

A microscopic image of a fungal culture, likely a mold, showing numerous hyphae and spores. The spores are small, oval-shaped, and appear to be arranged in chains or clusters. The hyphae are long, thin, and branching. The overall appearance is that of a typical mold culture.

# **MYCOLOGY PROFICIENCY TESTING PROGRAM**

**Critique**  
**May 2001**

**Wadsworth Center**  
**New York State Department of Health**

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## METHODS

A minimum of two strains of each of the proposed yeast specimens was examined for inclusion in the proficiency test. The morphology of all yeast strains was studied on cornmeal + Tween 80 agar plates inoculated by the Dalmau or streak-cut method. On occasion, the morphologic features were examined on corn meal + Tween 80 slide cultures. 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 investigated through the use of Durham tubes in 3 ml of Wickerham fermentation broth in 16 x 125 mm screw-cap tubes. Supplemental physiologic characteristics, such as nitrate assimilation, urease activity, cycloheximide sensitivity, etc., were investigated with the appropriate test media. The strain demonstrating best morphologic and physiologic characteristics of each of the proposed test specimens was used in the test.

## GRADING

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. Referee laboratories are selected at random from among hospital laboratories participating in the program. They represent all geographical areas of the New York State and have a record of excellent performance during the last three years.

The grading for each specimen is based on the following formula:

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

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

# ANSWER KEY

## MYCOLOGY - GENERAL & YEAST ONLY

<b>Specimen Key</b>	<b>Validated Specimen</b>	<b>Acceptable Answers</b>
<b>Y - 1</b> <i>Cryptococcus neoformans</i>	<i>Cryptococcus neoformans</i>	<i>Cryptococcus neoformans</i>
<b>Y - 2</b> <i>Malassezia pachydermatis</i>	<i>Malassezia pachydermatis</i>	<i>Malassezia pachydermatis</i>
<b>Y - 3</b> <i>Candida lipolytica</i>	<i>Candida lipolytica</i>	<i>Candida lipolytica</i> <i>Yarrowia lipolytica</i>
<b>Y - 4</b> <i>Prototheca wickerhamii</i>	<i>Prototheca wickerhamii</i>	<i>Prototheca wickerhamii</i>
<b>Y - 5</b> <i>Geotrichum candidum</i>	<i>Geotrichum candidum</i>	<i>Geotrichum candidum</i> <i>Geotrichum penicillatum</i> <i>Geotrichum capitatum</i> <i>Geotrichum klebahnii</i> <i>Geotrichum species</i>
Ed.sp. <i>Candida glabrata</i>		

# RESULTS

## MYCOLOGY – GENERAL & YEAST ONLY

		<b>CORRECT RESPONSES/ TOTAL # LABS(%)</b>	<b>REFEREE LABS (%)</b>
<b>Y - 1</b>	<i>Cryptococcus neoformans</i>	149/150 (99%)	10/10 (100%)
<b>Y - 2</b>	<i>Malassezia pachydermatis</i>	144/150 (96%)	10/10 (100%)
<b>Y - 3</b>	<i>Candida lipolytica</i>	137/150 (91%)	10/10 (100%)
<b>Y - 4</b>	<i>Prototheca wickerhamii</i>	150/150 (100%)	10/10 (100%)
<b>Y - 5</b>	<i>Geotrichum candidum</i>	149/150 (99%)	10/10 (100%)

# TEST STATISTICS

## MYCOLOGY – GENERAL & YEAST ONLY

Number of participating laboratories	150
Number of REFEREE laboratories	10
Number of laboratories responding by deadline	150
Number of laboratories responding after deadline [TEST NOT GRADED]	0
Number of laboratories not responding	0
Number of laboratories successfully completing this test	147
Number of laboratories unsuccessfully completing this test	3

### Commercial Identification Systems Used\*

AMS Vitek system	74
API 20C	41
API 20C AUX	28
Microscan	06
Remel Uni-Yeast-Tek	08
Other	14

(\* Includes multiple systems used by some labs.)

