATCATATTA TAACAATAAT

151
AAAACTTTCA ACAACGGATC TCTTGGCTTC CGCATCGATG AAGAACGCAG
AAAACTTTCA ACAACGGATC TCTTGGCTTC CGCATCGATG AAGAACGCAG

201
C
C

```

Figure 34. Alignment of primary sequences of the ITS1 regions of *Cr. neoformans* var. *neoformans* ATCC 32045 and PT specimen *Cr. neoformans* var. *neoformans* JEC21. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

```

1
ATCC 32045 (AF218975) GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
JEC21 (AY217026) GCATCGATGA AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA

51
TTCAGTGAAT CATCGAGTCT TTGAACGCAA CTTGCGCCCT TTGGTATTCC
TTCAGTGAAT CATCGAGTCT TTGAACGCAA CTTGCGCCCT TTGGTATTCC

101
GAAGGGCATG CCTGTTTGAG AGTCATGAAA ATCTCAATCC CTCGGGTTTT
GAAGGGCATG CCTGTTTGAG AGTCATGAAA ATCTCAATCC CTCGGGTTTT

151
ATTACCTGTT GGACTTGGAT TTGGGTGTTT GCCGCGACCT GCAAAGGACG
ATTACCTGTT GGACTTGGAT TTGGGTGTTT GCCGCGACCT GCAAAGGACG

201
TCGGCTCGCC TTAAATGTGT TAGTGGGAAG GTGATTACCT GTCAGCCCGG
TCGGCTCGCC TTAAATGTGT TAGTGGGAAG GTGATTACCT GTCAGCCCGG

251
CGTAATAAGT TTCGCTGGGC CTATGGGGTA GTCTTCGGCT TGCTGATAAC
CGTAATAAGT TTCGCTGGGC CTATGGGGTA GTCTTCGGCT TGCTGATAAC

301
AACCATCTCT TTTTGTTTTGA CCTCAAATCA GGTAGGGCTA CCCGCTGAAC
AACCATCTCT TTTTGTTTTGA CCTCAAATCA GGTAGGGCTA CCCGCTGAAC

351
TTAAGCATAT CAATAAGCGG AGGA
TTAAGCATAT CAATAAGCGG AGGA

```

Figure 35. Alignment of primary sequences of the ITS2 regions of *Cr. neoformans* var. *neoformans* ATCC 32045 and PT specimen *Cr. neoformans* var. *neoformans* JEC21. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

**Comments:** Originally, *Cryptococcus neoformans* consisted of two varieties: *Cr. neoformans* var. *neoformans* (serotypes A & D) and *Cr. neoformans* var. *gattii* (serotype B & C). Recently, *Cr. neoformans* var. *neoformans* was further subdivided into two varieties: *Cr. neoformans* var. *neoformans* (serotype D) and *Cr. neoformans* var. *grubii* (serotype A). The organism sent in this testing event labeled as Y-5 was *Cr. neoformans* var. *neoformans*. The organism sent in this testing event labeled as Y-Ed.sp. was *Cr. neoformans* var. *gattii*.

*Ed. Sp. Cryptococcus neoformans var. grubii*

Source: Blood

See Y-5. *Cryptococcus neoformans* var. *neoformans*

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 number AY217027.

```

1                               50
RAA (AF196311) TCCGTAGGTG AACCTGCGGA AGGATCAGTA GAGAATACTG GACTTTGGTC
H99 (AY217027) TCCGTAGGTG AACCTGCGGA AGGATCAGTA GAGAATATG GACTTCGGTC

51                               100
CATTTATCTA CCCATCTACA CCTGTGAACT GTTTATGTGC TTCGGCACGT
CATTTATCTA CCCATCTACA CCTGTGAACT GTTTATGTGC TTCGGCACGT

101  150
TTTACACAAA CTTCTAAATG TAATGAATGT AATCTTATTA TAACAATAAT
TTTACACAAA CTTCTAAATG TAATGAATGT AATCTTATTA TAACAATAAT

151                               200
AAAACTTTCA ACAACGGATC TCTTGGCTTC CACATCGATG AAGAACGCAG
AAAACTTTCA ACAACGGATC TCTTGGCTTC CACATCGATG AAGAACGCAG

201                               250
CGAAATGCGA TAAGTAATGT GAATTGCAGA ATTCAGTGAA TCATCGAATC
CGAAATGCGA TAAGTAATGT GAATTGCAGA ATTCAGTGAA TCATCGAATC

251                               300
TTTGAACGCA ACTTGCGCCC TTTGGTATTC CGAAGGGCAT GCCTGTTTGA
TTTGAACGCA ACTTGCGCCC TTTGGTATTC CGAAGGGCAT GCCTGTTTGA

301                               350
GAGTCATGAA AATCTCAATC CCTCGGGTTT TATTACCTGT TGGACTTGGA
GAGTCATGAA AATCTCAATC CCTCGGGTTT TATTACCTGT TGGACTTGGA

351                               400
TTTGGGTGTT TGCCGCGACC TGCAAAGGAC GTCGGCTCGC CTTAAATGTG
TTTGGGTGTT TGCCGCGACC TGCAAAGGAC GTCGGCTCGC CTTAAATGTG

401                               450
TTAGTGGGAA GGTGATTACC TGTCAGCCCG GCGTAATAAG TTTGCTGGG
TTAGTGGGAA GGTGATTACC TGTCAGCCCG GCGTAATAAG TTTGCTGGG

451                               500
CCTATGGGGT AGTCTTCGGC TTGCTGATAA CAACCATCTC TTTTTGTGTT
CCTATGGGGT AGTCTTCGGC TTGCTGATAA CAACCATCTC TTTTTGTGTT

501                               550
GACCTCAAAT CAGGTAGGGC TACCCGCTGA ACTTAAGCAT ATCAATAAGC
GACCTCAAAT CAGGTAGGGC TACCCGCTGA ACTTAAGCAT ATCAATAAGC

