Update of Medically Important Yeasts and a Practical Approach to Their Identification

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Abstract
While *Candida albicans* remains the major yeast pathogen, several other *Candida* species and other genera of yeasts are now considered medically important. Significant fungal infections caused by yeasts are seen in septicemia, the urinary tract, organ transplantation, meningitis, pneumonia, endocarditis, and hematologic malignancies. Because more patients are at risk for fungal infections, and because the rate of yeast isolation is increasing, hospital and reference laboratories face an important challenge: to select an approach for isolation and identification of yeasts that is not only accurate and cost effective but also provides results in a reasonable time period.

*Candida albicans* was first demonstrated as the etiologic agent of Thrush in 1839. By 1963, there were approximately 6 yeasts that were considered medically important: *C. albicans*, *C. stellatoidea*, *C. parapsilosis*, *C. tropicalis*, *C. guillermondii*, and *Cryptococcus neoformans*. Since that time, more *Candida* species and other yeast genera have been associated with significant morbidity and mortality. In 1995, Hazen reported at least 17 species of *Candida* that were known to cause disease in humans. Other yeasts, such as *Trichosporon*, *Saccharomyces*, *Blastochizomyces*, and *Rhodotorula*, have also emerged as pathogens.1 Pinner and colleagues reported in 1996 that fungal infections were the 7th leading cause of infection-related mortality—with 70% caused by *Candida*, *Cryptococcus*, and *Aspergillus*.2

Identification of yeasts to the species level is important information for the physician, in part because of the changing susceptibility patterns that are now seen. For example, amphotericin B resistance is documented for *C. lusitaniae* (5 cases), *C. tropicalis* (4 cases), *C. guillermondii* (2 cases), and *C. glabrata* (5 cases). Resistance to fluconazole is seen with *C. krusei*, *C. guillermondii*, *C. dubliniensis*, *C. inconspicua*, *C. norvegensis*, and *Rhodotorula rubra*. *Candida krusei* is intrinsically resistant to fluconazole. Variable susceptibility with fluconazole is noted for *C. glabrata*, *C. parapsilosis*, *C. rugosa*, *C. tropicalis*, *Saccharomyces cerevisiae*, and *Trichosporon cutaneum*.1,3-5 One study cited prominent decreased susceptibility to fluconazole, itraconazole, and amphotericin B in *C. glabrata* and *C. krusei* isolates.6 Fluconazole-resistant strains of any *Candida* species are occasionally observed, and while some *C. glabrata* may be dose-dependent susceptible, up to 15% may exhibit true resistance.3

Because yeasts that were once considered only saprophytic are now known to cause invasive disease, and because these yeasts can vary in their susceptibility to antifungal agents, the microbiology laboratory should have the ability to provide identifications in a timely manner.

Update on Yeasts of Medical Importance

Many yeasts have the ability to cause disease, in the right environment. Some of the risk factors for yeast infections are: I.V. catheters, burns, surgery, diabetes mellitus, use of broad spectrum antibiotics and steroids, neutropenia, HIV+, and cellular immune deficiencies.

*Candida* species overall remain the genus most frequently isolated. *Candida* is the 6th most common nosocomial pathogen in the United States.7,8 Pfaller and colleagues studied nosocomial blood stream infections occurring in 55 medical centers in the United States between 1995 and 1998. *Candida* was the fourth most frequent cause of bloodstream infections, following coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, and *Enterococcus* species. This data was identical to that generated by the Centers for Disease Control and Prevention (CDC) at the end of the 1980s and middle of the 1990s and showed the crude mortality rate of *Candida* as 40% (crude mortality reflects the sickness of the population).9,10

In solid-organ transplant recipients, *Candida* species are major pathogens associated with kidney, pancreas, liver, and small bowel transplantation.11

*Candida albicans* remains the most common yeast isolate in the clinical laboratory. It is important to remember that *C. albicans* is normal flora in the mucosal areas of the body—oral cavity, genitourinary tract, intestinal tract—where it is found in low numbers compared to bacteria. *Candida albicans* causes localized infections such as thrush, vaginitis, and skin and nail infections. It is also a major pathogen in immunocompromised persons, spreading systemically. In 2001, researchers reported that in the United States, *C. albicans* was the predominant yeast isolate in blood stream infections, accounting for 55% of yeast isolates. It was also number 1 in Canada, Latin America, and Europe. *Candida albicans* was followed by *C. glabrata* (21%), *C. parapsilosis* (11%), *C. tropicalis* (9%), *C. krusei* (2%), and other *Candida* sp. (2%).12
**Candida glabrata**, formerly called *Torulopsis glabrata*, has increased in incidence as a cause of blood stream infections in the United States. It is now the second most common yeast in the bloodstream. It is estimated to account for up to 21% of urinary yeast isolates and has also been associated with endocarditis, meningitis, and disseminated disease. The concern for *C. glabrata* is that it is less susceptible to amphotericin B and fluconazole, and can develop resistance to other azoles.

