The Journal of Microbiology, February 2005, p.65-84 Vol. 43, special issue (No. S)

Laboratory Diagnosis of Invasive Candidiasis

Arjuna N.B. Ellepola[1,2] and Christine J. Morrison[1,*]

[1]Mycotic Diseases Branch, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA
[2]Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya, Peradeniya, Sri Lanka

(Accepted July 7, 2004)

Invasive candidiasis is associated with high morbidity and mortality. Clinical diagnosis is complicated by a lack of specific clinical signs and symptoms of disease. Laboratory diagnosis is also complex because circulating antibodies to *Candida* species may occur in normal individuals as the result of commensal colonization of mucosal surfaces thereby reducing the usefulness of antibody detection for the diagnosis of this disease. In addition, *Candida* species antigens are often rapidly cleared from the circulation so that antigen detection tests often lack the desired level of sensitivity. Microbiological confirmation is difficult because blood cultures can be negative in up to 50% of autopsy-proven cases of deep-seated candidiasis or may only become positive late in the infection. Positive cultures from urine or mucosal surfaces do not necessarily indicate invasive disease although can occur during systemic infection. Furthermore, differences in the virulence and in the susceptibility of the various *Candida* species to antifungal drugs make identification to the species level important for clinical management. Newer molecular biological tests have generated interest but are not yet standardized or readily available in most clinical laboratory settings nor have they been validated in large clinical trials. Laboratory surveillance of at-risk patients could result in earlier initiation of antifungal therapy if sensitive and specific diagnostic tests, which are also cost effective, become available. This review will compare diagnostic tests currently in use as well as those under development by describing their assets and limitations for the diagnosis of invasive candidiasis.

***Key words*:** *candida*, diagnosis, candidiasis, laboratory tests

The availability of modern and sophisticated medical care to prolong and improve the lives of the severely debilitated is becoming increasingly more common in today's health care system. Ironically, such medical advances have resulted in a group of patients more vulnerable to fungal infections. Such patients include those who receive therapy that produces prolonged neutropenia, such as some stem cell transplant recipients, or those undergoing solid organ transplantation, particularly liver transplant recipients. In these patient populations, *Candida* spp. are a prominent cause of invasive disease (Rees *et al*., 1998; Pappas *et al*., 2004). In addition, *Candida* spp. are the fourth most common agent of all hospital-acquired bloodstream infections (BSIs) in the United States; such infections have an attributable mortality of up to 49% (Edmond *et al*., 1999; Centers for Disease Control and Prevention, 2000; Gudlaugsson *et al*., 2003). Although sentinel surveillance studies in the 1980s indicated that the overall incidence of *Candida* BSIs increased among patients in hospital intensive care units (ICUs) (Banerjee *et al*., 1991), the incidence of infections caused by *Candida albicans*, historically the most common cause of *Candida* BSIs, significantly decreased in this population in the 1990s (Trick *et al*., 2002). During the same period, a significant increase in *Candida glabrata* BSIs occurred (Trick *et al*., 2002). Changes in *Candida* spp. distributions in the bloodstream of patients outside of the ICU were also reported (Price *et al*., 1994; Nguyen *et al*., 1996; Abi-Said *et al*., 1997; Hajjeh *et al*., 2004). Identification of the infecting organism to the spp level has therefore become increasingly important for several reasons. First, not only has the *Candida* spp. distribution changed in recent years, but *Candida* spp. differ in their susceptibility to antifungal agents. For instance, *Candida krusei* is often innately azole resistant, *C. glabrata* has been reported to acquire resistance *in vitro* and *in vivo*, and *Candida dubliniensis* isolates have been observed to rapidly develop resistance to fluconazole (Wingard, 1995; Moran *et al*., 1997, 1998; Pfaller *et al*., 1999; Trick *et al*., 2002). *Candida lusitaniae* can be resistant to amphotericin B, and *Candida parapsilosis* and *Candida guilliermondii* have higher MICs to the echinocandins than other *Candida* spp.; *C. glabrata* and *C. krusei* require maximum doses of amphotericin B to be effective, are resistant to itraconazole, and have high MICs to voriconazole. Therefore, knowledge of the infecting species is highly predictive of likely drug susceptibility and can be used as a guide to therapy (Pappas

* To whom correspondence should be addressed.
(Tel) 1-404-639-3098; (Fax) 1-404-639-3546
(E-mail) cjm3@cdc.gov

et al., 2004). Second, species-specific identification is relevant for epidemiological purposes; for example, repeated identification of a particular species in a given hospital ward or locale may indicate a point source outbreak, particularly if a species occurs at a higher rate than that found historically, or if the species is unusual (i.e., *C. lusitaniae*, *Candida lipolytica*) (Denning *et al*., 2003). Third, the risk of developing deep organ involvement, and the severity of clinical manifestations, differs depending on the infecting species. For example, *Candida tropicalis* is likely to become invasive in neutropenic patients but not in liver transplant patients (Rabkin *et. al*, 2000; Denning *et al*., 2003). Identification of relevant *Candida* spp., however, has traditionally required isolation of the infecting organism from a normally sterile site, such as blood, peritoneal fluid, or intravenous lines. However, in cases of hepatosplenic candidiasis, blood cultures are frequently negative; indeed, blood cultures were reported to be negative in up to 50% of all autopsy-proven cases of invasive candidiasis in such patients (Berenguer *et al*., 1993). Although newer blood culturing systems have been reported to increase the recovery of *Candida* spp. (Fuller *et al*., 2001), particularly if sufficient amounts of blood (20 ml are recommended for aerobic culture) are inoculated (Washington, 1994), recovery is not 100%. In addition, blood cultures may only become positive late in infection. Therefore, in the absence of a positive blood culture, what other laboratory tools are available to aid in the early diagnosis of invasive candidiasis? Serological tests have been the subject of much study but can be difficult to interpret. For example, circulating antibodies to *Candida* spp. may occur in normal individuals as the result of commensal colonization of mucosal surfaces and antibody production in the immunocompromised patient population varies according to immune status. *Candida* spp. antigens and metabolites are often rapidly cleared from the circulation so that antigen detection tests may lack the desired level of sensitivity required for an unequivocal diagnosis. Histopathological evidence of tissue invasion can confirm a diagnosis but commercial reagents to identify and differentiate *Candida* spp. are not currently available. In addition, some subpopulations of patients at-risk for invasive candidiasis may be thrombocytopenic making tissue biopsy procedures hazardous. Newer molecular biological methods, employing polymerase chain reaction (PCR) technology, hold promise but are not yet standardized or readily available in most clinical laboratories. Also, large clinical trials to determine the sensitivity and specificity of such molecular tests are nonexistent. Ultimately, a combination of serological, microbiological, histological, and molecular biological tests may be required for the best possible diagnosis. This article will review currently available methods, as well as newer methods not yet commercially available, and discuss their assets and limitations for the diagnosis of invasive candidiasis.

