

Sample A***Aspergillus terreus***

Score: 95%.

Pathogenicity:

This species causes allergic or invasive bronchopulmonary aspergillosis. About 10% of all cases of aspergillosis may be ascribed to this species. Nosocomial infections have been reported. It is regularly implicated in a wide variety of infections in humans, such as cutaneous, ophthalmic, pulmonary and disseminated infections. This species is noteworthy for its refractoriness to amphotericin B therapy.

Distribution:

Worldwide

Lab diagnosis:

1. Macroscopic morphology on Sabouraud agar at 30°C
Suede-like cinnamon-buff to sand brown colonies. Reverse yellow to deep dirty brown (Fig. 1). Isolates that are initially white and producing only accessory conidia may be mistaken for *Histoplasma capsulatum*.
2. Microscopic morphology
Hyphae are septate and hyaline. Conidial heads are biserial (containing metula that support phialides, metula as long as phialides). Conidiophores are smooth-walled and hyaline, terminating in mostly globose vesicles. Conidia are small, globose, and smooth (Fig. 2).



Fig. 1 Macroscopic morphology *A. terreus* on Sabouraud agar



Fig. 2 Microscopic morphology *A. terreus*

Difference between *A. terreus* and other species are shown below.

Strain	Macroscopic morphology	Microscopic morphology	Supplementary test(s)
<i>A. terreus</i>	Suede-like cinnamon-buff to sand brown; reverse yellow to deep dirty brown.	Biseriate, conidiophores smooth-walled, conidia small, globose and smooth.	
<i>A. flavus</i>	Olive to lime green, cream reverse, woolly to cottony to somewhat granular	Biseriate (some isolates remain uniseriate), conidiophores coarsely roughened, conidia smooth to very finely roughened, globose to subglobose	

Sample B

Candida albicans and *Candida glabrata*

Score: 67%

This time the sample is not scored. We have included 2 *Candida* species in this sample; on routine Sabouraud agar mixed cultures are hardly visible and only recognizable using chromogenic media. It is important to culture clinical samples on these media in case of expecting mixed fungal populations because of the evolving antifungal drug susceptibility.

Candida is the most common cause of opportunistic mycoses worldwide. It is also a frequent colonizer of human skin and mucous membranes. *Candida* is a member of normal flora of skin, mouth, vagina, and stool. As well as being a pathogen and a colonizer, it is found in the environment, particularly on leaves, flowers, water, and soil.

C. albicans is the most abundant and significant species. It has been implicated in both superficial and systemic disease.

C. glabrata is after *C. albicans* one of the most common yeast species. It is often isolated as an incidental finding from skin and urine. It has been implicated as an "opportunistic" cause of both superficial and systemic infections, especially in immunocompromised patients, and it has been isolated from patients with septicemia, pyelonephritis, pulmonary infections, endocarditis and hyperalimentation.

Pathogenicity:

C. albicans: Recent reports of infections include corneal, nail, ear, endocarditis, and bloodstream. Risk factors for infections with *C. albicans* include age of 65 years or above, immunosuppression prior to steroid use, leukocytosis, intensive care unit stays, or presence of intravascular or urinary catheters.

For those patients who have undergone cancer chemotherapy and who often appear less critically ill, infections are most likely to be caused by *Candida* species other than *C. albicans*. Although this species continues to be the most common species isolated in bloodstream infections, reports show that the incidence is decreasing and the resistance is rare in neonatal populations. *C. albicans* is also a predominate species in fungal biofilms on medical devices.

C. glabrata is known for its decreased susceptibility to azoles but good susceptibility patterns to candins. Despite the decreased susceptibility to azoles, fluconazole prophylaxis has not contributed to an increased incidence of bloodstream infection caused by *C. glabrata*. This species does, however, contribute to mortality in approximately 21% of pediatric patients with bloodstream infections.

Although this species may be recovered from virtually all infection sites, recent reports of infection include corneal, endocarditis, vaginitis, and oral.

Distribution:

Worldwide in man and other mammals, birds, fruit juice and insects.

Lab diagnosis:

1. Macroscopic morphology

On Sabouraud agar at 30°C:

C. albicans: cream colored, glistening or somewhat waxy, usually smooth; some strains may become wrinkled and have a mycelial border.

C. glabrata: white, glossy and smooth.

On CandiSelect 4 agar (CS4):

C. albicans: shiny, purple (Fig. 3)

C. glabrata: shiny, white with turquoise center (Fig. 3)

2. Microscopic morphology on rice cream agar 48-72 hours at 30°C

C. albicans: budding cells. Abundant branched pseudohyphae and true hyphae with blastoconidia are present. The blastoconidia are formed in grape-like clusters along the length of the hyphae. Terminal chlamydoconidia.

C. glabrata: Ovoid, budding yeast-like cells or blastoconidia. No pseudomycelium produced. Yeast cells of *C. glabrata* are quite small compared to *C. albicans*.