*Candida dubliniensis*, a newly described yeast species in 1995, refers to Dublin, Ireland, where it was first recognized. It is now seen in many countries and causes oral thrush in HIV infected persons. *Candida dubliniensis* may also cause oral and vaginal candidiasis and blood stream infections in HIV negative persons. *Candida dubliniensis* phenotypically resembles *C. albicans* and is misidentified as such. It produces germ tubes and chlamydospores, just like *C. albicans*. However, the chlamydospores appear in groups rather than single as in *C. albicans*. Because of the concern over its resistance to fluconazole as well as the need to further determine its prevalence and role in disease, it is important for laboratories to recognize this yeast. One approach to separating *C. dubliniensis* from *C. albicans* is growth at 45°C (*C. albicans* is positive), utilization of xylose (*C. albicans* is positive), and colony color on initial isolation on CHROMagar *Candida* (*C. albicans* is light green and *C. dubliniensis* is dark green).

Other *Candida* species that have been associated with disease:

- *C. guilliermondii*
- *C. kefyr*
- *C. krusei*—emerges in settings where fluconazole is used for prophylaxis.
- *C. lipolectica*
- *C. lusitaniae*—resistant to amphotericin B; usually causes fungemia in patients with malignancies or other serious conditions.
- *C. parapsilosis*
- *C. rugosa*
- *C. tropicalis*
- *C. chiropterum*
- *C. ciferrii*
- *C. famata*
- *C. haemulonii*
- *C. humicola*
- *C. norvegensis*
- *C. pintolopesii*
- *C. pulcherrima*
- *C. utilis*
- *C. viswanathii*
- *C. zeylanoides*

**Cryptococcus neoformans** has long been considered a pathogen in immunocompromised patients, causing meningitis. In patients without HIV infection, it is seen in association with lupus erythematosus, leukemia, lymphomas, and other immunologic disorders and malignancies. *Cryptococcus* meningitis is one of the AIDS-defining diseases. Almost all AIDS patients with cryptococcosis are infected with the variety *neoformans*. *Cryptococcus neoformans* variety *gattii* (serotype B) is recovered in the environment where river red gum trees flower (*Eucaliptus camaldulensis*) and is sometimes found in AIDS patients, especially in California. Unlike *Candida* species, *Cryptococcus neoformans* is not part of the human mucosal flora but is associated with fowl excreta, particularly pigeon droppings. It has also been isolated from the droppings of canaries, parrots, swallows, and other birds. The infection with *C. neoformans* begins with inhalation of the yeast into the lungs and is then spread via the blood stream to the central nervous system. *Malassezia furfur*, also known as taenia versicolor, is a lipid-requiring yeast that inhabits the skin. It was once considered to cause only mild chronic infection of the stratum corneum layer of the skin, a condition called pityriasis versicolor. However, it has emerged as an etiologic agent of intravascular catheter related sepsis. These systemic infections with *M. furfur* have been associated with I.V. administration of fat emulsions for caloric supplementation. Of particular interest is that it colonizes the skin of hospitalized infants in neonatal intensive care units. *Malassezia furfur* is unique because of its absolute growth requirement for long chain fatty acids. To recover *M. furfur*, media with lipid sources or Tween compounds are required. *Malassezia pachydermatis* is a much less common cause of outbreaks in neonatal ICUs and, unlike *M. furfur*, is not dependent on lipids for growth.