Culture

Empiric antifungal treatment is recommended following recovery of even one positive blood culture (Edwards 1992; Lecciones *et al*., 1992; Rex *et al*., 1994). Multiple blood cultures on successive days, especially after removal of or in the absence of central lines, are compelling evidence for invasive disease. Several advances in blood culturing techniques have been developed which appear to have improved the sensitivity and/or reduced the time required to obtain a positive blood culture. These include the development of lysis centrifugation tubes and automated monitoring of blood culture bottles. The lysis centrifugation system (Wampole Laboratories, USA) increases the yield of *Candida* spp. recovered from blood by using a detergent to release fungi trapped within host phagocytic cells. The lytic mixture disrupts the host cells and also inactivates both complement and some antimicrobial agents which could be harmful to the viability of the fungus. Tubes are centrifuged and the resultant pellet plated onto a solid medium, such as chocolate agar, for rapid recovery of most yeasts (Archibald *et al*., 2000; Procop *et al*., 2000). The mean time to obtain a positive blood culture is faster for patients with multiple organ involvement than for those with single organ involvement, suggesting a correlation with severity of disease (Berenguer *et al*., 1993). Others have not advocated the routine use of lysis centrifugation tubes because of a high rate of contamination discovered during their use (Cregar *et al*., 1998). However, the main disadvantages of this system are that it is expensive and labor-intensive, precluding routine use in many laboratories. In addition, it appears to be more useful for the recovery of more fastidious fungi such as *Histoplasma capsulatum*, rather than for *Candida* species (Vetter *et al*., 2001).

Recent advances in the formulation of blood culture media, especially those containing resins to absorb out residual antimicrobial drugs found in the blood of patients as well as substances normally found in blood which are inhibitory to fungal growth, have significantly improved the recovery of *Candida* spp. from blood culture bottles (Petti *et al*., 1996; Doern *et al*., 1998; Archibald *et al*., 2000; McDonald *et al*., 2001). Perhaps the single most important improvement, however, was the institution of automated blood culturing systems with continuous growth monitoring. Either colorimetric (BacT/ALERT 3D, Organon Teknika Corp., Durham, NC) or fluorescent (BACTEC 9240, Becton Dickinson, USA) monitoring can now be conducted automatically and electronically at approximately 10 min intervals. In a recent study, a direct comparison of these two automated blood culture systems was conducted to compare detection of *Candida* growth (Horvath *et al*., 2004). Aerobic, anaerobic, and mycology medium, all of which are normally used in each system, were evaluated: Plus Aerobic/F, Plus Anaerobic/F, and

Myco/F lytic bottles for the BACTEC system and FA, SN, and MB bottles for the BacT/ALERT system. Each blood culture bottle was inoculated with fresh blood from healthy donors and spiked with 10^3 yeast cells per bottle. Both systems gave comparable results for the growth of all *Candida* spp. tested when aerobic culture media were used: the BACTEC and BacT/ALERT detected 90% and 100% of *Candida* spp. isolates, respectively. In addition, both systems detected 50 of 50 *Candida* spp. isolates using specialty mycology medium. The major difference noted between these two culturing systems was for growth in anaerobic media: the BACTEC system only detected 10% of the isolates inoculated compared to 70% detected by the BacT/ALERT system (Horvath *et al*., 2004). It must be taken into consideration, however, that anaerobic cultures are not generally inoculated without also inoculating aerobic cultures so the overall detection rate by combining aerobic and anaerobic culture results would not be much different between these two systems. However, the mean time to growth detection in aerobic culture medium was significantly faster for *C. glabrata* isolates in the BacT/ALERT system than in the BACTEC system (Horvath *et al*., 2004). Given the reduced susceptibility of *C. glabrata* to several antifungal drugs (Pappas *et al*., 2004), a more rapid identification of this species could be advantageous for clinical management of disease.

Other blood culture systems which use continuous manometric monitoring, instead of colorimetric or fluorescent monitoring, also exist (ESP, Difco, Detroit, Mich. and O.A.S.I.S., Unipath, Inc.). A comprehensive review of all these systems can be found in Reimer *et al.* (1997).

Body fluids other than blood may also be culture positive for *Candida* spp.. Although the majority of patients with candiduria are asymptomatic, positive urine cultures, in the absence of indwelling urinary catheters, which yield $>1\times10^4$ cfu/ml should raise suspicion of infection. Positive urine cultures from some patient populations (e.g., those in ICUs or neonatal ICUs, burn unit patients, and transplant patients) are particularly important and organisms recovered should be identified to the species level; these species are usually the same as those found in blood or at other sites in multiply colonized patients. For example, positive urine cultures from infants in neonatal ICUs often represent invasive disease and may precede candidemia (Denning *et al*., 2003). However, *Candida* spp. may be absent from the urine even in disseminated infection and *vice versa* (Tiraboschi *et al*., 2000). Although candiduria may not be a specific marker for disseminated candidiasis in all cases, it has been proposed that it is an indicator of poor prognosis in patients with advancing age because of the multiple serious underlying diseases found in this population (Tiraboschi *et al*., 2000).

Phenotypic species identification

Identification of the most common *Candida* spp. is often accomplished by using carbohydrate assimilation and fermentation tests along with Dalmau plate (corn meal-Tween 80 agar) morphology. In addition, more rapid and less laborious phenotypic identification methods have been developed. These include tests such as the RapID Yeast Plus System (Innovative Diagnostic Systems, Norcross, Ga) which contains conventional and chromogenic substrates and requires only 4 to 5 h to complete (Kitch *et al*., 1996; Heelan *et al*., 1998; Wadlin *et al*., 1999), the Fongiscreen test (Sanofi Diagnostics Pasteur, France) (Quindos *et al*., 1993; Hoppe and Frey, 1999), and the automated Rapid Yeast Identification Panel (Dade Microscan, USA) (Land *et al*., 1991; St-Germain and Beauchesne, 1991). Although such tests can identify an isolate in as little as one day, most of these tests are more accurate for the identification of common than uncommon yeast pathogens. For instance, in one study, the RapID Yeast Plus System correctly identified 96% of common yeasts but only 79% of rarer *Candida* spp. and 75% of other uncommon yeasts (Espinel-Ingroff *et al*., 1998).