551
GGAGGA
GGAGGA

```

Figure 35. Alignment of primary sequences of the ITS1 and ITS2 regions of of *Cr. neoformans* var *grubii* RAA and PT specimen *Cr. neoformans* var. *grubii* H99. Unmatched nucleotide bases are shaded. GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) accession numbers are in parentheses.

## 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 (3). It includes two methods, broth microdilution and broth macrodilution. Various commercial systems are also being developed for antifungal susceptibility testing of yeasts, such as Sensititre YeastOne Colorimetric Panel and Etest. The disk diffusion testing method is another good method for antifungal susceptibility testing of yeast. In it the results are read after 24 hr incubation rather than after 48 hr (1). Interpretive criteria for fluconazole disk susceptibility testing are based on US-NCCLS recommended MIC breakpoints (2, 5).

## Materials & Methods

Nineteen microbiology laboratories within the United States and one reference lab each from Canada and United Kingdom participated in this event. Two NCCLS quality control strains, *Candida krusei* ATCC 6258 (S-2) and *Candida parapsilosis* ATCC 22019 (S-4), and three NCCLS reference strains, *Candida albicans* ATCC 90028 (S-1), *Candida parapsilosis* ATCC 90018 (S-3), and *Candida tropicalis* ATCC 750 (S-5) (3, 4), were included in the October 2, 2002 antifungal proficiency testing event. These isolates have been well characterized, and their MIC ranges against amphotericin B and fluconazole have been published (4, 6). MICs within  $\pm 2$  dilutions of the reference result (range of MICs for a particular yeast described in NCCLS, M27-A2) are the acceptable results in this event (3).