## Y-1 *Cryptococcus neoformans*

Source: CSF

### Correct answers

Referee Labs: 10/10

Labs with correct ID: 149

### Incorrect answers:

Unidentified: 1

### **Clinical Significance:**

Prior to AIDS epidemic, cryptococcosis was commonly reported from patients with underlying cancer. *Cr. neoformans* consisted of two varieties: *Cr. neoformans* var. *neoformans* (serotypes A & D) and *Cr. neoformans* var. *gattii* (serotypes B & C). Recently, *Cr. neoformans* var. *neoformans* was further subdivided to two varieties: *Cr. neoformans* var. *neoformans* (D) and *Cr. neoformans* var. *grubii* (A) (4). *Cr. neoformans* var. *gattii* causes meningoencephalitis and pulmonary cryptococcosis in patients with normal immune status. *Cr. neoformans* var. *neoformans* and *Cr. neoformans* var. *grubii* infect patients with AIDS or other underlying immune dysfunctions. *Cr. neoformans* var. *neoformans* infections are more likely to have cutaneous involvement, and infect older patients as compared to infections caused by *Cr. neoformans* var. *grubii* (9).

### **Ecology:**

*Cr. neoformans* var. *neoformans* and *Cr. neoformans* var. *grubii* are commonly found in avian (pigeon) droppings while *Cr. neoformans* var. *gattii* is commonly found on *Eucalyptus camaldulensis* (red river gum tree) mainly in tropical areas.

### **Epidemiology:**

Infections caused by *Cr. neoformans* var. *neoformans* are more common in Europe. *Cr. neoformans* var. *grubii* is worldwide in distribution while infections caused by *Cr. neoformans* var. *gattii* are restricted to tropical and subtropical regions, and in non - AIDS patients.

### **Laboratory Diagnosis:**

- a. Culture – At 25<sup>o</sup> C, on Sabouraud's dextrose agar, mucoid, smooth, moist, initially cream later turning tan colonies seen in 3 – 5 days (**Fig. 1**).
- b. Microscopic morphology – On corn meal agar with Tween – 80, round yeast cells with no pseudohyphae and in India-ink preparation encapsulated yeasts are seen (**Fig. 2**).
- c. Differentiation from other yeasts - *Cr. neoformans* does not ferment any carbohydrates, does not grow on the media containing cycloheximide, and grows at 37<sup>o</sup> C. *Cr. neoformans* produces dark brown colonies on niger seed agar. It produces urease enzyme and it is negative on nitrate reaction. On the API 20C AUX, a specific assimilation biocode identifies this organism. The three varieties are differentiated: 1) Growth and color change by *Cr. neoformans* var. *gattii* on canavanine-glycine-bromthymol blue (CGB) medium to blue-green after 2 – 5 days at 25<sup>o</sup> C, 2) PCR technique can differentiate *Cr. neoformans* var. *gattii* from the other two varieties (3).
- d. Molecular tests - *Cr. neoformans* is one of the most intensely studied pathogenic fungus. 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 (3).
- e. In vitro susceptibility testing – Most isolates are susceptible to amphotericin B, 5 FC, and to azoles like fluconazole, itraconazole, etc. Few isolates with high MIC to fluconazole have been isolated from AIDS patients (1).

### **Comments:**

The organism sent in this testing event was *Cr. neoformans* variety *gattii* ATCC 32609.

serotype A isolates. J Clin Microbiol. 37(3): 838-40.

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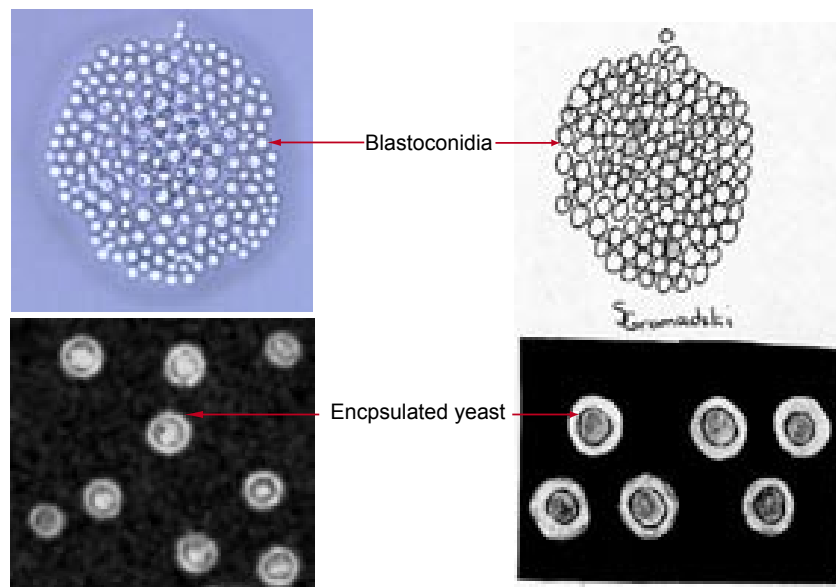
**Figure 1.**

Four-day-old, creamish colony of *Cryptococcus neoformans* on Sabouraud's dextrose agar.