Perhaps the most convenient and popular methods for *Candida* species identification consist of strips or plates for carbohydrate assimilation and/or enzyme detection which are commercially available in an assortment of different formats. These systems are readily and easily inoculated with yeasts and include, but are not limited to, the API 20C AUX (bioMerieux-Vitek, Hazelwood, Mo.), the API Candida (bioMerieux, France), the Auxacolor (Sanofi Diagnostics Pasteur, France), and the Uni-Yeast-Tek kit (Remel Laboratories, Lenexa, Kan.). These tests use an increase in turbidity (API 20C AUX) or the production of color (API Candida, Auxacolor, Uni-Yeast-Tek) in each of a series of wells containing different substrates to produce a particular biochemical profile. The profile produced is then translated into a numerical code that is deciphered using the manufacturer's reference manual. These tests give good results for the more common species of *Candida* and genera of yeasts (99.8% for Uni-Yeast-Tek, 98% for API 20C AUX, 81% to 91% for Auxacolor, and 79% to 92% for API Candida) (Bernal *et al*., 1998; Campbell *et al*., 1999; Moghaddas *et al*., 1999; Verweij *et al*., 1999). The Auxacolor and API 20C AUX tests are also relatively useful for identifying common germ tube negative *Candida* spp. (i.e., the accuracy of these tests ranged from 75% to 93%) (Davey *et al*., 1995; Verweij *et al*., 1999). However, identification of less common *Candida* spp. and genera are not as accurate (i.e., the Auxacolor and API Candida tests failed to identify *Candida norvegensis, Candida catenulata, Candida haemulonii,* and *C. dubliniensis* and the API 20C AUX had only a 90% accuracy for isolates belonging to genera of *Cryptococcus, Trichosporon,* and *Geotrichum*) (Campbell *et al*., 1999; Verweij *et al*., 1999). Further, although the API Candida system correctly identified 92% of 146 clinical isolates, 23 required supplemental biochemical or mor-

phological tests for unequivocal confirmation (Campbell *et al*., 1999).

A recent study to differentiate *C. dubliniensis* from *C. albicans* revealed that the API 20C AUX carbohydrate assimilation system incorrectly identified *C. dubliniensis* as *C. albicans* in all but 3 of 22 cases: remaining isolates were misidentified as *C. albicans/C. tropicalis*, *C. tropicalis/C. albicans*, and *C. lusitaniae/C. albicans* (Ellepola *et al*., 2003). In addition, 82% of *C. albicans* isolates tested, and 100% of *C. dubliniensis* isolates, assimilated trehalose; the latter finding was opposite to that expected for *C. dubliniensis* according to the API 20C AUX reference manual. Xylose and α-methyl-D-glucoside assimilation, respectively, were negative for 100% and 95% of *C. dubliniensis* isolates and positive for 100% and 91% of *C. albicans* isolates, confirming earlier reports that assimilation results for xylose and α-methyl-D-glucoside may be helpful in the discrimination of these two species (Ellepola *et al*., 2003).

It was suggested that a novel medium referred to as Pal's medium (sunflower seed agar) could be used to differentiate *C. albicans* from *C. dubliniensis* (Mosaid *et al*., 2003). Growth on this medium for 48 to 72 h resulted in smooth creamy-grey colonies for both species, but only *C. dubliniensis* isolates exhibited a hyphal fringe surrounding the colonies. However, as some isolates belonging to other *Candida* species (*C. tropicalis*, *C. parapsilosis* and *C. krusei*) also produce a fringe on Pal's agar, this medium might best be used only for germ tube positive and/or chlamydospore positive isolates.

The development of a chromogenic medium (CHROMagar Candida, France) which incorporates substrates linked to chemical dyes in a solid medium to differentiate *Candida* spp. by the color and/or texture of the growth produced (*C.albicans* = green/blue green; *C. tropicalis* = blue/purple with halo; *C. krusei* = pink/ruffled) has been particularly helpful for the presumptive identification of *C. albicans*, *C. tropicalis*, and *C. krusei*, especially from fresh cultures (Odds and Bernaerts, 1994; San-Millan *et al*., 1996). CHROMagar Candida medium is also valuable for the differentiation of mixed cultures which would ordinarily be missed during conventional plating on solid medium (Pfaller *et al*., 1996; Powell *et al*., 1998). Some reports have suggested that this medium can also be used to differentiate *C. glabrata* from other yeast species (Pfaller *et al*., 1996; Willinger and Manafi, 1999). In contrast, others reported that it can not be used for this purpose because *Candida kefyr, C. lusitaniae, Candida guilliermondii, Candida famata, Candida rugosa, Candida utilis, Candida robusta,* and *Candida pelliculosa* all produce the same type of glossy pink colonies as *C. glabrata*, leading to misidentification (Baumgartner *et al*., 1996; Freydiere, 1996; Freydiere *et al*., 1997a).

CHROMagar Candida was recently reformulated (Becton Dickinson) but no significant differences in growth rate or colony size were observed for most species and no differences in the capacity to differentiate among colonies of *C. albicans, C. tropicalis,* and *C. krusei* were reported for the new formulation compared to the original one. However, all *C. albicans* isolates gave a lighter shade of green on this medium compared to the old formulation whereas *C. dubliniensis* isolates gave the same typical dark green color on agar made with both the old and new formulations (Jabra-Rizk *et al*., 2001). Hence, it was proposed that the new medium could not only differentiate between *C. albicans, C. tropicalis,* and *C. krusei* but could also differentiate *C. albicans* from *C. dubliniensis*. In contrast, in a recent study, it was demonstrated that *C. albicans* rather than *C. dubliniensis* was more likely to demonstrate a dark green or blue green colony color on CHROMagar Candida medium made with the new formulation (i.e. 100% of *C. albicans* isolates were dark green or blue green versus only 64% of *C. dubliniensis* isolates) (Ellepola *et al*., 2003). Fluconazole has also been incorporated into CHROMagar Candida medium in some studies so as to not only identify the *Candida* spp. present but to also identify antifungal drug resistant isolates during initial isolation (Patterson *et al*., 1996; Verghese *et al*., 2000).

Preliminary studies have demonstrated that a newly-formulated chromogenic medium (Oxoid Chromogenic Candida Agar, OCCA; Oxoid, Inc., USA) allows differentiation of *C. tropicalis* (dark blue colonies), *C. albicans*/*C. dubliniensis* (green colonies) and *C. krusei* (dry, irregular, pink-brown colonies) from colonies of *C. glabrata*, *C. kefyr, C. parapsilosis* or *C. lusitaniae* (beige/yellow/brown colonies), and it has been suggested that experienced users may be able to differentiate among the latter four species. This medium incorporates X-NAG (5-bromo-4-chloro-3-indolyl-N-acetyl-β-D-glucosaminide) and BCIP (5-bromo-6-chloro-3-indolyl-phosphate-p-toluidine salt) as chromogenic substrates, which detect the activity of hexosaminidase and alkaline phosphatase, respectively. Chloramphenicol is also incorporated into the medium to inhibit bacterial growth (De Caux *et al*., 2004). More extensive evaluations in the clinical microbiology laboratory setting are needed to determine the full potential of this medium.