## Results

A total of 19 labs participated in this antifungal susceptibility testing event, and the performances of all of the labs were satisfactory. Of the 19 participating laboratories, 6 labs used the broth microdilution method, 1 lab used broth macrodilution, and 3 labs used Etest. Of the 19 labs, 9 labs used the commercially prepared YeastOne Colorimetric microdilution method, while the other 7 labs performed testing according to NCCLS M27-A2 guidelines. The supplementary information on antifungal susceptibility testing procedures is summarized in Table 1. The MIC results submitted by the 19 participants are illustrated in Figure 36. For amphotericin B, good performance was noted for *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 irrespective of the methodology used by the laboratories. For fluconazole, good performance was seen for *C. albicans* ATCC 90028, *C. krusei* ATCC 6258, and *C. parapsilosis* ATCC 90018. Overall, agreement with the NCCLS reference ranges was 95% against amphotericin B and 98% against fluconazole for all five isolates, after with the expansion of the reference range by  $\pm 2$  dilutions. 87% answers for fluconazole and 81% of answers for amphotericin B were within the NCCLS reference range.

## **Further Reading:**

1. Barry, A. and Brown, S. 1996. Fluconazole disk diffusion procedure for determining susceptibility of *Candida* species. *J. Clin. Microbiol.* 34: 2154-2157.
2. National Committee for Clinical Laboratory Standards. 1996. Minutes US-NCCLS antifungal susceptibility subcommittee meeting on interpretive breakpoints. NCCLS, Villanova, PA.
3. National Committee for Clinical Laboratory Standards. 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard – Second Edition. NCCLS document M27-A2 (ISBN 1-56238-469-4). National Committee for Clinical Laboratory Standards, Wayne, Pa.