**Figure 2**

Microscopic morphology of *Cryptococcus neoformans* on cornmeal agar showing round blastoconidia (left; 200X magnification, right; line drawing not to scale) and India-ink preparation demonstrating capsule (400X magnification).



## Y-2 *Malassezia pachydermatis*

**Source:** Rectal swab

### Correct answers:

Referee Labs: 10/10  
Labs with correct ID: 144

### Incorrect answers:

Bacteria: 1  
*Candida inconspicua*: 1  
*Cryptococcus laurentii*: 1  
*Malassezia furfur*: 1  
*Malassezia* species: 1  
*Prototheca zopfii*: 1

### **Clinical Significance:**

*Malassezia pachydermatis* causes catheter induced sepsis in premature neonates receiving intravenous lipid administration. It has also been associated with skin infections causing tinea versicolor and folliculitis.

### **Ecology:**

*M. pachydermatis* is commonly found in animals like dogs, cats, and elephants rarely in humans. It causes otitis externa in dogs.

### **Laboratory Diagnosis:**

- a. **Culture** – At 25<sup>o</sup> C, colonies on Sabouraud's dextrose agar are yellowish cream in color, dry and small in 2 – 5 days (**Fig. 3**).
- b. **Microscopic morphology** – On corn meal agar with Tween-80, oval yeast cells with budding from phialides are seen (**Fig. 4**).
- c. **Differentiation from other yeasts** - *Malassezia pachydermatis* grows on media containing cycloheximide, grows well at 37<sup>o</sup> C, but poorly at 25<sup>o</sup> C and is urease positive. It is differentiated from *M. furfur* and other *Malassezia* species by its ability to grow without olive oil.
- d. **Molecular tests** – Seven species of *Malassezia* were distinguished based on ribosomal gene

(large subunit) and ITS (internal transcribed spacer) region PCR and restriction endonuclease analysis (4). Genetic diversity in *Malassezia pachydermatis* was observed by multilocus enzyme electrophoresis (6). RAPD (random amplified polymorphic DNA) (1), partial sequencing of the large subunit ribosomal RNA (2) was also used for epidemiological analysis of *M. pachydermatis*.

- e. **In vitro susceptibility testing** – Most isolates of *M. pachydermatis* were susceptible to azoles like ketoconazole, itraconazole, fluconazole. Strains tested for terbinafine ranged from susceptible to resistant (5).

### **Comments:**

This clinical strain has been isolated from an outbreak of blood stream infections in neonatal intensive care unit. The Mycology Laboratory at the Wadsworth Center received 21 blood and rectal isolates from 9 patients. All patients' *M. pachydermatis* isolates showed identical band patterns and were genetically related.

### **Further Reading:**

1. Boekhout, T., M. Kamp, and E. Gueho. 1998. Molecular typing of *Malassezia* species with PFGE and RAPD. *Medical Mycology*. 36(6): 365–372.
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4. Gupta, A. K., Y. Kohli, and R. C. Summerbell. 2000. Molecular differentiation of seven *Malassezia* species. *J Clin Microbiol*. 38(5): 1869-75.
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6. Midreuil, F., J. Guillot, E. Gueho, F. Renaud, M. Mallie, and J. M. Bastide. 1999. Genetic diversity in the yeast species *Malassezia pachydermatis* analysed by multilocus enzyme electrophoresis. *Int J Syst Bacteriol*. 49 Pt 3:1287-94.
7. Nakamura, Y., R. Kano, T. Murai, S. Watanabe, and A. Hasegawa. 2000. Susceptibility testing of *Malassezia* species using the urea broth microdilution method. *Antimicrob Agents Chemother*. 44(8): 2185-6.