Additional methods for *Candida* spp. identification include automated biochemical systems such as the ID 32C strip system (bioMerieux), the Vitek Yeast Biochemical Card system (bioMerieux Vitek, Inc., Hazelwood, Mo.), the Vitek 2 ID-YST card system (bioMerieux Vitek, Inc.), the Quantum II (Abbott Laboratories, USA), and the Biolog YT MicroPlate system (Biolog, USA), to mention only a few. Details of a variety of other colorimetric and enzymatic systems, as well as fatty acid analysis and physicochemical spectroscopic identification methods can be found in a review by Freydiere *et al.* (2001).

Molecular biological identification of Candida species isolated in culture

The limitations described above for phenotypic identification techniques have led to the development of nucleic acid-based identification systems. These systems offer the potential for more rapid and specific identification of *Candida* spp. compared to traditional phenotypic methods. Most nucleic acid-based systems use polymerase chain reaction (PCR) techniques to amplify fungal DNA as the first step in the identification process. Before PCR amplification can occur, appropriate DNA targets and PCR primers must be selected. Highly conserved regions of ribosomal DNA have been the most popular targets for PCR amplification and have included the 5.8S, 18S, and 28S rRNA genes (Sandhu *et al*., 1995; Kurtzman and Robnett, 1997; Mannarelli and Kurtzman 1998; Hui *et al*., 2000; Jaeger *et al*., 2000; Loeffler *et al*., 2000a; Guiver *et al*., 2001). Other targets have included the more variable internal transcribed spacer (ITS) regions between these genes (Elie *et al*., 1998; Chen *et al*., 2000; Ellepola *et al*., 2003; Coignard *et al*., 2004; Massonet *et al*., 2004) or the intergenic spacer (IGS) region (Cirak *et al*., 2003).

The main advantage of using amplification targets from regions of DNA which are conserved among all *Candida* species is that a PCR product can be obtained from all species using a single set of PCR primers and conditions optimal for that set of primers. Following amplification, species-specific probes can be designed from the more variable DNA regions, located between primer binding sites, for the identification of specific organisms (Elie *et al*., 1998; Ellepola *et al*., 2003; Coignard *et al*., 2004) or amplicons can be subjected to gel electrophoresis, with or without the use of restriction enzyme digestion, followed by ethidium bromide staining to differentiate among the species (Buchman *et al*., 1990; Crampin and Matthews 1993; Hopfer *et al*., 1993; Rand *et al*., 1994; Wildfeuer *et al*., 1996; Morace *et al*., 1997; Irobi *et al*., 1999; Cirak *et al*., 2003; Graf *et al*., 2004). Increased sensitivity can be achieved by Southern blotting of such gels onto nylon membranes and by detection with radiolabeled or colorimetric probes (Crampin and Matthews, 1993; Miyakawa *et al*., 1993; Burgener-Kairuz *et al*., 1994; Holmes *et al*., 1994; Jordan, 1994; van Deventer *et al*., 1995; Loeffler *et al*., 2000a). Others have used line-probe, reverse cross blot or slot blot detection methods (Loeffler *et al*., 2000a). More sophisticated detection methods include analysis by direct fluorescent capillary automated DNA sequencing, amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), or single strand conformational polymorphisms (SSCP) (Sandhu *et al*., 1995; Walsh *et al*., 1995a; Hui *et al*., 2000; Loeffler *et al*., 2000b; Martin *et al*., 2000; Posteraro *et al*., 2000; Bautista-Munoz *et al*., 2003; Borst *et al*., 2003).

Sequence polymorphisms in the ITS2 region of the rRNA genes have also been used as a means to identify yeasts. Chen *et al*. (2000) used 434 isolates, representing 34 species of yeasts to determine the length of the PCR-amplified ITS2 region by automated capillary electrophoresis. Unique, species-specific PCR products were obtained from 92% of the clinical isolates tested; the remaining 8% contained DNA sequences that required restriction enzyme analysis for differentiation. These data, and the specificity of length polymorphisms for identifying yeasts, were confirmed by DNA sequence analysis of the ITS2 region for 93 isolates. Phenotypic and ITS2-based identifications were concordant for 427 of 434 yeast isolates examined. The remaining seven clinical isolates contained ITS2 sequences that did not agree with their phenotypic identification, and ITS2-based phylogenetic analyses indicated the possibility that these isolates represented new or clinically unusual species among the *Rhodotorula* and *Candida* genera. Whereas it was suggested that size estimation and restriction enzyme analysis of PCR-amplified ITS2 region DNAs were rapid and reliable methods to identify clinically significant yeasts, including potentially new or emerging pathogenic species (Chen *et al*., 2000), few clinical laboratories have the capability or budget to conduct DNA sequence-based methods and, in particular, restriction enzyme fragment length analyses.

The application of an enzyme immunoassay (EIA) format, using a colorimetric substrate, is perhaps the easiest and least costly method available for amplicon detection (Elie *et al*., 1998; Loeffler *et al*., 1998). It is also user-friendly, does not require any dangerous radioactive reagents, and can be automated. One example of such an assay uses universal fungal primers flanking the ITS2 region of ribosomal DNA. Species-specific DNA probes, directed to the ITS2 region and labeled with digoxigenin, are hybridized to the PCR amplicons along with a biotinylated all-*Candida* spp. probe which functions to capture amplicons onto streptavidin-coated wells of a 96-well microtiter plate. Amplicons are then detected in an EIA format (PCR-EIA) using horseradish peroxidase-labeled, anti-digoxigenin antibodies, H_2O_2, and a colorimetric substrate. The assay can be completed in a single day and can identify up to 18 medically important *Candida* spp., including *C. dubliniensis* (Elie *et al*., 1998; Ellepola *et al*., 2003; Coignard *et al*., 2004). This detection format is particularly amenable to the identification of *Candida* spp. in a clinical laboratory.

Other PCR-based identification methods to specifically differentiate *C. albicans* from *C. dubliniensis* have included comparisons of *ACT-1* intron amplicons, PCR amplicons obtained using primers derived from the pH-regulated *PHR1* gene of *C. albicans*, or from flanking regions of the *CaLSU* intron (Haynes *et al*., 1995; Boucher *et al*., 1996; Donnelly *et al*., 1999; Kurzai *et al*., 1999). Each of these non-EIA methods have limitations in that they either lack a specificity control and, as such, can be prone to potential misidentifications (*PHR1* gene method), require large amounts of DNA template (*PHR1*

gene method), have fluctuating reproducibility regarding agarose gel detection of the larger of two required DNA bands (*ACT-1* intron method), or require multiple internal controls. These methods also require gel electrophoresis of the PCR amplicons for isolate identification. Molecular mass can be difficult to interpret in an agarose gel and can vary depending upon the composition of the agarose used, the length of run time, the size of the gel format, and the molecular markers employed. Few clinical laboratories are equipped to use electrophoresis gels on a regular basis whereas an EIA detection format is more readily adaptable.