4. Pfaller, M.A., Bale, M., Buschelman, B., Lancaster, M., Espinel-Ingroff, A., Rex, J.H., Rinaldi, M.G., Cooper, C.R., and McGinnis, M.R. 1995. Quality control guidelines for National Committee for Clinical Laboratory Standards recommended broth macrodilution testing of amphotericin B, fluconazole, and flucytosine. *J. Clin. Microbiol.* 33: 1104-7.
5. Rex, J.H., Pfaller, M.A., Galgiani, J., Bartlett, M., Espinel-Ingroff, A., Ghannoum, M., Lancaster, M., Odds, F., Rinaldi, M., Walsh, T., Barry, A. 1997. Development of interpretive breakpoints for antifungal susceptibility testing; Conceptual framework and analysis of *in vivo* and *in vitro* correlation data for fluconazole and itraconazole and *Candida* infections. *Clin. Infect. Diseases.* 24: 235-247.

### Individual Isolates:

#### S-1 *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

Thirteen labs reported values within the NCCLS reference range, 3 labs reported values within the expanded range, and 1 lab reported a MIC value lower than the expanded range for amphotericin B. Fifteen labs reported values within the NCCLS reference range and 4 labs reported values within the expanded range for fluconazole.

#### 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

All of the participating labs reported values within the NCCLS reference range for amphotericin B. Sixteen labs reported values within the NCCLS reference range and 3 labs reported values within the expanded values for fluconazole.

#### S-3 *Candida parapsilosis* ATCC 90018

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

Nine labs reported values within the NCCLS reference range, 7 labs reported values within the expanded range, and 1 lab reported a MIC value lower than the expanded range for amphotericin B. Sixteen labs reported values within the NCCLS reference range and 3 labs reported values within the expanded range for fluconazole.