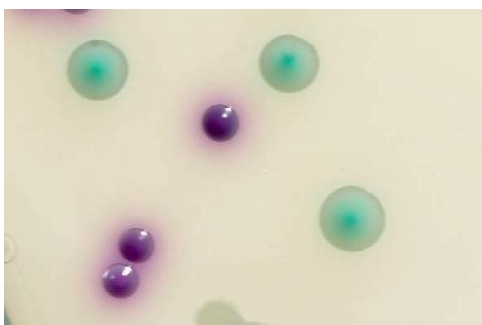


Fig. 3 Macroscopic morphology on CandiSelect 4 agar

Difference between *C. albicans* and *C. glabrata* and other species are shown below.

Strain	Macroscopic morphology	Microscopic morphology	Supplementary test(s)
<i>C. albicans</i>	Cream colored, glistening or somewhat waxy, usually smooth Purple on CS4.	Budding; abundant branched pseudohyphae and true hyphae with blastoconidia; blastoconidia formed in grape-like clusters along the length of the hyphae; terminal chlamydoconidia.	Germ tube test: + Growth 45 °C: +
<i>C. glabrata</i>	White, glossy and smooth shiny, white with turquoise center on CS4.	Ovoid, budding yeast-like cells or blastoconidia; no pseudomycelium.	Germ tube test: -
<i>C. dubliniensis</i>	Cream colored, glistening or somewhat waxy, usually smooth Purple on CS4.	Abundant branched pseudohyphae and true hyphae with blastoconidia and abundant chlamydoconidia in, pairs, chains, and clusters.	Germ tube test: + Growth 45 °C: -
<i>C. tropicalis</i>	Cream colored, off-white, smooth and creamy or wrinkled near the margin Dark turquoise on CS4.	Oval blastospores located along the long pseudohyphae; blastoconidia may appear singly or in clusters; pseudohyphae branch abundantly.	Germ tube test: -

Sample C

Exophiala dermatitidis

Synonym: *Wangiella dermatitidis*

Score: 67%

Pathogenicity:

E. dermatitidis is an occasional causative agent of phaeohyphomycosis. Subcutaneous phaeohyphomycosis is the most common clinical picture. The infection develops after traumatic implantation of the fungus through the skin. *E. dermatitidis* is a neurotropic fungus. Central nervous system infections have been reported. It may also cause keratitis, otitis, pneumonia, and endocarditis. Disseminated infections may develop particularly in immunocompromised patients.

E. dermatitidis can be cultured from cystic fibrosis patients. Isolation rates from sputum samples range between 1.8 and 15.7%.

Distribution:

Worldwide

Lab diagnosis:

1. Macroscopic morphology on Sabouraud agar at 30°C
Moist, yeast-like, and shiny. Aerial hyphae develop after 3 to 4 weeks of incubation. Color is black from the front and the reverse (Fig. 4)
2. Microscopic morphology
Abundant yeast cells. Flask-shaped to cylindrical phialides without distinctive collarettes. Conidia are hyaline to pale brown, one-celled, round to obovoid, smooth-walled and accumulate in slimy balls (glioconidia) at the apices of the phialides or down their sides (Fig. 5). Cultures grow at 42°C



Fig. 4 Macroscopic morphology
E. dermatitidis on Sabouraud agar

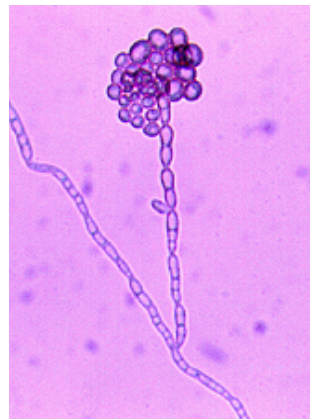


Fig. 5 Microscopic morphology
E. dermatitidis

Difference between *E. dermatitidis* and other species are shown below.

Strain	Macroscopic morphology	Microscopic morphology	Supplementary test(s)
<i>E. dermatitidis</i>	Black at front and reverse, moist, yeast-like, and shiny. Aerial hyphae after 3 - 4 weeks.	Abundant yeast cells. Flask-shaped to cylindrical phialides without collarettes. Conidia hyaline to pale brown, one-celled, round to obovoid, smooth-walled and in slimy balls at apices of phialides or down their sides.	Growth 40°C and 42°C: + Raffinose: +
<i>E. jeanselmei</i>	Smooth, greenish-grey to black, mucoid and yeast-like, becoming raised and developing tufts of aerial mycelium with age, often becoming dome-shaped and suede-like. Reverse: olivaceous-black.	Subspherical cells with annellidic butt producing conidia in long chains. Conidiogenous cells rocket shaped, brown.	Growth 40°C: - Raffinose: -
<i>E. bergeri</i>	Black, slimy at centre with dry marginal zone. No brown diffusible pigment.	Abundant yeast cells. Hyphae dark olivaceous, conidiogenous cells arising as part of long, branched chains of subhyaline, ellipsoidal cells.	Growth 40°C: - Raffinose: +
<i>Scedosporium apiospermum</i>	Greyish-white, suede-like to downy with a greyish-black reverse.	Numerous single-celled, pale-brown, broadly clavate to ovoid conidia, rounded above with truncate bases. Conidia borne singly or in small groups on elongate, simple or branched conidiophores or laterally on hyphae.	
<i>Aureobasidium pullulans</i>	Smooth, covered with slimy masses of conidia, cream or pink to brown or black	Hyphae hyaline, septate, frequently dark-brown with age and undergoing holothallic transformation to form chains of 1- to 2-celled, thick-walled, darkly pigmented arthroconidia.	