The PCR-EIA method has also been used to resolve discrepant phenotype-based *Candida* spp. identifications between the Centers for Disease Control and Prevention (CDC) laboratories and referring hospitals participating in an active, population-based surveillance for candidemia (Coignard *et al*., 2004). Species identifications were performed at these institutions and then confirmed at the CDC by phenotype-based methods using CHROMagar Candida medium and the API 20C AUX system. Discrepancies in species identification between the referring institution and the CDC were noted for 43 of 935 isolates (4.6%). The PCR-EIA results were identical to the CDC phenotypic identification method for 98% of the isolates tested. Discrepancies between the CDC phenotypic method and the PCR-EIA results were resolved by DNA sequence analysis of the ITS1 rRNA gene, which confirmed the PCR-EIA result. Among all misidentifications, the referring institutions most frequently misidentified *C. glabrata* (37% of all discrepant identifications). Such misidentifications could lead to the administration of inappropriate therapy given the propensity of *C. glabrata* to develop resistance to azole antifungal drugs. Hence, use of the PCR-EIA for species identification might circumvent such problems.

Most recently, detection methods have been developed which are referred to as "real-time" PCR. Detection of the amplicon occurs as the PCR product is produced and quantitative results can be graphically displayed during the process. Therefore, post-amplification manipulation of the product is not required. The TaqMan system (Perkin-Elmer; Applied Biosystems, USA) is a fluorogenic assay which uses a reporter and a quencher dye in proximity to each other on the detector probe. As DNA amplification occurs, the 5'→3' endonuclease activity of the *Taq* DNA polymerase separates the quencher dye from the reporter dye allowing signal to be detected. Guiver *et al.* (2001) used the TaqMan system and species-specific primer and probe sets for the identification of *C. albicans, C. glabrata, C. kefyr, C. krusei*, and *C. parapsilosis*. The probes were labeled with three fluorescent dyes to enable differentiation between species when three primer and probe sets were combined in two multiplex reactions. The primer and probe sets were shown to be 100% specific for their respective species; there was no cross-reaction between any set and human, fungal, bacterial or viral DNA tested (Guiver *et al*., 2001). The TaqMan assay has the advantage of reducing post-amplification manipulation steps but the initial cost for the equipment and laboratory set up to perform such assays is prohibitive in many laboratory settings.

Another "real time" PCR method is the LightCycler system (Roche Molecular Systems, Indianapolis, Ind.) which allows rapid amplification of DNA in glass capillaries and simultaneous fluorescent detection of amplicons using fluorescence energy transfer or "FRET" (Loeffler *et al*., 2000b; Schmidt *et al*., 2001). One DNA probe is labeled at the 3' end with fluorescein and another probe is labeled at the 5' end with Light Cycler Red 640 fluorophore. The fluorescein is excited by the light source of the Light Cycler instrument and the energy emitted by the fluorescein is transferred to the Light Cycler Red 640 fluorophore. The light emitted by the fluorophore is then measured and is proportional to the amount of DNA amplified. Selvarangan *et al.* (2002) used this method in an attempt to distinguish *C. albicans* from *C. dubliniensis*. Fluorescently-labeled nucleic acid probes, specific for *C. albicans* and *C. dubliniensis*, were bound to PCR amplicons. Subsequent denaturation produced characteristic peak melting temperatures (T_m) that identified each of the two species. No signal was generated when the *C. albicans* or *C. dubliniensis* probes were tested against DNA from other *Candida* spp.. However, both probes also reacted with *C. tropicalis* DNA, but the average T_m values were sufficiently different to allow differentiation of *C. tropicalis* from *C. albicans* and *C. dubliniensis*. Hsu *et al*. (2003) also used a "real-time" LightCycler assay to detect and identify *Candida* spp.. In this instance, template DNA from different *Candida* species was amplified and detected in real time by employing SYBR Green fluorescent dye and specific target sequences from the 18S and 28S regions of rDNA. *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, and *C. tropicalis* could be discriminated by species-specific primers and confirmed by melting-curve analyses (Hsu *et al*., 2003).

A DNA sequence-based approach for the identification of medically important fungi, including *Candida* spp., by automated capillary electrophoresis of PCR products has also been developed (Pryce *et al*., 2003). In this study, the entire ITS1, 5.8S, and ITS2 ribosomal DNA region was sequenced using automated dye termination sequencing on 89 clinical isolates. Eighty eight of the 89 isolates had DNA sequences that were highly homologous to those of reference strains accessioned in GenBank and 87 of 89 gave a sequence-based identification result that correlated with the traditional phenotypic identification. Sequence-based identification of *Candida* spp. in pure culture was obtained within 24 h of DNA extraction. Another study describing sequenced-based yeast identification used a

commercially available MicroSeq D2 large-subunit ribosomal DNA sequencing kit (Applied Biosystems, USA). The use of this method, together with the API 20C AUX system, revealed that of 100 isolates sequenced (representing 19 species of *Candida*), 98% gave results concordant with identifications made by the API 20C AUX system. *C. dubliniensis*, however, was not included in the MicroSeq database and was identified as *C. albicans* (Hall *et al*., 2003). Several other less common yeasts were also not included in the MicroSeq database.

In general, nucleic acid sequencing may provide the greatest benefit to the laboratory by identifying organisms whose identities are questionable or cannot be determined by phenotypic methods. However, a cost analysis, comparing phenotypic testing to nucleic acid sequencing, showed that the cost of sequencing was $29.50 higher than the cost of using the API 20C AUX system (Hall *et al*., 2003). The MicroSeq D2 library was also somewhat limited and did not include sequences for all clinically important yeast species. However, the potential exists to allow each laboratory to construct a custom database for the most common species found in a given laboratory, thereby making the system more useful. Constructing a database of species not already included in the MicroSeq D2 library and of isolates that show some genetic diversity among their sequences would enhance the flexibility of the MicroSeq D2 sequencing kit. Other limitations of direct sequencing systems are in the inaccuracies and incompleteness of some sequence databases. For instance, commercially available databases often contain sequences from cultures which may not have been accurately identified initially (Hall *et al*., 2003). Public databases such as GenBank are not refereed and contain sequencing errors as well as nomenclature errors. Selection of gene sequencing targets can also greatly alter identification results (i.e., use of conserved gene regions tend to "lump" some species together whereas more variable gene targets, such as the ITS and IGS regions of rDNA, tend to "split" species apart). Direct sequencing also requires technical expertise and sophisticated equipment which is not available in many clinical laboratories.