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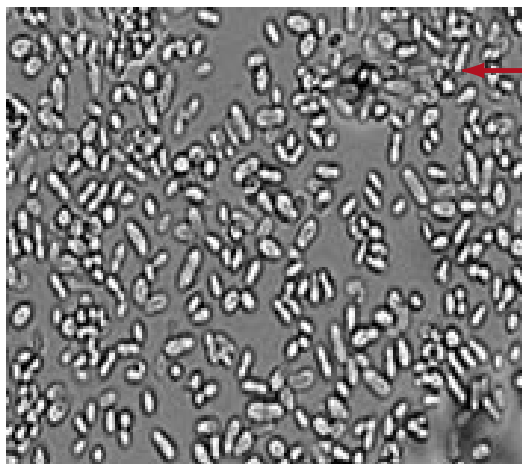
**Figure 3.**

Six-day-old dry, colony of *Malassezia pachydermatis* on Sabouraud's dextrose agar.

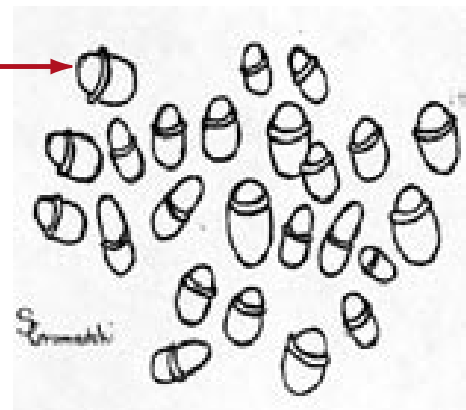


**Figure 4.**

Microscopic morphology of *Malassezia pachydermatis* on cornmeal agar showing oval yeast cells with budding from phialides (left; 400X magnification, right; line drawing not to scale).



Oval yeast cells



### Y-3 *Candida lipolytica*

Source: Blood

Correct answers:

Referee Labs: 10/10  
Labs with Correct ID: 137

Incorrect answer:

*Blastoschizomyces capitatus*: 4  
*Candida krusei*: 2  
*Candida norvegensis*: 2  
*Geotrichum capitatum*: 1  
*Candida albicans*: 1  
*Candida zeylanoides*: 1  
*Trichosporon beigelii*: 1  
Unidentified: 1

**Clinical Significance:**

*Candida lipolytica* causes fungemia and sinusitis in immunocompromised patients. It is also reported in traumatic ocular infections. It has been isolated from human vagina.

**Ecology:**

*Candida lipolytica* has been isolated from humans, lower mammals and plants.

**Laboratory Diagnosis:**

- a. Culture – At 25<sup>o</sup> C, colonies on Sabouraud's dextrose agar are soft, initially smooth later becoming wrinkled in 3 – 5 days (**Fig. 5**).
- b. Microscopic morphology – Colonies on corn meal agar with Tween-80 show abundant, multibranched true hyphae and infrequent blastoconidia along the hyphae (**Fig. 6**). The teleomorph (sexual form) of *C. lipolytica* is *Yarrowia lipolytica*, which forms ascospores on yeast – malt agar in 3 –7 days at 25<sup>o</sup> C.
- c. Differentiation from other yeasts–*Candida lipolytica* grows on media containing cycloheximide, grows well at 25<sup>o</sup> C, urease positive and negative on nitrate reactions. Sugars

are not fermented by *C. lipolytica*. No growth at 42<sup>o</sup> C and positive growth on media containing cycloheximide differentiates it from *C. krusei*. *C. lipolytica* is differentiated from *Geotrichum* species by negative urease reaction by the later. On the API 20C AUX, a specific assimilation biocode differentiates this organism from the Genus *Trichosporon*.

- d. Molecular tests – Comparisons of partial rRNA/ rDNA sequences analysis demonstrated the *C. lipolytica* is distinctly related to selected members of Genus *Candida* (4). Randomly amplified polymorphic DNA (RAPD) PCR has been used for the identification of *C. lipolytica* isolated from dairy products (1, 2).
- e. In vitro susceptibility testing – Isolates of *C. lipolytica* are less susceptible to amphotericin B. Most isolates are susceptible to azoles like fluconazole and ketoconazole and 5FC but resistant to itraconazole.

**Comments:**

This clinical isolate was not identified by 13 of the participating labs. This could be because *C. lipolytica* produces true hyphae, grows on media containing cycloheximide, thereby resembling *B. capitatus* and *T. beigelii*.