**Saccharomyces cerevisiae** is brewer’s or baker’s yeast and is a great example of an emerging yeast pathogen. Aucott and colleagues reported 3 cases of invasive infection caused by *S. cerevisiae*—pneumonia in a leukemic patient, liver abscesses in a patient with liver cancer, and endocarditis in a patient with prosthetic cardiac valves. Recently, it was reported that treatment with the probiotic ultrallevra may lead to fungemia in immunocompromised or critically ill patients. Ultrallevra contains *Saccharomyces boulardii*, which is considered to be a synonym for a particular strain of *S. cerevisiae*. The mortality rate among 60 reported cases of *S. cerevisiae* fungemia was 28%. *Trichosporon* is considered normal flora on the skin, especially in the perigenital region, and it causes a harmless infection of the hair shaft called white piedra. The genus is now well recognized as an agent of invasive mycosis, with disseminated infections seen in neutropenic patients and patients with hematologic malignancies, burns, and organ transplants. The genus was revised in 1992 and *T. beigelii* is now called *T. cutaneum*. Gueho and colleagues proposed that *T. beigelii* no longer be used and that human disease is caused by 6 species of *Trichosporon*: *T. asahii*, *T. asteroides*, *T. cutaneum*, *T. inkin*, *T. mucoides*, and *T. ovoides*. *Trichosporon cutaneum* and *T. asteroides* cause superficial skin infections. *Trichosporon ovoides* causes white piedra of the scalp, and *T. inkin* causes white piedra of pubic hair. The species responsible for most deep and systemic infections is *T. asahii* followed by *T. mucoides*. The mortality rate from these serious infections is incredibly high at >80%, 1,3,20

**Blastoschizomyces capitatus** (previously called *Trichosporon capitatum* and *Geotrichum capitatum*) is ubiquitous in nature and only considered a minor component of normal skin flora. It disseminates in immunocompromised patients and is known to cause endocarditis. There have been cases of fluconazole-resistant strains of this yeast responsible for nosocomial infections in cancer patients.

**Laboratory Procedures for Identification of Yeasts**

**Direct Smears of Clinical Specimens**

Direct microscopy is important because it can provide tentative diagnosis prior to growth in culture, and it may give enough information for the clinician to begin correct immediate patient management. There are several "fungal" stains that...
have been used. Some of these are not used regularly in the clinical microbiology laboratory but may be available in the histology laboratory. These include stains such as Gomori Methenamine Silver (GMS) and Periodic Acid Schiff (PAS). There are other stains already used routinely in microbiology or are rapidly and easily incorporated into a microbiology laboratory.

**Gram stain:** Yeast cells stain purple and should be easy to differentiate from bacteria. It should be noted that yeasts often do not stain evenly with the Gram stain. Microscopically, most yeasts appear round or oval, often with “buds.” They can easily be seen on 40× magnification. Under oil immersion yeasts appear as giant gram-positive cocci (as described and identified incorrectly by many microbiology students). The size of the organism and “budding” are not consistent with most bacterial cells. There are some yeasts that also form rectangular shaped arthroconidia from true hyphae.

**Potassium hydroxide (KOH), a wet mount:** A 10% or 15% solution can be used, which acts as a clearing agent of the tissues and cellular debris but does not damage the fungal cells. Specifically, the KOH digests proteinous debris, bleaches pigment, and dissolves the “cement” that holds keratinized cells together. The difficulties in using KOH for direct smears are: (1) the fungal cells are not stained, making them difficult to see; (2) it is a wet mount and is viewed at 10× and 40× instead of 100×; (3) if it is allowed to dry, crystals form and make the reading difficult; and (4) KOH can damage the stage and objectives of the microscope.

**Calcofluor white + KOH wet mount:** Calcofluor white is a fluorescent dye that binds to the cellulose and chitin in the cell walls of yeasts (and molds). When viewed under a fluorescent microscope, it is easy to see the intense yellow-green fluorescent yeast and mold elements. This is still a wet-mount preparation, and the KOH is necessary for its clearing ability. The calcofluor white greatly enhances the ability to detect fungi.

**Potassium hydroxide (KOH) + Lactophenol Cotton Blue (LPCB) wet mount:** This is used for the same purpose as the other KOH preparations. The LPCB stains the fungi, the lactic acid is a clearing agent, and phenol kills the fungi.

**India ink:** This is another rapid wet-mount procedure usually performed on cerebrospinal fluid (CSF). Yeasts that produce capsules will appear as cells surrounded by clear halos. The capsules repel the carbon particles of the India ink procedure and is widely used.26 The India ink procedure is closely related to Cryptococcus and can give a positive serum cryptococcal latex antigen test in patients with disseminated Trichosporon infection.