Sample D

Rhodotorula mucilaginosa

Score: 95%

Rhodotorula is a yeast found in air, soil, lakes, ocean water, and dairy products. It may colonize plants, humans, and other mammals. While being considered as a common contaminant, *Rhodotorula* may infect individuals with predisposing risk factors.

As summarized by Kurtzman & Fell, The Yeasts, 2000, page 822, *R. mucilaginosa* is now given priority over *R. rubra*. Basically, the type strain of *R. rubra* was found to actually be a strain of *R. glutinis*. Due to the naming rules, the epithet *R. rubra* was thus no longer available. The type strain of *R. mucilaginosa* was found to be identical to the type strain for this species and thus now provides the name for the species.

Pathogenicity:

Rhodotorula sp. are rarely isolated as causative agents of opportunistic mycoses in vulnerable hosts, such as patients with AIDS or acute leukemia. It has also been reported as causing fungemia, endocarditis and meningitis in patients undergoing chemotherapy for cancer.

Catheter-related fungemia appears to be one of the more common *Rhodotorula*-related diseases. Rarely, a septic picture may develop. As with other possible environmental contaminants, multiple positive cultures and relevant clinical signs or symptoms suggest real fungemia. Cases of endocarditis, meningitis, ocular infections and peritonitis have been reported. In a recent review of central venous catheter-associated *Rhodotorula* fungemia (Tuon et al 2007), 87 of 88 patients had a known immunocompromised state. Specifically, 78% of patients had underlying malignancy. The most frequently isolated species was *R. mucilaginosa* (75%), and the reported overall mortality was 9.1%. Therefore, while typically less virulent than more common pathogenic yeasts such as *Cryptococcus* and *Candida* species, *Rhodotorula* infections may have serious implications.

Distribution:

Worldwide

Lab diagnosis:

1. Macroscopic morphology on Sabouraud agar at 30°C
Colonies are coral red to pink, glistening, smooth or rough, mucous (Fig. 6).
2. Microscopic morphology on rice cream agar 48-72 hours at 30°C
Spherical to elongate budding yeast-like cells or blastoconidia.



Fig. 6 Macroscopic morphology *R. mucilaginosa* on Sabouraud agar

Identification is based on the pigment, urease activity, nonfermenter, lack of utilization of inositol and nitrate utilization.

Difference between *Rhodotorula mucilaginosa* and other species are shown in subjoined table. The species are difficult to distinguish with biotyping tests, therefore every *Rhodotorula* is scored.

Strain	Macroscopic morphology	Microscopic morphology	Supplementary test(s)
<i>R. mucilaginosa</i>	Coral red to pink, glistening, smooth or rough, mucous	Spherical to elongate budding yeast-like cells or blastoconidia; pseudomycelium may be present.	Growth 37°C: + Lactose: - Maltose: +/- Rhamnose: +/- Nitrate: +/- Nitrite: +/- Urease: +
<i>R. glutinis</i>	Coral red to salmon or slightly orange, smooth or wrinkled often with fine transverse striations, glossy, later dull.	Budding cells (ellipsoidal), often with rudimentary pseudomycelium.	Growth 37°C: v Lactose: - Maltose: +/- Rhamnose: +/- Nitrate: +/- Nitrite: + Urease: +
<i>R. minuta</i>	Pink, smooth, glistening	Budding cells spherical to broadly ellipsoidal; pseudomycelium absent.	Growth 37°C: v Lactose: +/- Maltose: - Rhamnose: - Nitrate: - Nitrite: - Urease: +/-

Literature

1. de Hoog GS, Guarro J, Gené J, Figueras MJ. Atlas of clinical fungi. 2nd ed. Nederland: Centraal bureau voor schimmelcultures, 2000
2. Andreoni S., Farina C., Lombardi G. Medical mycology atlas. GRAFIK@rt srl – Paderno Dugnano, 2004
3. Larone DH. Medically important fungi. ASM Press, 2002
4. Kane J., Summerbell R., Sigler L., Krajden S., Land G. Laboratory handbook of dermatophytes. Star publishing company, 1997
5. Merzr Wg, Hay RJ. Topley & Wilson's Microbiology & Microbial Infections - Medical Mycology 10th edition, 2005
6. <http://www.doctorfungus.org>
7. <http://www.mycology.adelaide.edu.au/>

The macroscopic descriptions are according to the literature above. This may differ from your results that can depend on the composition of the Sabouraud agar plates.

Brun S, Bouchara JP, Bocquel A, Basile AM, Contet-Audonneau N, Chabasse D. Evaluation of five commercial Sabouraud gentamicin-chloramphenicol agar media. *Eur. J. Clin. Microbiol. Dis.* 2001; 20:718-723