Histopathology

Histopathological examination of tissue sections is one of the most reliable methods of establishing a diagnosis of systemic fungal infection. However, the ease with which a fungal pathogen can be recognized in tissue depends on its abundance and the distinctiveness of its appearance. The presence of typical blastospores and pseudohyphae of *Candida* spp. in histochemically-stained tissue sections can be used as a diagnostic parameter for invasive candidiasis. However, the production of fluorescent antibodies specific for the identification of individual *Candida* spp. has proved extremely difficult. Generic reagents which react across *Candida* spp. have been used to differentiate infections by *Candida* spp. from those of other fungi (Kaufman *et al*., 1997). A recent study described the use of an IgG1 monoclonal antibody, 3H8, directed against *C. albicans* cell wall mannoprotein, to specifically recognize *C. albicans* in culture and in paraffin-embedded tissue sections using immunofluorescent and immunohistochemical staining (Marcilla *et al*., 1999). This antibody preferentially detected mycelial forms and, to a lesser extent, blastospores of *C. albicans* and did not react with any other *Candida* spp. tested. This monoclonal antibody was originally produced for use in a latex agglutination kit (Bichro-latex albicans, Fomouze Diagnostics, Asnieres, France) for the rapid identification of *C. albicans* in culture (Freydiere *et al*., 1997b; Quindos *et al*., 1997; Marcilla *et al*., 1999). In addition, differentiation of *C. albicans* from *C. dubliniensis* has been reported by indirect immunofluorescence (Bikandi *et al*., 1998). In this study, anti-*C. dubliniensis* serum was adsorbed with *C. albicans* blastospores so that no reactivity was observed against *C. albicans* or several other *Candida* species, except *C. krusei*.

Fluorescent in-situ hybridization (FISH), using oligonucleotide probes directed against 18S rRNA has been used to differentiate *C. albicans* from *C. parapsilosis* in tissues of infected mice (Lischewski *et al*., 1997). The *C. albicans* probe detected fungal cells in tissue sections of the kidney, spleen, and brain of mice infected with *C. albicans*, but not in tissues from mice infected with *C. parapsilosis* and *vice versa* for the *C. parapsilosis* probe. In addition, the *C. albicans* probe could detect as few as three *C. albicans* cells per 500 µl of spiked human blood after a lysis-filtration assay and subsequent FISH (Lischewski *et al*., 1997).

A new FISH method, using fluorescein-labeled peptide nucleic acid (PNA) probes targeting 26S rRNA, detects *C. albicans* directly in smears taken from positive blood culture bottles (Rigby *et al*., 2002). The PNA probe is added and hybridized. Unhybridized probe is removed by washing, and the smears are examined by fluorescence microscopy. The performance of the *C. albicans* PNA FISH method as a diagnostic test was evaluated with 33 routine and 25 simulated yeast-positive blood culture bottles and showed 100% sensitivity and 100% specificity (Rigby *et al*., 2002). Oliveira *et al*. (2001) also used a PNA FISH assay but to differentiate *C. albicans* from *C. dubliniensis*. Samples from liquid cultures were smeared onto microscope slides, heat fixed, and then hybridized with probes for 30 min. Unhybridized probe was removed by washing, and slides were examined by fluorescence microscopy. Evaluation of the PNA FISH method using 79 *C. dubliniensis* and 70 *C. albicans* isolates showed 100% sensitivity and specificity for both probes.

Antibody detection

The clinical usefulness of antibody detection for the diag-

nosis of systemic candidiasis has been limited by false-negative results in immunocompromised patients who produce low or undetectable levels of antibody and by false-positive results in patients with superficial colonization. A study was conducted to evaluate the usefulness of antibody detection by double immunodiffusion (ID) in 214 patients admitted to the intensive care unit of a university hospital (Pallavicini *et al*., 1999). Patients were followed over a period of 42 months for the development of invasive candidiasis. Seventeen percent of patients developed invasive candidiasis but the sensitivity and specificity of the ID test was only 29% and 67%, respectively, indicating that the ID test is not very useful for the diagnosis of invasive candidiasis in this patient population.

In an attempt to reduce false-positive results, several researchers have developed tests to detect antibodies directed against cytoplasmic antigens, assuming that the host would not be exposed to intracellular antigens except during invasive disease. Unfortunately, in a study of patients undergoing induction chemotherapy for acute leukemia, antibody to a major 54 kDa cytoplasmic antigen was detected in only 25% of patients with disseminated candidiasis (Jones 1980a; 1980b; Greenfield and Jones, 1981; Greenfield *et al*., 1983) and others found increases in antibody titers in 10% of patients without candidiasis (Fujita *et al*., 1986). In contrast, El Moudni *et al*. (1998) described highly successful detection of antibodies to a high-performance liquid chromatographically purified 52 kDa metalloprotein of *C. albicans* in an enzyme-linked immunosorbent assay (ELISA). However, they failed to specify whether the patients studied were immunocompromised. Nonetheless, at a cutoff absorbance of 0.425, test sensitivity and specificity was reported to be 83% and 97%, respectively. It was therefore suggested that this aminopeptidase may be a useful antigen for the detection of antibodies formed during invasive candidiasis.

Na and Song (1999) described an ELISA assay for the detection of antibodies to the secreted aspartyl proteinase (Sap) of *C. albicans*, a putative virulence factor released during tissue invasion (Ray *et al*., 1991; Hube, 1996). In a retrospective evaluation of 33 patients with invasive candidiasis, the sensitivity and specificity, respectively, for this test was only 70% and 76%, making it less desirable for the diagnosis of invasive candidiasis than Sap antigen detection tests (for a description of the latter tests, please see the antigen detection section of this paper).

Some researchers have also examined the usefulness of combined antigen and antibody detection tests for the diagnosis of invasive candidiasis. A retrospective study (Sendid *et al*., 1999), using 162 serum samples from 43 patients with mycologically and clinically proven candidiasis, was conducted to evaluate the usefulness of two commercial ELISA tests: one to detect antibodies to *C. albicans* cell wall mannan (Platelia *Candida* Antibody test, Bio-Rad Laboratories, France) and the other to detect mannan serum antigen using a monoclonal antibody (Platelia *Candida* Antigen test, Bio-Rad). The sensitivity and specificity for the antibody test alone was reported to be 53 and 94%, respectively, whereas these values changed to 80 and 93% when a combination of both antibody and antigen detection tests were employed. It was suggested that a combination of both tests may be more useful for the diagnosis of invasive candidiasis than either test alone. In a later study, Yera *et al.* (2001) examined the contribution of these same two tests for the diagnosis of invasive candidiasis in relation to the time of positive blood culture. In patients who presented with at least one positive blood culture (n=45), one or both serological tests were positive in 73% of patients at least 2 days, and in some patients, up to 15 days before blood cultures became positive. It was suggested that serological surveillance of patients at-risk for invasive candidiasis could therefore result in earlier implementation of antifungal therapy than blood culture if both serological tests were employed simultaneously. It is of particular interest, however, that in this same study, patients were generally either positive for antibodies or for antigen but generally not for both simultaneously. In addition, the kinetics of test positivity was dependent upon the hospital ward in which the patient was housed: i.e., hematology and ICU patients were more likely to have had positive antigen tests whereas surgical patients were more likely to have had positive antibody tests. These results are not surprising given the more immunosuppressed state that might be associated with patients from the former two hospital units.