**Primary Culture of Yeasts**

Having read many textbook chapters and review papers on this subject and attended several mycology workshops, the author has come to the conclusion that choice of culture media varies greatly among laboratory professionals. Therefore, this section will identify media for the recovery of yeasts and give comments or “pearls” about each.

**Sheep blood agar (SBA)—** *Candida* species grow well on SBA in 24 to 48 hours, and *C. neoformans*, *Trichosporon* species, *M. furfur*, and other yeasts may require longer incubation times or supplements. Many isolates of *C. albicans* produce “colonies with feet or extensions.” One must remember that a few other yeasts can do this also—25% of *C. tropicalis* and *C. krusei*.31

**Sabouraud dextrose agar (SDA)—** pH 5.0 and 4% glucose. This has been used for isolation of fungi for many years, and the growth of many species has been described on it. However, some no longer recommend its use unless for the isolation of dermatophytes.

**Emmons sabouraud dextrose agar—** pH 6.8 to 7.0 and 2% glucose. This formula is recommended over the regular Sabouraud agar because of better recovery at the neutral pH.

**Sabouraud dextrose agar with chloramphenicol and cycloheximide—** Chloramphenicol inhibits bacterial growth, and cycloheximide inhibits the growth of many fungi that can be both saprophytic and pathogenic. These include *C. neoformans* and some *Candida* species, *Aspergillus* species, *Fusarium* species, and *Pseudallescheria boydii*. Therefore, when this medium is used, the laboratory should also include media without cycloheximide.

**Inhibitory mold agar (IMA)—** IMA is considered an enriched media that also has chloramphenicol. It supports the growth of most molds and yeasts while inhibiting bacterial growth. It is a popular medium.

**CHROMagar Candida—** This culture medium selects for yeasts and differentiates colonies of *C. albicans*, *C. tropicalis*, and *C. krusei*, simply by the different colors of colonies that grow on the media. CHROMagar contains a chromogenic mix substrate along with chloramphenicol. Colony growth and color development should be read after 48 hours, but some yeast colonies give characteristic color development at 24 hours. This medium is particularly helpful when performing surveillance cultures and probable mixed cultures from cancer or other immunocompromised patients. The guidelines for color differentiation and identification are as follows:

- *C. albicans* appear as green, smooth colonies.
- *C. tropicalis* appear as blue, smooth colonies with pink halos.
- *C. krusei* appear as rough, spreading pale pink colonies with white borders.

A study by Pfäffer and colleagues showed the effectiveness of CHROMagar Candida (Hardy Diagnostics, Santa Maria, CA) in making rapid presumptive identification of these 3 yeasts. More than 95% of stock and clinical isolates of *C. albicans*, *C. tropicalis*, and *C. krusei* were correctly identified. In addition, the study reported 94% of *C. glabrata* isolates were correctly identified on CHROMagar Candida. *Candida glabrata* colonies appear as dark pink colonies with pale edges.32 However, the manufacturer’s directions for CHROMagar Candida media do not include *C. glabrata* as 1 of the yeasts that is identified because it does not provide a “clear-cut” color differentiation for *C. glabrata*.

**Algorithms and Testing Methodologies for the Identification of Yeasts**

Algorithm 1 using CHROMagar (Figure 1):

The most rapid algorithm for identifying 3 of the most common yeasts—*C. albicans*, *C. tropicalis*, and *C. krusei—is
when CHROMagar is used. After 48 hours incubation, the yeast colonies can be identified based on color production, as previously outlined. Any variations from this should not be used for identification. As discussed, some studies report identification of *C. glabrata* from CHROMagar; however, it can be difficult to differentiate the pink colonies of *C. glabrata* from the pink colonies produced by several other yeasts. A rapid trehalose test can be done on any yeast from CHROMagar that shows small yeast cells on wet mount. Within 2 hours, *C. glabrata* can be identified using this procedure. The procedure is discussed in algorithm 2. If the yeasts growing on CHROMagar are not *C. albicans*, *C. tropicalis*, *C. krusei*, or *C. glabrata*, then regular identification methods (kits or instruments) must be used, and the technologist should also streak a cornmeal or rice agar for morphologies. This is also discussed in algorithm 2.