#### S-4 *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

All of the participating labs reported values within the NCCLS reference range for amphotericin B. Eighteen labs reported values within the NCCLS reference range and 1 lab reported a MIC value lower than the expanded range for fluconazole.

#### 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	0.5-8.0 µg/ml

Fourteen labs reported values within the NCCLS reference range, 1 lab reported values within the expanded range, and 2 labs reported a MIC value lower than the expanded range for amphotericin B. Eighteen labs reported values within the NCCLS reference range and 1 lab reported a MIC value higher than the expanded range for fluconazole.

Table 1. Summary of supplementary information on antifungal susceptibility testing by participating laboratories

	<b>No. Participant Labs</b>
<b>Test Method</b>	
NCCLS broth microdilution	6
NCCLS broth macrodilution	1
Sensititre YeastOne Colorimetric	9
Etest	3
<b>Medium employed</b>	
RPMI 1640	11 *
RPMI 1640 w / alamar blue	1
Antibiotic medium 3	1
Casitone agar	1
Sabouraud dextrose	2
YeastOne broth	5
<b>Inoculum preparation</b>	
Spectrophotometric	9
MacFarland	10
<b>Inoculum size</b>	
0.5-2.5 × 10 <sup>3</sup>	10
1.5-8 × 10 <sup>3</sup>	7
0.5-1.0 × 10 <sup>4</sup>	2
<b>Incubation temperature</b>	
35°C	17
37°C	2
<b>Incubation duration</b>	
24 hr	10 *
48 hr	10
<b>Endpoint reading</b>	
Visual	13
Spectrophotometric	1
Colorimetric	5
<b>Scoring endpoint<sup>1</sup></b>	
100% inhibition	9 *
95% inhibition	1
80% inhibition	8
50% inhibition	3
Other (color change)	4
<b>QC organism</b>	
NCCLS recommended strains	19
Unknown	0

<sup>1</sup>Most labs used 100% inhibition for amphotericin B and either 80 or 50% for azoles.

\* More than one value reported by individual laboratories

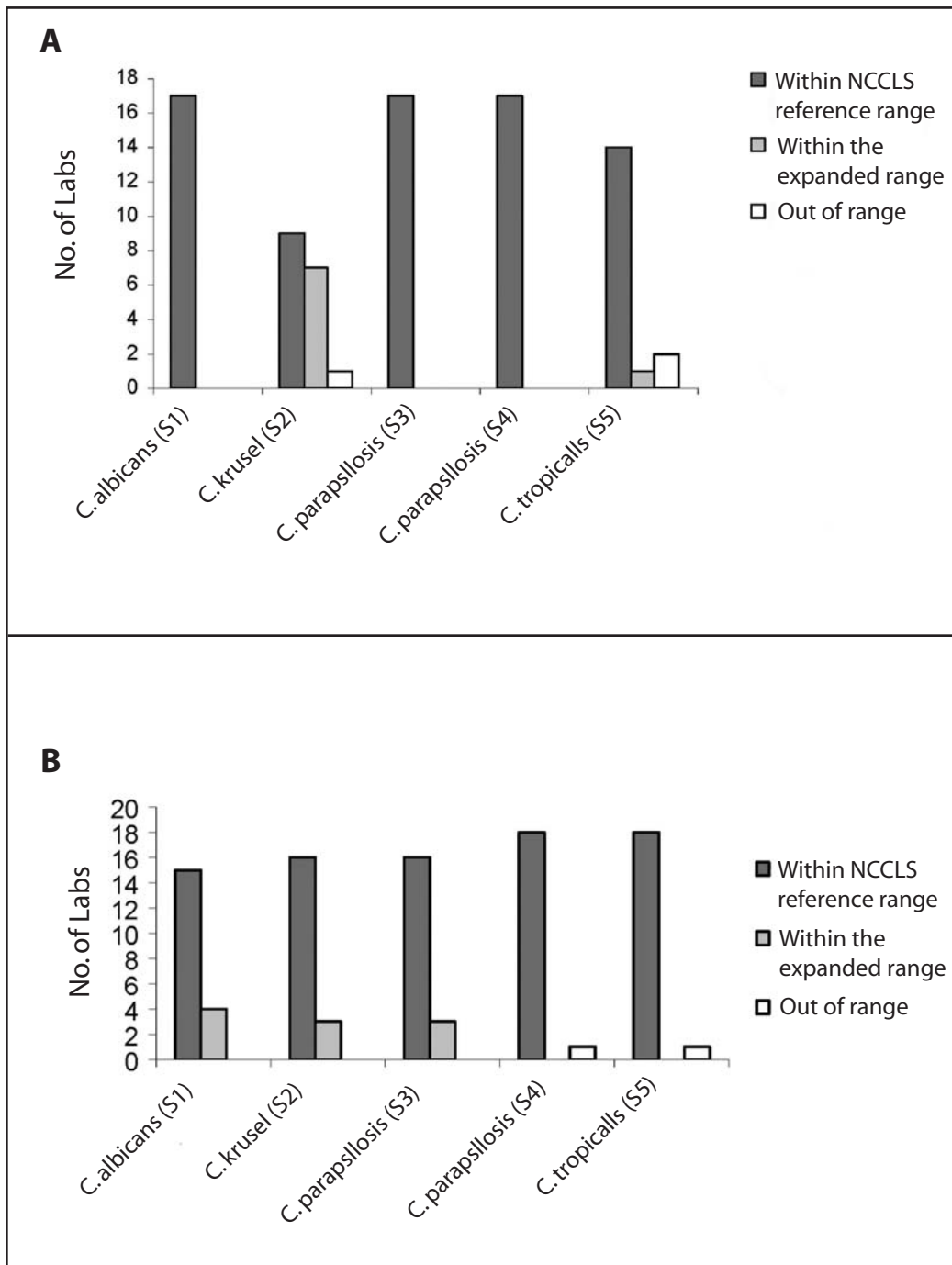