Indeed, the fact that antibody production can be variable or nonexistent in immunocompromised patients make tests to detect antibodies for the diagnosis of systemic candidiasis less useful in this patient population. However, immunosuppressed patients may be in antigen excess, making the detection of antigens a potentially more successful strategy for the diagnosis of invasive candidiasis in these patients groups.

Antigen detection

Numerous circulating antigens have been used as potential targets for the diagnosis of disseminated candidiasis. One such antigen is an inducible, extracellularly secreted aspartyl proteinase (Sap) produced by *C. albicans* and some other *Candida* spp.. It has been studied extensively as a virulence factor in the invasion and dissemination of *C. albicans* in animal models of infection (Staib, 1965; Kwon-Chung *et al*., 1985; Ruchel, 1992; Hube 1996; Morrison *et al*., 2003). The theoretical usefulness of Sap as a diagnostic antigen stems from the hypothesis that because Sap is an inducible enzyme, produced during active tissue invasion (MacDonald and Odds, 1983; Ray and Payne, 1988), its production should correlate with

invasive disease and not simple colonization.

Ruchel *et al.* (1988) examined serum samples from patients for the utility of Sap detection as an aid to the diagnosis of invasive disease. Using anti-Sap antibodies in an ELISA format, the sensitivity of detection was low (positive in 50% of suspected plus confirmed cases) and may have been the result of complex formation between Sap and alpha-2-macroglobulins in the circulation (Ruchel and Boning-Stutzer, 1983).

More recently, Na and Song (1999) compared two different ELISA assays for their efficacy to detect Sap antigen in serum: an inhibition ELISA and an antigen capture ELISA. Both antigen detection tests used a monoclonal antibody, CAP1, which was reported to be specific for *C. albicans* Sap. The sensitivities and specificities of these tests, respectively, were 94% and 92% for the antigen capture ELISA and 94% and 96%, for the inhibition ELISA. These data suggest that the inhibition ELISA could be useful for the diagnosis of invasive *C. albicans* infections. However, further studies are warranted to validate these findings in a well-controlled, prospective study.

The potential use of proteinases as markers of invasive candidiasis has led to the development of a competitive binding inhibition ELISA to detect Sap in serum and urine obtained from a rabbit model of disseminated candidiasis (Morrison *et al*., 2003). Although ELISA inhibition was observed when serum specimens were used, more significant inhibition, which correlated with disease progression, occurred when urine specimens were tested. Urine collected as early as 1 day after infection resulted in significant ELISA inhibition compared with preinfection control urine, and inhibition increased until experimental termination on day 5. The overall test sensitivity and specificity was 83% and 92%, respectively. The specificity could be increased to 97% if at least two positive test results were required to define a positive result. The use of Sap detection in urine could therefore provide a noninvasive means to diagnose disseminated candidiasis (Morrison *et al*., 2003).

Mannan, the major cell wall mannoprotein of *C. albicans*, is another diagnostic antigen. Dissociation of antigen-antibody complexes is necessary for the optimal detection of mannan in the circulation. This antigen is heat stable and resists boiling, proteinase treatment, and acidic pH (Reiss and Morrison, 1993). Therefore, antigen-antibody complexes are routinely dissociated by boiling in the presence of EDTA or by enzymatic treatment. Bailey *et al.* (1985) detected mannan in the serum of 17 of 21 patients with disseminated candidiasis when specimens were treated with pronase and heat, whereas only 3 of 21 patients were positive if no dissociation step was included. Mannan is cleared rapidly from the circulation, resulting in low serum concentrations, and multiple serum sampling is required for optimal detection. Mannanemia occurs in approximately 31 to 90% of patients with disseminated candidiasis depending on the frequency of sampling, the underlying disease, the degree of immunosuppression, the serotype of *C. albicans*, the *Candida* species involved, the definition of disseminated candidiasis, the specificity and titer of the capture antibodies, and the immunoassay method used. Numerous laboratories have attempted to use radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), latex agglutination (LA) or reverse passive latex agglutination (RPLA) to detect circulating mannan (Bougnoux *et al*., 1990; Lemieux *et al*., 1990; Reiss and Morrison, 1993).

Methods such as the sandwich ELISA (Bougnoux *et al*., 1990; Lemieux *et al*., 1990; Fujita and Hashimoto, 1992) and RPLA (Bailey *et al*., 1985; Kahn and Jones, 1986), have moderate sensitivity but good specificity for disseminated disease. In a retrospective study of patients with malignancy, the sensitivity and specificity of the sandwich ELISA was 65% and 100%, respectively (de Repentigny *et al*., 1985). Fujita and Hashimoto (1992) compared the sensitivity of the sandwich ELISA format to that of the RPLA format using the same capture antibodies for both. They found the sensitivity of the RPLA to be 38% whereas that of the sandwich ELISA was 74% (Fujita and Hashimoto 1992). In other studies, the RPLA test detected serum mannan in 78% of leukemic patients with disseminated candidiasis (Kahn and Jones, 1986) and in 72% of patients for whom disseminated candidiasis was confirmed by biopsy, autopsy, or persistent candidemia during granulocytopenia (Bailey *et al*., 1985). In a more recent study, cerebrospinal fluid samples from five patients from whom *Candida* cells were cultured were tested for the presence of mannan using an ELISA (Verduyn Lunel *et al*., 2004). Samples from four of five patients determined to have proven *Candida* meningitis reacted positively in the assay. Samples from the remaining patient and from patients with other central nervous system infections were negative. Although sample numbers in this study were small, test results are encouraging.

A sandwich ELISA, commercially available as the Platelia *Candida* antigen test (Bio-Rad), was developed for the diagnosis of systemic candidiasis based on the detection of α-linked oligomannose residues (α-Man) released from *Candida* cells into the serum. This test has been shown to have good specificity but must be repeated frequently because of the rapid clearance of this form of mannan from the circulation (Sendid *et al*., 2004). Therefore, a second ELISA, based on the detection of β-linked oligomannoses (β-Man), was developed. It was suggested that because β-Man are linked to different *Candida* molecules and interact differently with the host immune system and endogenous lectins than does α-Man, that β-Man may therefore possess different serum clearance kinetics. In a guinea pig model of systemic *C. albicans* infection, the relative amounts of detectable α- and β-Man differed considerably according to the virulence of the strain, the

infecting dose, and the time after challenge that serum samples were drawn. Although detection of α-Man was more sensitive per serum sample than detection of β-Man, the sensitivity to detect the combination of both reached 90%. The same tests were then applied to 90 sera from 26 patients selected retrospectively for having been infected with *C. albicans*, *C. tropicalis*, and *C. glabrata*. A total of 22 patients had positive antigenemia: 4 had α-mannanemia, 4 had β-mannanemia, and 14 showed the presence of both types of mannan. For patients showing the presence of both forms of mannan, the use of both tests enhanced the duration of mannanemia detection. A combined use of both tests had a cumulative specificity of 95%, and positive and negative predictive values of 79% and 97%, respectively. These findings indicate that detection of both epitopes may contribute to increased test sensitivity (Sendid *et al*., 2004).