**Further Reading:**

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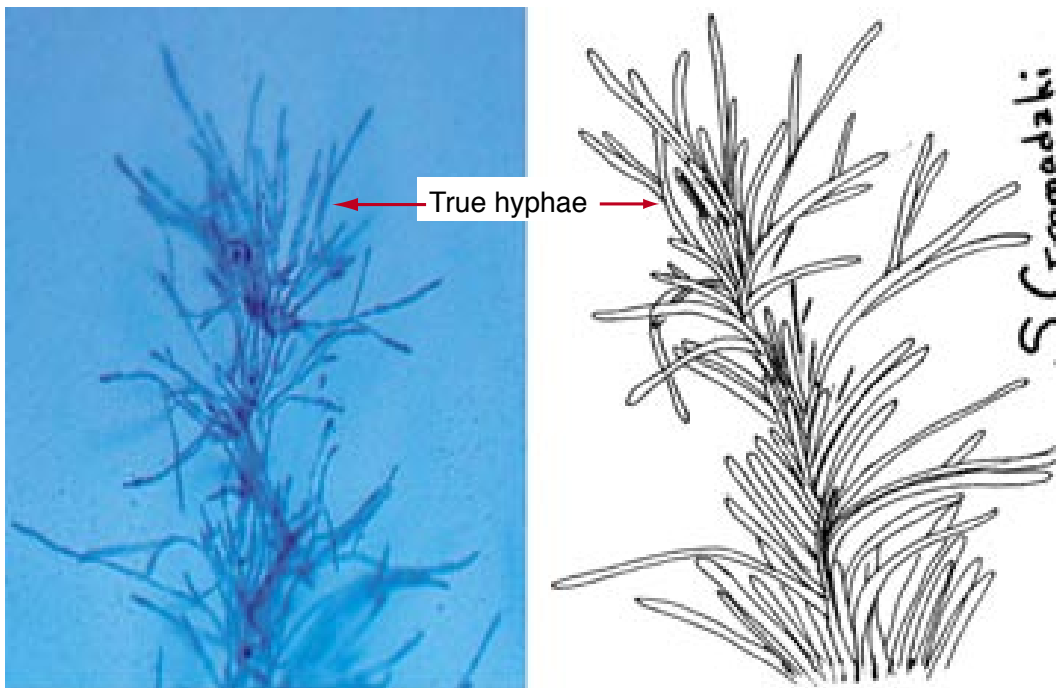
**Figure 5.**

Three-day-old, wrinkled colony of *Candida lipolytica* on Sabouraud’s dextrose agar.



**Figure 6.**

Microscopic morphology of *Candida lipolytica* on cornmeal agar showing multibranched, true hyphae and few blastoconidia (left:200X magnification, right; line drawing not to scale).



## Y-4 *Prototheca wickerhamii*

Source: Wound

Correct answers:

Referee Labs: 10/10  
Labs with Correct ID: 150

### Clinical Significance:

*P. wickerhamii* causes protothecosis in humans. Most commonly, these yeast-like algae cause cutaneous lesions and subcutaneous lesions like bursitis. Rarely, *P. wickerhamii* causes systemic infections. Most of the time, the organism is acquired through traumatic implantation in subcutaneous tissue .

### Ecology:

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

### Laboratory Diagnosis:

- a. Culture – At 25<sup>o</sup> C, on Sabouraud’s dextrose agar colonies are moist, cream – colored, yeast - like in 2 – 4 days (**Fig.7**).
- b. Microscopic morphology – On cornmeal agar with Tween-80, sporangia of various sizes, some filled with sporangiospores (endospores), are seen (**Fig. 8**). There is no budding, true or pseudo hyphae formation.
- c. Differentiation from other yeasts - *P. wickerhamii* requires thiamine for growth, does not grow on media containing cycloheximide, grows well at 25 and 37<sup>o</sup> C. The cells of *P. wickerhamii* are smaller than those of *P. zopfii*. On the API 20C AUX, a specific assimilation biocode differentiates it from other *Prototheca* species. The isolates of *P. zopfii* are resistant to 50-µg clotrimazole disk at 37<sup>o</sup> C while *P. wickerhamii* isolates produced a zone of inhibition (2).

- d. Molecular tests – Sequence analysis of the mitochondrial small subunit rRNA from *P. wickerhamii* showed higher homology with mitochondrial sequence from plants (4, 9).
- e. In vitro susceptibility testing – Almost all isolates are susceptible to amphotericin B but resistant to fluconazole and 5FC, variably susceptible to itraconazole and ketoconazole.