Figure 36. Summary of the results submitted by the participating labs for 5 isolates, for amphotericin B (A) and fluconazole (B).

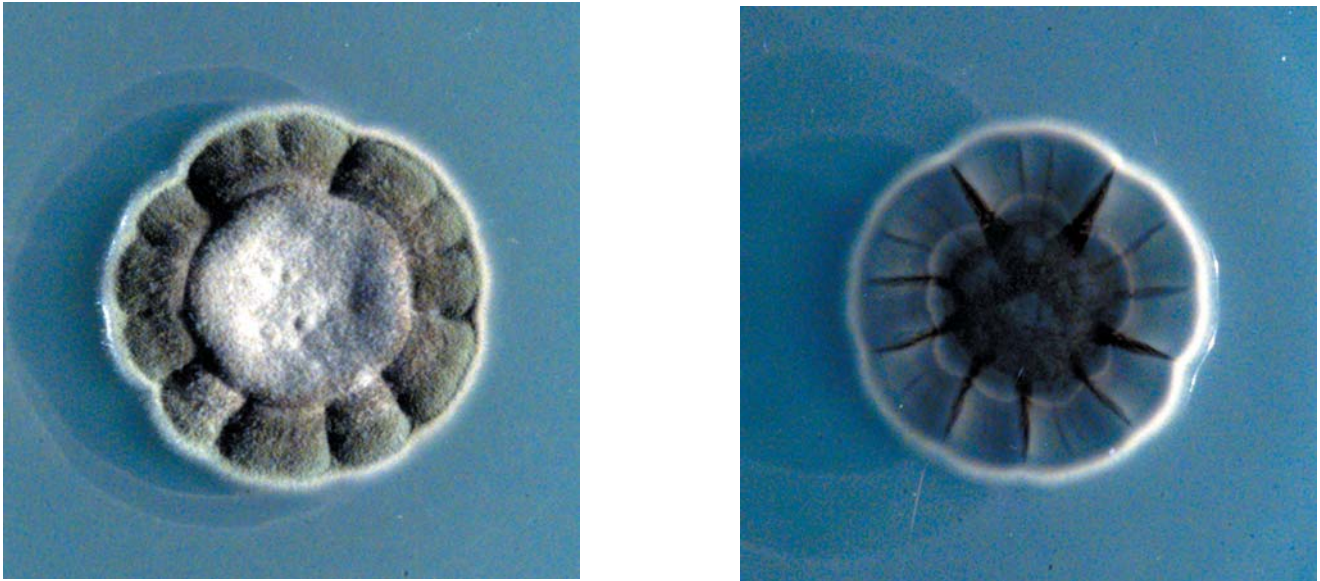


Figure 1. (L) Eight-day-old, grayish green colony of *Cladosporium* sp. on Sabouraud's dextrose agar. (R) The reverse shows greenish-black to brownish-black pigmentation.

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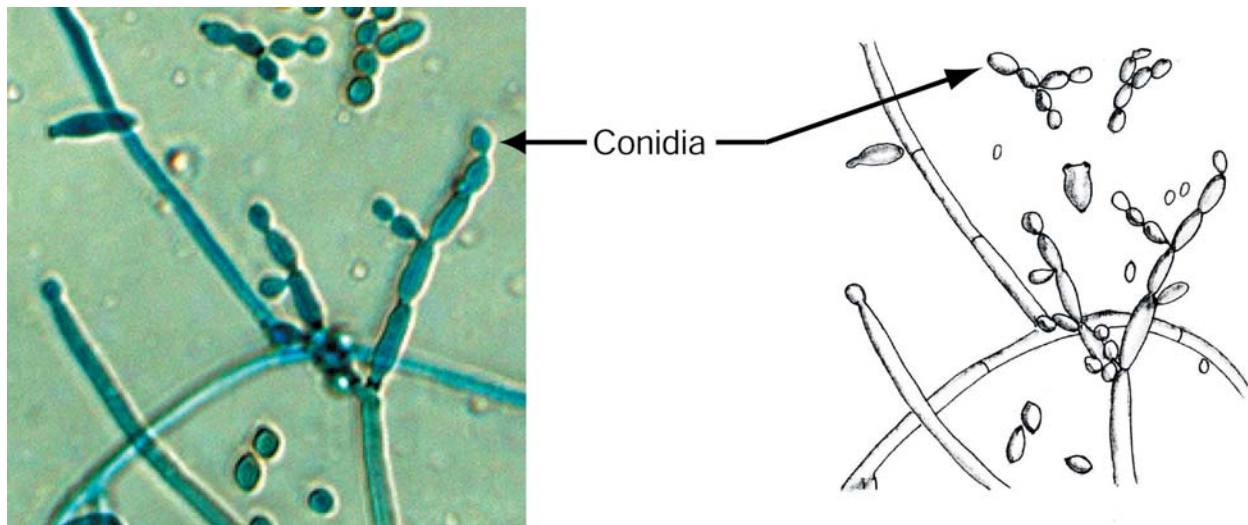


Figure 2. Microscopic morphology of *Cladosporium* sp. conidia occur in long, branched chains with variable size. The scars at the points of attachment of conidia are evident (left; 400× magnification, right; line drawing not to scale).

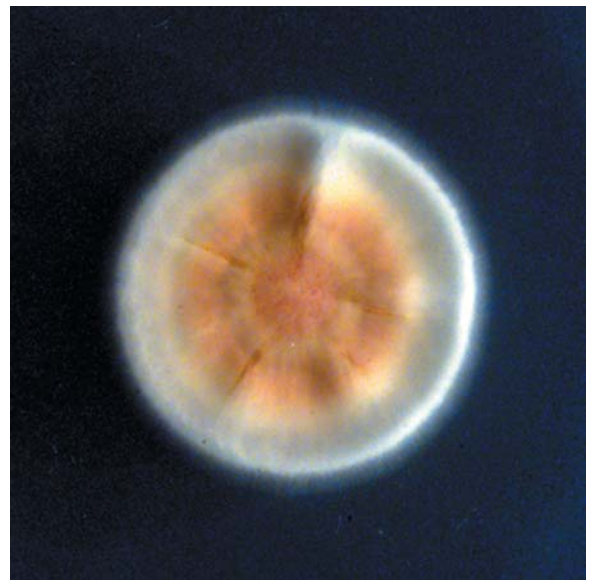


Figure 3. (L) Eight-day-old, olive-green colony of *Aspergillus versicolor* on Sabouraud's dextrose agar. (R) The reverse of the colony shows yellowish to light brown or orange pigment.

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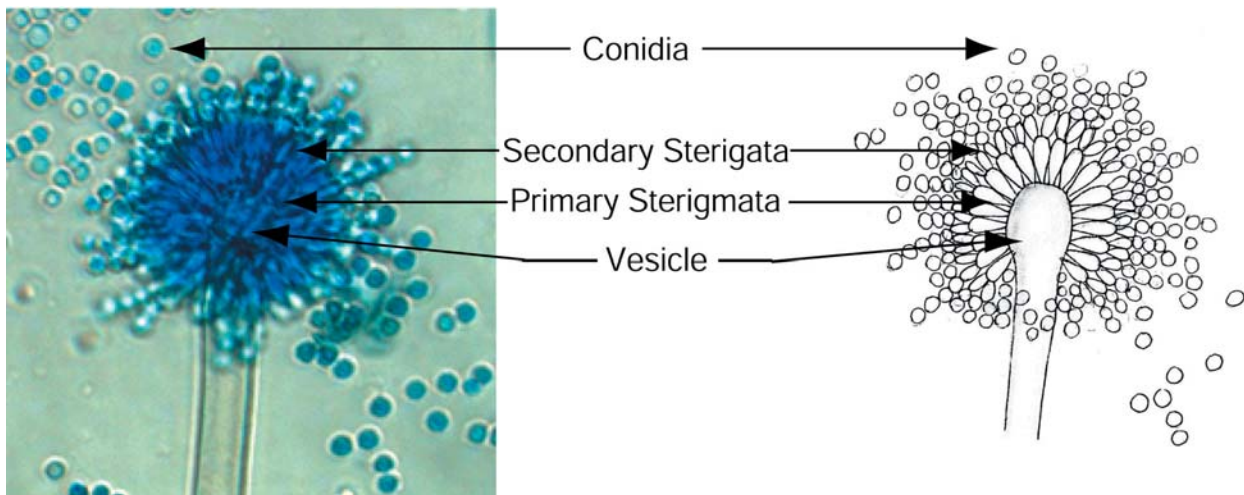


Figure 4. Microscopic morphology of *Aspergillus versicolor* showing typical radiate conidial heads with biseriata phialides and round, smooth, or rough conidia (left; 400x magnification, right; line drawing not to scale).

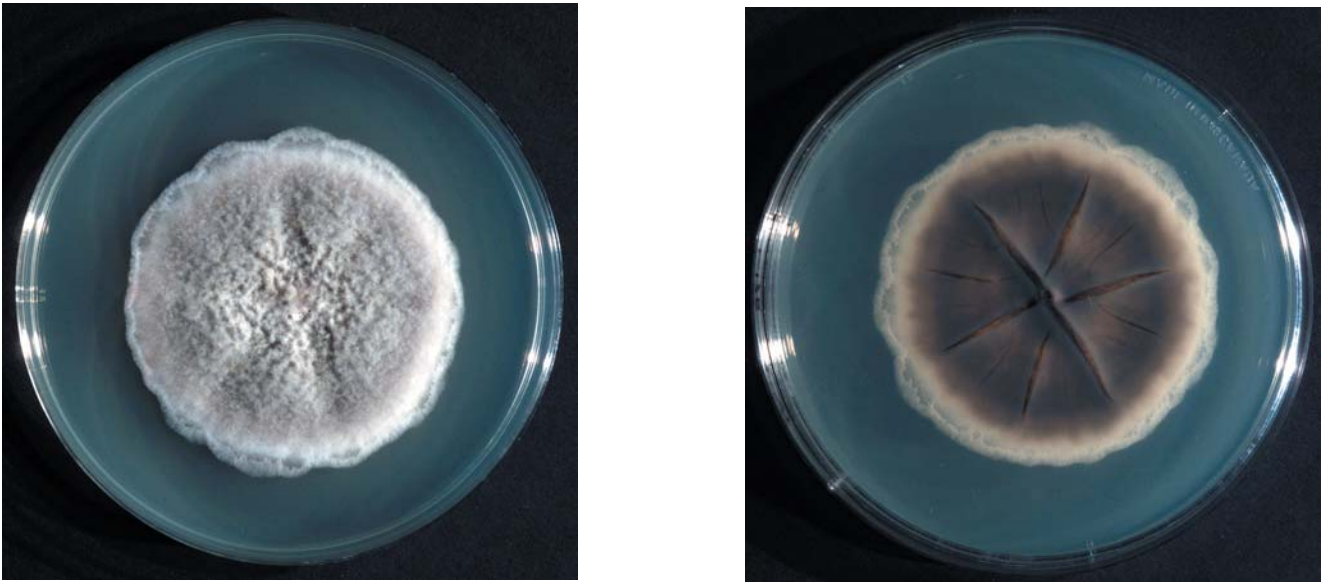


Figure 5. (L) Eight-day-old, pale gray colony, with white fringe and wooly surface, of *Alternaria* sp. on Sabouraud's dextrose agar. (R) The reverse side of the colony is brown to black.

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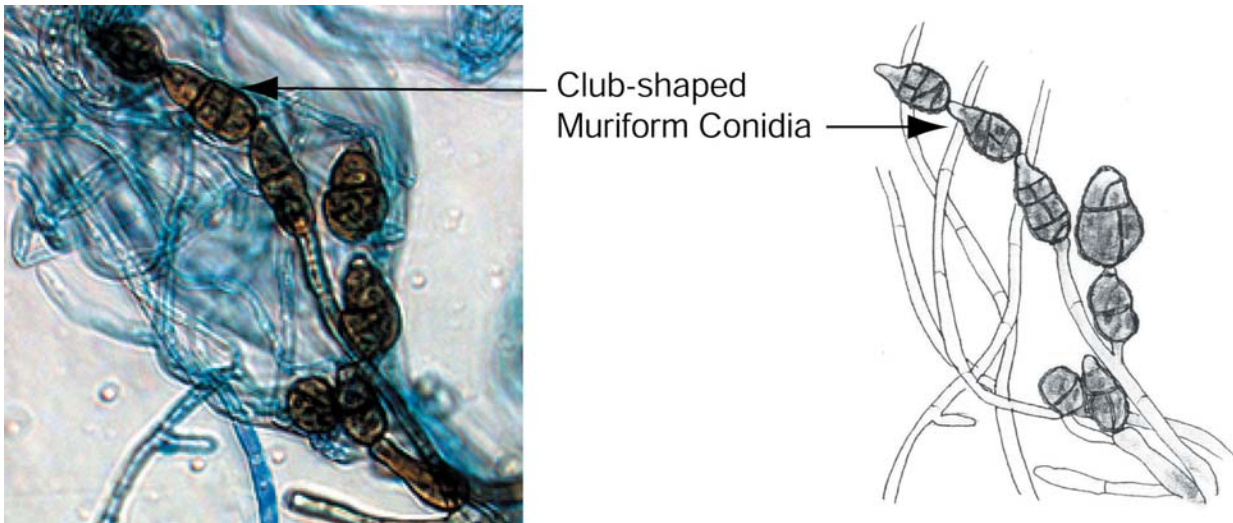


Figure 6. Microscopic morphology of *Alternaria* sp. showing septate hyphae, darkly pigmented. Club-shaped conidia with both horizontal and transverse septa are seen in chain (left; 200× magnification, right; line drawing not to scale).

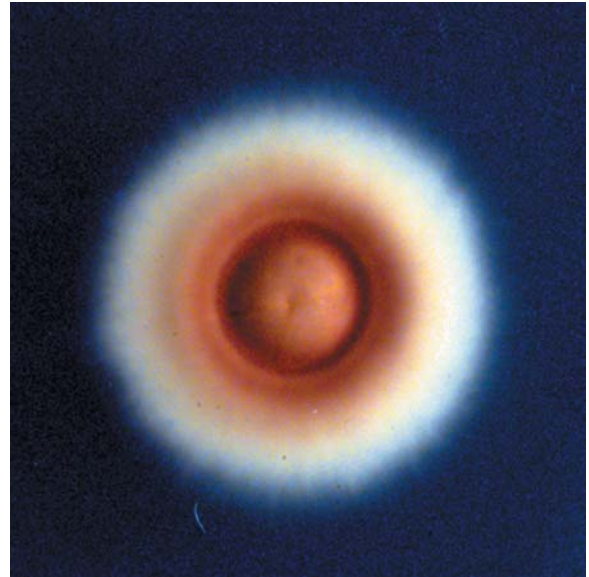