**Algorithm 2 using the more traditional approach (Figure 2):**

**Wet mount or Gram stain**—Technologists should not discount the importance of performing initial microscopy on the colony. How many yeast colonies are considered gram-positive cocci, because a stain or wet mount was not done? After further testing, the isolate would most likely be identified as coagulase negative *Staphylococcus*, which, of course, changes the approach for therapy.

**Germ tube**—*Candida albicans* is germ tube positive in 2 to 3 hours. A germ tube is different from a “bud.” There is no constriction at the site of attachment; it is literally a “blowing out” of the cell wall or a germination that occurs under specific conditions (ie, 35°C in animal serum or albumin for 2 to 3 hours). It is important to remember the circumstances that enhance or interfere with germ tube production: (1) optimal media for selecting a yeast colony are those without antibiotics; (2) a neutral pH (7.4 being optimum) is preferred because acidic or basic conditions decrease germination; (3) bacterial contamination interferes with germ tube production; (4) a heavy inoculum decreases the percentage of cells producing germ tubes; use a “light” inoculum or < 10⁶ cells/mL; and (5) younger cultures favor germ tube production.⁴⁻³³

**Rapid trehalose**—If the isolate is germ tube negative and the yeast cells appear small, a rapid trehalose can be done. The commercial Remel test requires a 2-hour incubation at 42°C. Rapid trehalose broth can be made in-house and then frozen at −70°C until used. In the author’s experience, a heavy inoculum is required, almost “pasty,” and control organisms must be included with each “run.” The Mayo Clinic’s original method requires only a 1-hour incubation at 37°C.³⁴

**Morphology agars (cornmeal/rice agars):** Because the interpretation of morphology agars requires careful evaluation and expertise, using a kit or instrument that does not require morphology agars is certainly appealing. The purpose of morphology agars is to support or aid in the identification provided by the kit or instrument. Sometimes morphology agars provide the most rapid way to differentiate 1 yeast species from another. Morphology agars are inoculated according to the recommended technique and incubated at room temperature (30°C) for 48 to 72 hours. Several different structures can be produced, depending on the genus and species of yeast.¹⁹⁻²⁸

1. **Pseudohyphae**—a chain of elongated “buds” or cells that remain attached to the “mother” cell. They constrict at the points of attachment to each other. Several genera of yeasts produce pseudohyphae, the most common being *Candida*.
2. **True hyphae**—the filamentous structure of a fungus, mostly associated with molds; however, some yeasts produce true hyphae and pseudohyphae, such as *Candida* and *Trichosporon* species. *Geotrichum* produces true hyphae only.
3. **Chlamydospore**—a swollen, thick-walled cell that neither germinates nor produces conidia when mature.

4. **Blastoconidia**—conidia or cells that are formed by “budding” along the true hyphae, pseudohyphae, or single yeast cell.

5. **Arthroconidia**—rectangular or barrel-shaped conidia formed by true hyphae breaking apart at points of septation.

Table 1 categorizes yeasts according to the structures they produce on morphology agar. For example, only *C. albicans* and *C. dubliniensis* produce chlamydospores. *Geotrichum*, *Trichosporon*, and *Blastoschizomyces* produce true hyphae and arthroconidia. In addition, *Trichosporon* also produces blastoconidia, while *Blastoschizomyces* produces anelloconidia. If a yeast produces blastoconidia only, it could be a *Cryptococcus*, *Candida glabrata*, *Rhodotorula*, or *Saccharomyces*. Typically, a yeast that produces pseudohyphae and blastoconidia is a *Candida* sp. Some *Candida* can also produce true hyphae along with pseudohyphae (*C. albicans*, *C. tropicalis*, *C. lusitaniae*, *C. lipolytica*, and *C. krusei*).

The structures produced on morphology agars allow us to narrow the identification to a genus or a short list of genera and sometimes a species.

The interpretation of morphology agars can be tedious, especially for a person with little experience. Is it worth the effort and the time? In 1994, Fenn and colleagues published a paper showing the value of morphology agars in contributing to the correct identification of yeasts. A total of 409 germ-tube-negative yeasts and *Geotrichum* were tested using 2 identification methodologies—API 20C (bioMérieux Vitek, Hazelwood, MO) and the Vitek System (bioMérieux Vitek). Each of the 409 isolates were also inoculated to a biplate of cornmeal and rice agars and the Vitek System (bioMérieux Vitek). Each of the 409 isolates were also inoculated to a biplate of cornmeal and rice agars.