### Comments:

All the participating labs have correctly identified this organism to genus and species level.

### Further Reading:

1. Boyd, A. S., M. Langley, and L. E. King, Jr. 1995. Cutaneous manifestations of *Prototheca* infections. J Am Acad Dermatol. 32(5 Pt 1): 758-64.
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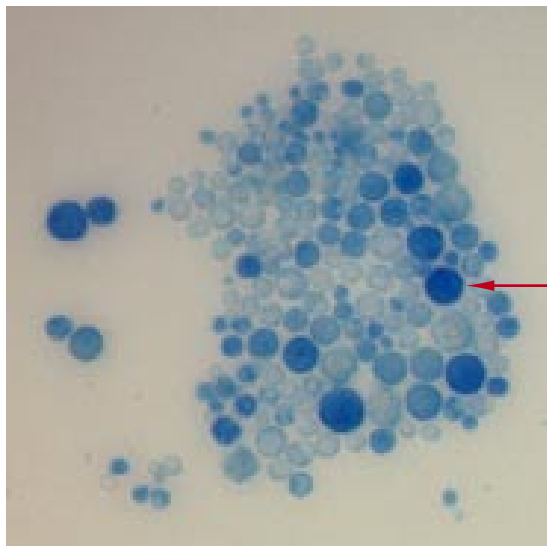
**Figure 7.**

Five-day-old, smooth colony of *Prototheca wickerhamii* on Sabouraud's dextrose agar.

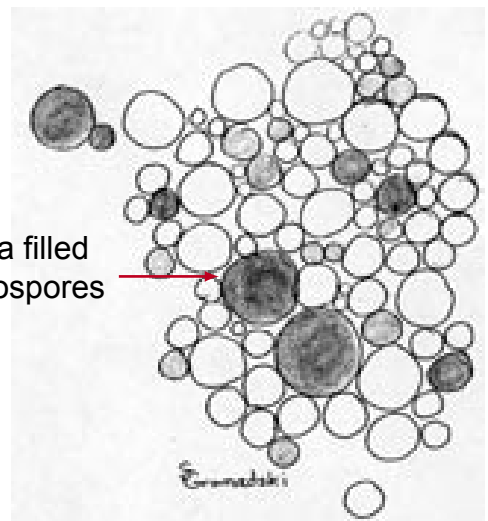


**Figure 8.**

Microscopic morphology of *Prototheca wickerhamii* on cornmeal agar. Sporangia some filled with endospores are seen (left; 200X magnification, right; line drawing not to scale).



Sporangia filled  
with Endospores



## Y-5 *Geotrichum candidum*

Source: Sputum

Correct answers:

Referee Labs: 10/10  
Labs with Correct ID: 149

Incorrect answer:

*Blastoschizomyces capitatus*: 1

### Clinical Significance:

*Geotrichum candidum* commonly cause pulmonary infections in immunocompromised patients. It also produces lesions in alimentary tract, vagina, and skin. *G. candidum* has also been reported to cause fungemia and disseminated infection.

### Ecology:

*Geotrichum candidum* is cosmopolitan in distribution. It has been isolated from air, water, plants, milk and milk products. It is found as a normal commensal in pulmonary and gastrointestinal tract of humans.

### Laboratory Diagnosis:

- a. Culture – At 25° C, on Sabouraud’s dextrose agar colonies are cream colored, flat with aerial mycelium, rapidly growing, covering the entire plate in 3 – 4 days (**Fig. 9**).
- b. Microscopic morphology – On corn meal agar with Tween-80, true hyphae with arthroconidia are seen (**Fig. 10**). Arthroconidia formation is by the fragmentation of hyphae, no disjunctor cells (empty cells between the arthroconidia) and no blastoconidia are formed.
- c. *Geotrichum candidum* grows on the media containing cycloheximide, negative on urease reaction, grows sparingly at 37° C. It is differentiated from *Trichosporon* species by absence of blastoconidia, no growth at higher temperatures (40, 42 & 45° C). *Blastoschizomyces capitatus* can be differentiated from *G. candidum* by the lack of growth on a medium containing

D-xylose as a carbon source and its growth at 45° C. *G. candidum* is differentiated from arthroconidia forming molds by its colony morphology. Microscopically *Arthrographis* and *Odioidendron* have conidiophores while *Malbranchea* and pathogenic fungi *Coccidioides immitis* have disjunctor cells.