Figure 8. (L) Nine-day-old, white to cream colored powdery colony of *Trichophyton mentagrophytes* on Sabouraud's dextrose agar. (R) The reverse of the colony appears yellowish to tan.

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Round, clustered  
Microconidia

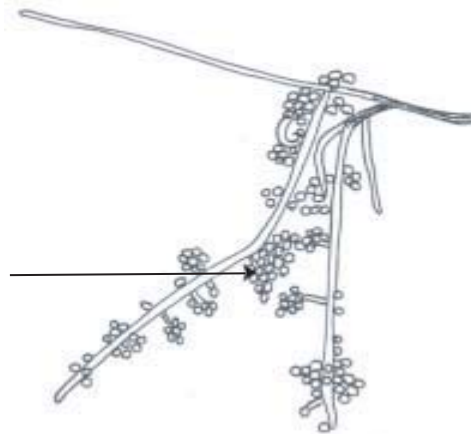
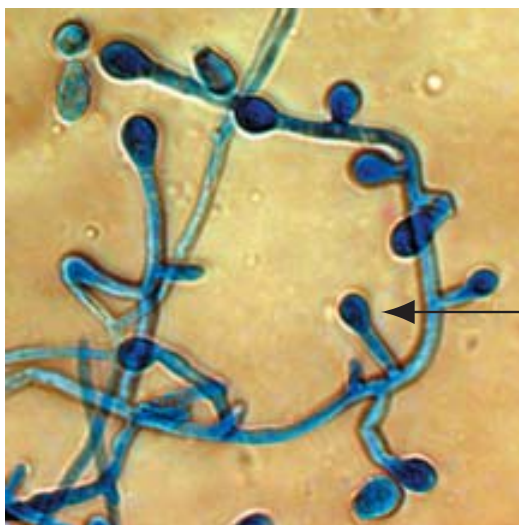


Figure 9. Microscopic morphology of *Trichophyton mentagrophytes*. Round microconidia clustered on conidiophores are seen (left; 200× magnification, right; line drawing not to scale).



Figure 11. (L) Nine-day-old, white to cream colored powdery to granular colony of *Chryso sporium* sp. on Sabouraud's dextrose agar. (R) The reverse of the colony is yellow or buff.

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Conidia with  
Truncated Bases

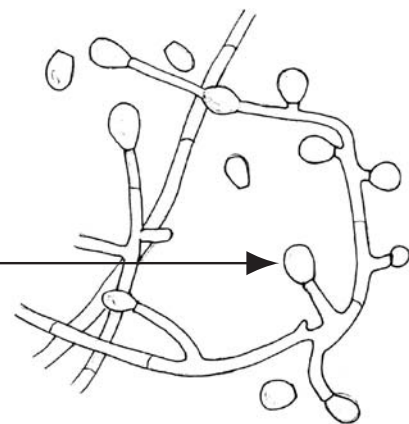


Figure 12. Microscopic morphology of *Chryso sporium* sp. showing hyaline septate hyphae. Ovoid or club-shaped conidia with broad truncated bases are seen either singly or in short chains borne directly on hyphae or in short conidiophores (left; 200× magnification, right; line diagram not to scale).

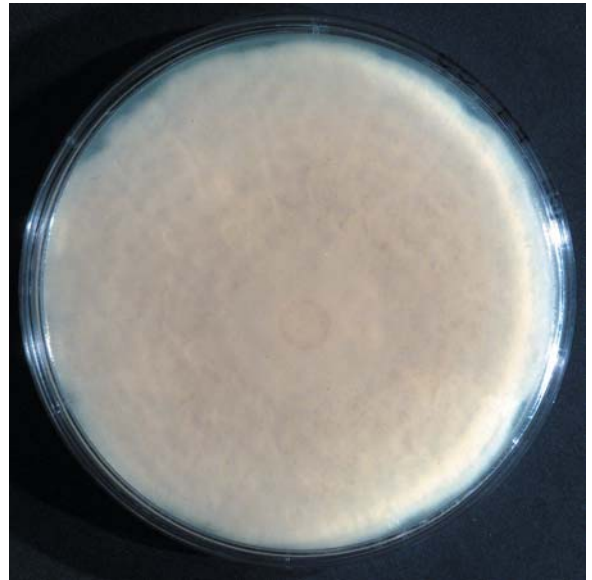


Figure 14. (L) Nine-day-old, grayish and very woolly colony of *Mucor racemosus* on Sabouraud's dextrose agar. (R) The reverse of the colony appears pale yellow.

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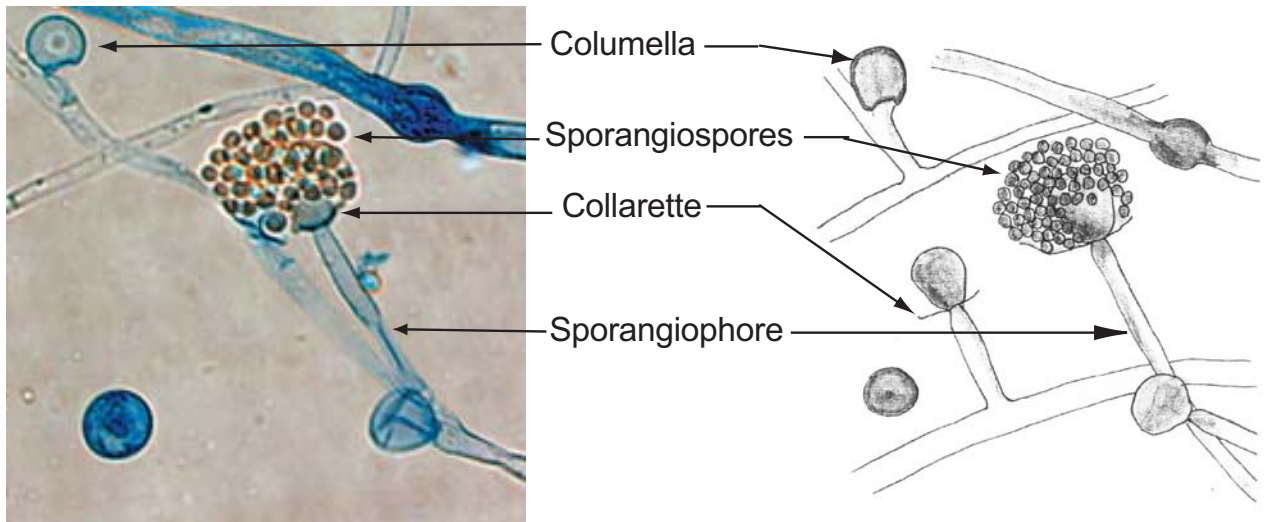


Figure 15. Microscopic morphology of *Mucor racemosus* showing hyaline aseptate hyphae. Sporangia with columellas lack apophyses (left; 200× magnification, right; line drawing not to scale).



Figure 16. Seven-day-old, white to yellowish, wrinkled and velvety colony of *Trichosporon asabii* on Sabouraud's dextrose agar.

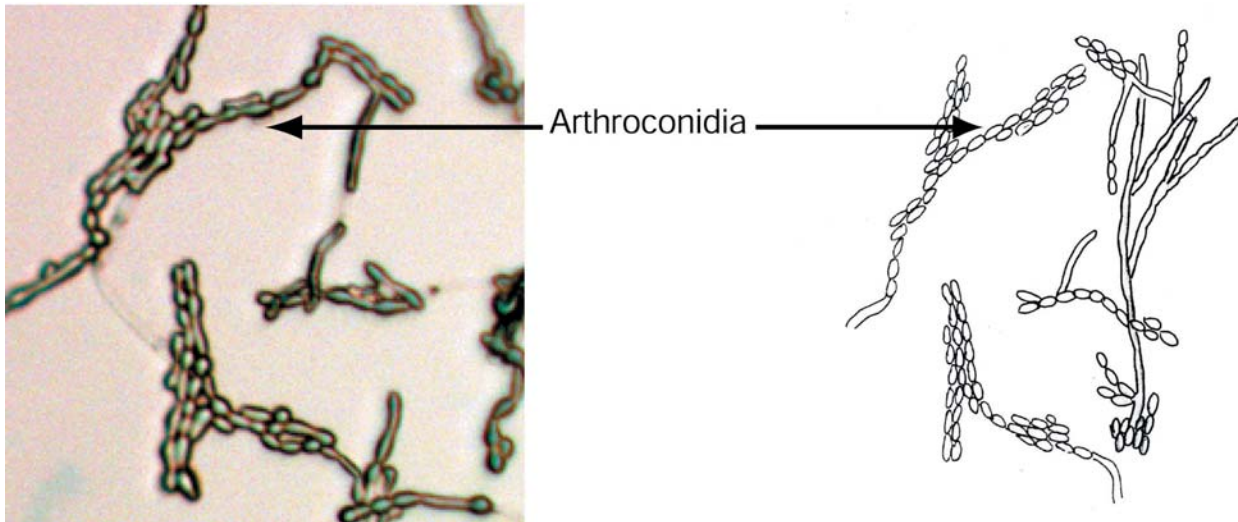