- d. Molecular tests – Randomly amplified polymorphic DNA (RAPD) PCR has been used for the identification of *G. candidum* isolated from cheese (7). Using DNA/DNA reassociation techniques, de Hoog et al (1 & 2) found the relatedness between *G. candidum* and its teleomorph (sexual state) *Galactomyces geotrichum*.
- e. In vitro susceptibility testing – Limited studies suggested that most isolates are susceptible to amphotericin B and to azoles like fluconazole and itraconazole.

### Comments:

All the participating labs except one have identified this organism.

### Further Reading:

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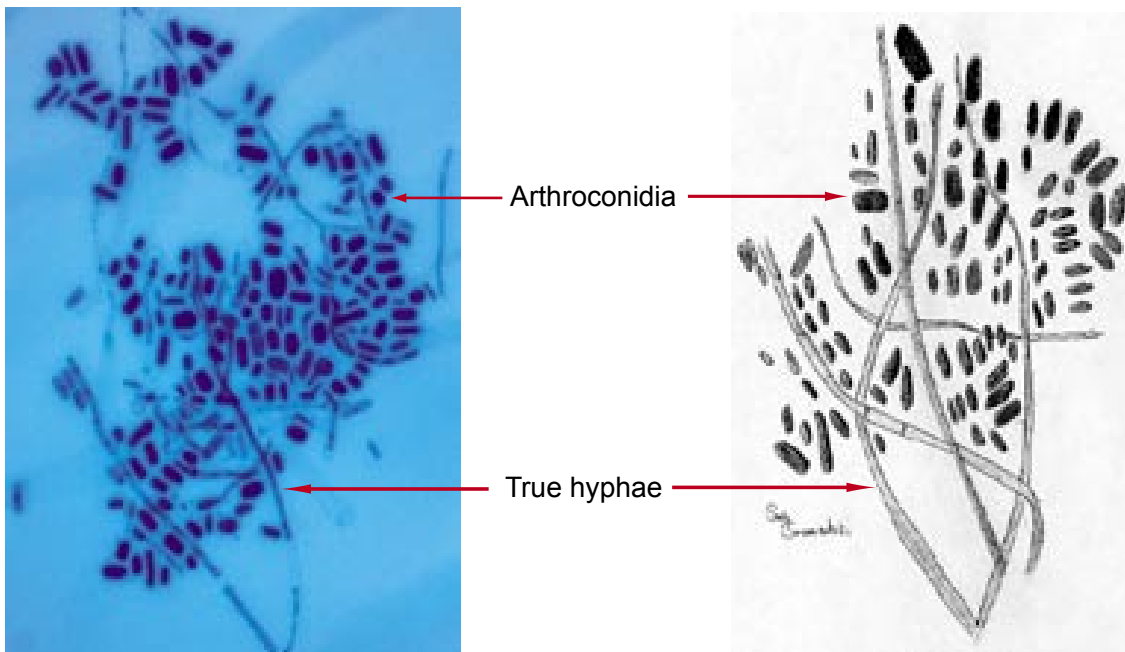
**Figure 9.**

Two-day-old, mold like colony of *Geotrichum candidum* on sabouraud's dextrose agar.



**Figure 10.**

Microscopic morphology of *Geotrichum candidum* on cornmeal agar showing true hyphae and arthroconidia (left; 200X magnification, right; line drawing not to scale).



## EDUCATIONAL SPECIMEN:

### *Candida glabrata*

Source: Urine

Correct answer:

*Candida glabrata:* 139

Incorrect answer:

*Candida norvegensis:* 1

*Candida pintolopseii:* 1

*Klocekera* species: 1

*Pichia polymorpha:* 1

*Saccharomyces cerevisiae:* 2

Unknown: 5

### Clinical Significance:

*Candida glabrata* causes fungemia especially in immunosuppressed patients, urinary tract infections and vaginitis. It is increasingly reported as nosocomial pathogen.

### Ecology:

*Candida glabrata* is cosmopolitan in distribution. It is found in humans, lower mammals and birds.