Figure 17. Microscopic morphology of *Trichosporon asabii* on corn meal agar with Tween 80, showing arthroconidia (left; 400× magnification, right; line drawing not to scale).



Figure 20. Seven-day-old, smooth, creamy, soft colony of *Hansenula anomala* on Sabouraud's dextrose agar.

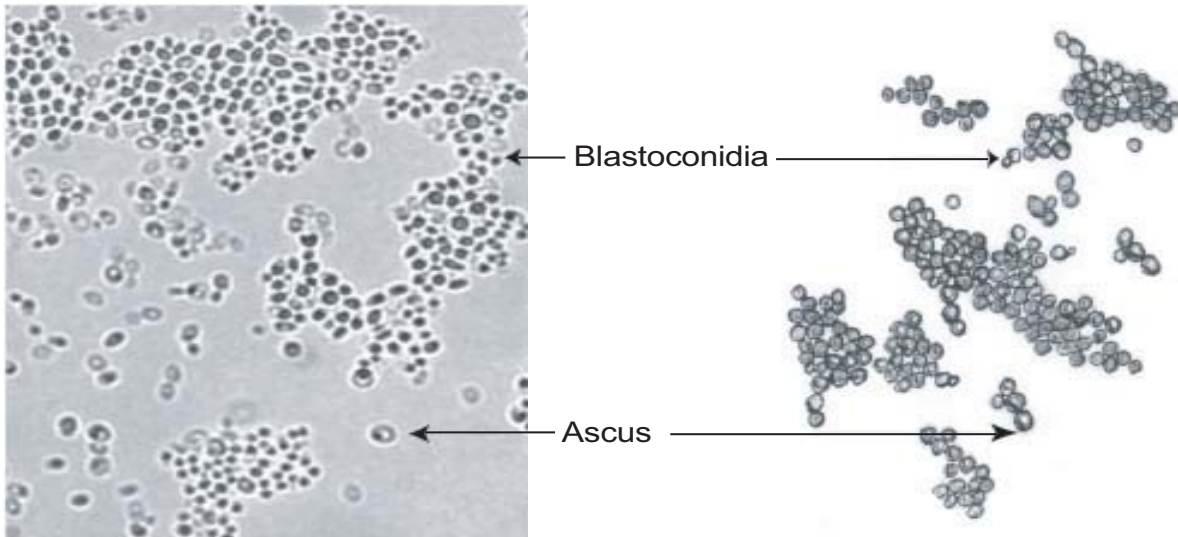


Figure 21. Microscopic morphology of *Hansenula anomala*. On corn meal agar with Tween 80 culture, blastoconidia with ascus are seen (left; 400× magnification, right; line drawing not to scale).



Figure 24. Seven-day-old, white to cream, smooth colony of *Candida parapsilosis* on Sabouraud's dextrose agar.

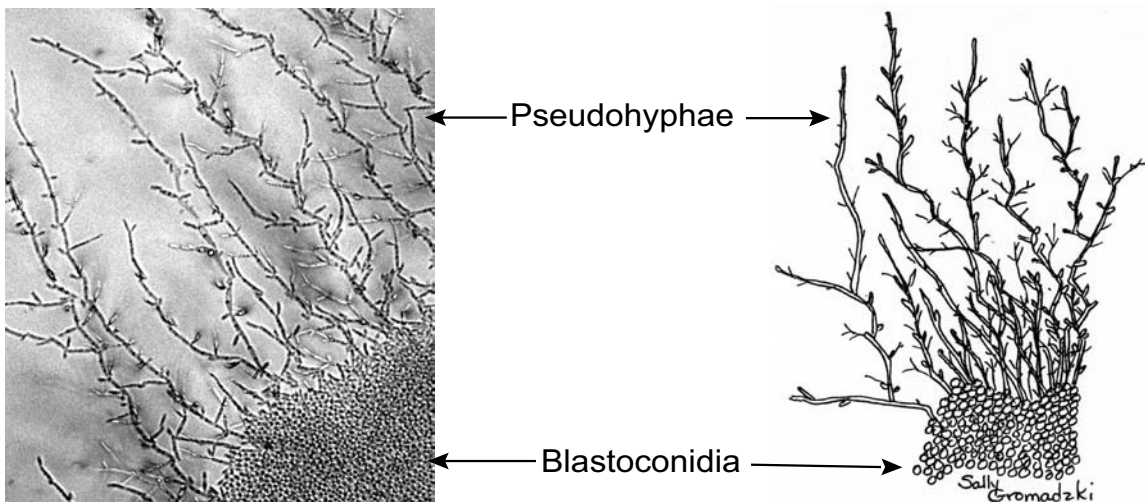


Figure 25. Microscopic morphology of *Candida parapsilosis*, on corn meal agar with Tween 80, shows long, multibranched pseudohyphae together with small cluster of elongated blastoconidia (left; 400× magnification, right; line drawing not to scale).



Figure 28. Seven-day-old, white to cream, glossy, smooth, and soft colony of *Candida stellatoidea* on Sabouraud's dextrose agar.

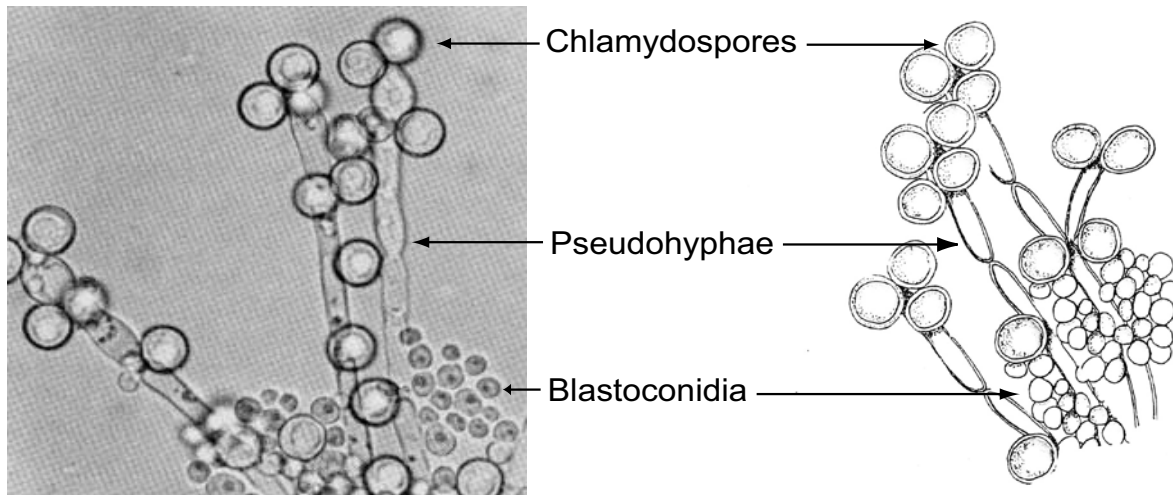


Figure 29. Microscopic morphology of *Candida stellatoidea*, on corn meal agar with Tween 80, shows clusters of chlamydospores and blastoconidia (left; 400× magnification, right; line drawing not to scale).

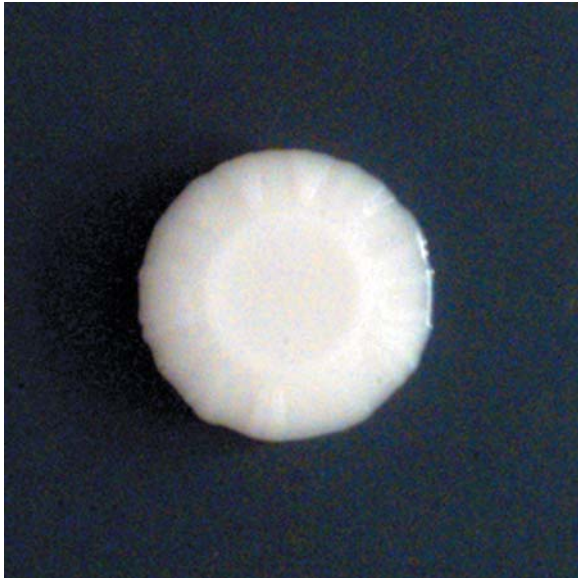


Figure 32. Seven-day-old, cream to tan colored, smooth, moist, and soft colony of *Cryptococcus neoformans* on Sabouraud's dextrose agar.

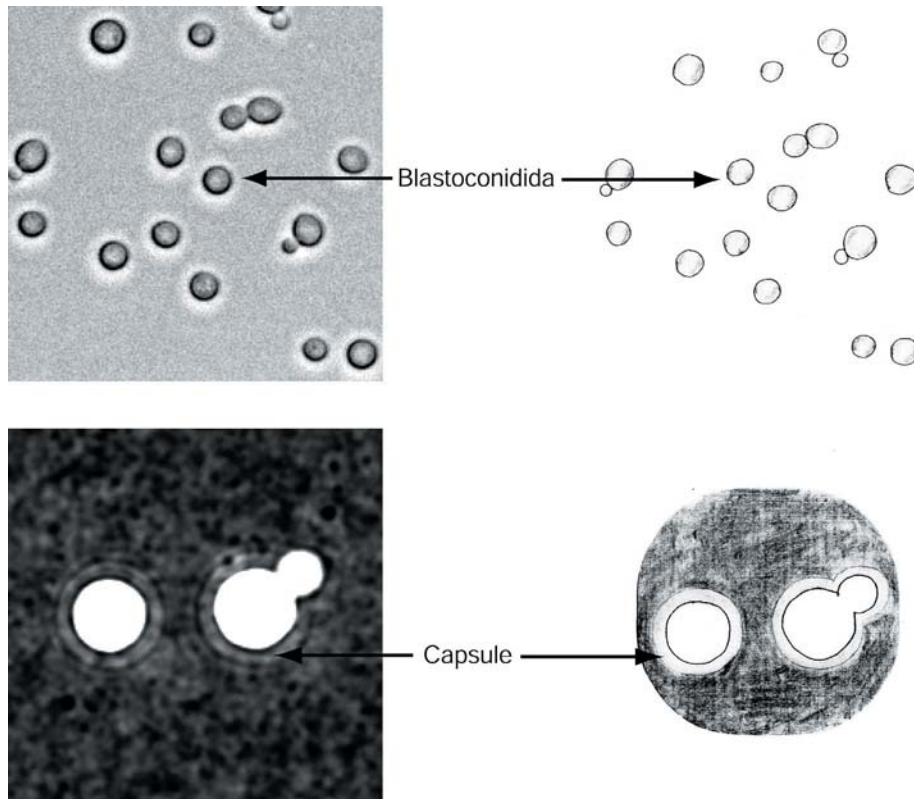


Figure 33. Microscopic morphology of *Cryptococcus neoformans* on corn meal agar with Tween 80. (U) Round, large blastoconidia. (L) India-ink preparation revealing capsules (left; 1000× magnification, right; line drawing not to scale).

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