### Laboratory Diagnosis:

- a. Culture – At 25° C, on Sabouraud's dextrose agar, colonies are smooth, glistening, rapidly growing in 2 days (**Fig. 11**).
- b. Microscopic morphology – On corn meal agar with Tween-80, *Candida glabrata* forms small, oval to round blastoconidia and no pseudohyphae (**Fig. 12**).
- c. Differentiation from other yeasts – *Candida glabrata* does not grow in presence of cycloheximide, ferments glucose and trehalose. Microscopically, it forms only blastoconidia and no pseudohyphae. On the API 20C AUX, a specific assimilation biocode differentiates it from other yeasts.

d. Molecular tests – Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) has been used to identify *Candida glabrata* (3, 5, and 7). Molecular typing of *Candida glabrata* using electrophoretic karyotyping, random amplified polymorphic DNA (RAPD) and comparative sequence analysis of cytochrome oxidase gene have been reported (2, 11 and 12). Recently fingerprinting probes (Cg6 and Cg12) have been used for typing of *C. glabrata* (8).

e. In vitro susceptibility testing – *C. glabrata* is susceptible to amphotericin B and 5FC but resistant to azoles like fluconazole and itraconazole.

### Comments:

The isolate sent in the current test event is an atypical one. Originally, it had two-colony morphologies: small and large. Initially, it did not grow well at 25° C, but grew well at 37° C. Biochemically, it did not ferment glucose and trehalose. On the API 20C AUX, this organism did not grow in 48h and the specific biocode generated after 72h matched low discrimination biocode for *C. glabrata*. However, 463 bp ITS1, 383 bp ITS2, and a 800 bp actin gene fragment showed 100% identity with *C. glabrata* sequences. Subsequent subcultures in the laboratory seems to have selected against atypical cells in favor of more typical *C. glabrata* population.

### Further Reading:

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2. Gumbo, T., C. M. Isada, G. Hall, M. T. Karafa, and S. M. Gordon. 1999. *Candida glabrata* Fungemia. Clinical features of 139 patients. *Medicine (Baltimore).* 78(4): 220-7.
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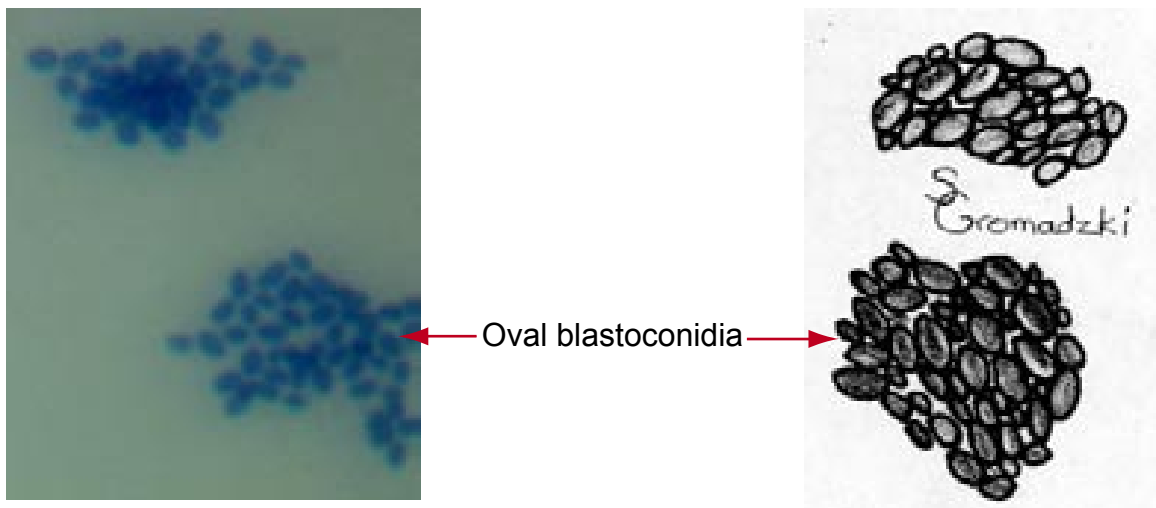
**Figure 11.**

Six-day-old, colonies (left) and two-day-old, colony (right) of atypical and typical *Candida glabrata* on Sabouraud's dextrose agar, respectively.



**Figure 12.**

Microscopic morphology of *Candida glabrata* on cornmeal agar showing oval blastoconidia (left; 400X magnification, right; line drawing not to scale)



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