Detection of cytoplasmic proteins of *C. albicans* has also been used diagnostically employing a variety of test formats (Stevens *et al*., 1980; Araj *et al*., 1982; Strockbine *et al*., 1984a; Matthews *et al*., 1987; Matthews and Burnie, 1988). The two predominant cytoplasmic proteins described to date include a 47-kDa protein which is thought to be a breakdown product of a 90-kDa heat-shock protein (HSP-90) and a 48 kDa protein, which was later found to be a *C. albicans* enolase (Strockbine *et al*., 1984b; Matthews *et al*., 1987; Mason *et al*., 1988; Sundstrom and Aliaga, 1992). Antigens in the 47 to 52 kDa range are not resolved by Western blot analysis unless monoclonal antibodies that specifically recognize the enolase antigen is applied (Strockbine *et al*., 1984b). The 47 kDa antigen could be detected in the sera of 77% of neutropenic patients with disseminated candidiasis using an enzyme-linked dot immunobinding assay (Matthews and Burnie, 1988). This assay proved to be more sensitive than an RPLA test for the same antigen (Burnie, 1985).

Early animal studies in mice and rabbits, using an ELISA format, revealed that the presence of the 48 kDa antigen in serum correlated with disseminated disease, was positive in the absence of candidemia, and declined following antifungal therapy (Walsh and Chanock, 1998). The assay was commercialized as a double-sandwich liposomal assay using murine IgA monoclonal antibody adsorbed to a nitrocellulose membrane (Directigen$_{1\text{-}2\text{-}3}$ Disseminated Candidiasis Test, Becton Dickinson, USA). Patient serum was added and polyclonal rabbit anti-*C. albicans* enolase was applied and detected with a liposome coated with goat anti-rabbit IgG and containing a rhodamine dye (Walsh and Chanock, 1998). A multicenter study conducted at cancer centers over a two year period revealed a sensitivity per sample of 54%; when multiple samples were tested, detection of antigenemia was improved to 85% (Walsh *et al*., 1991). Hence, multiple sampling was recommended to optimize the detection of antigenemia. Unfortunately this test is no longer available commercially.

In contrast to previously described antigens which had been identified and chemically purified, Gentry *et al*. (1983) described detection of a structurally uncharacterized, 56°C-labile antigen, by RPLA. Latex particles were sensitized with serum from rabbits immunized with whole, heat-killed *C. albicans* blastoconidia. The test was commercialized as the Cand-Tec test (Ramco Laboratories, Houston, Tex.). The circulating antigen was heat-sensitive and susceptible to pronase, 2-mercaptoethanol, and sodium periodate treatment. The sensitized latex particles could not agglutinate mannan and it has been suggested that the assay may detect a neoantigen derived from *C. albicans* after host processing or a host antigen which cross-reacts with those of *C. albicans*. The test was relatively easy to perform. However, it lacks sensitivity when an antigen titer of 1:8, which excludes most false-positive results, is used (Bougnoux *et al*., 1990; Lemieux *et al*., 1990; Phillips *et al*., 1990) and test specificity is also low (Lemieux *et al*., 1990). More recently, Pallavicini *et al*. (1999) conducted a study to evaluate the usefulness of the Cand-Tec latex agglutination test. Over a period of 42 months, 214 patients admitted to the ICU of a university hospital were followed for the development of invasive candidiasis. Although 17% of the patients developed invasive candidiasis, the positive predictive value of the Cand-Tec test was low (13-17%). Most studies to date suggest that the Cand-Tec test does not provide adequate predictive value for a reliable diagnosis of disseminated candidiasis.

Although the Cand-Tec test is not a reliable indicator of invasive candidiasis, one study found this test to be a useful predictor for timing the initiation of antifungal drug therapy (Iwasaki *et al*., 2000). The Cand-Tec assay was performed serially on 10 patients with acute leukemia during 12 febrile episodes following post-remission chemotherapy. Febrile neutropenia after antileukemic chemotherapy and an increased Cand-Tec titer relative to that measured before antileukemic chemotherapy were used as indicators to administer intravenous azole antifungal drug therapy. In 9 of the 11 evaluated cases, antifungal therapy was effective and the Cand-Tec titers declined to less than or equal to baseline. In contrast, for the remaining two cases, where antifungal drug therapy failed, the Cand-Tec titers did not decline. The Cand-Tec test was therefore suggested to provide a means to prevent excess use of antifungal agents and to reduce the potential development of azole-resistant *Candida* spp. infections. Recently, a modification of the Cand-Tec test was described which uses a microtiter plate format (Cand-Tec MT, Ramco, Japan) and which expresses the *Candida* antigen level as a cutoff index (CI) by colorimetric analysis. The sensitivity and specificity of the Cand-Tec MT assay to diagnose deep-seated *Candida* infection in 25 patients with hematologic diseases were 100% and 80%, respectively.

The CI value was followed during antifungal therapy and it decreased in 75% of cases that responded to antifungal therapy, but did not show this tendency in non-responsive cases. Therefore, this new format may improve the sensitivity of the Cand-Tec test but specificity issues remain. This study also supports the potential usefulness of this test to monitor response to therapy (Misaki *et al*., 2003).

In yet another study, the combined detection of *Candida* antigen and antibody for the determination of systemic *Candida* infections was investigated (Bar and Hecker, 2002). One hundred and four patients from an intensive care unit were analyzed in this study. Seventeen of the patients were suspected to have systemic *Candida* infections based on clinical and laboratory criteria. In these patients, *Candida* antigens (Cand-Tec LA assay) and antibodies were analyzed. The sensitivity and specificity were 58.8% and 97.6% for antigenemia, and 52.9% and 85.7% for antibody detection. These values reached 100% and 83.3%, respectively, when the results of both tests were combined, indicating that the combined use of antigen and antibody detection might improve the usefulness of the Cand-Tec test.

Detection of D-arabinitol

Invasive candidiasis can also be diagnosed by detecting a metabolite, D-arabinitol, in serum or urine, produced by most medically important *Candida* species except for *C. krusei* and perhaps *C. glabrata* (Christensson *et al*., 1999). It is uncertain whether the human host produces one or both enantimers of arabinitol or if baseline levels in serum and urine are the result of dietary or microbial arabinitol absorbed by the gut (Wong *et al*., 1990; Christensson *et al*., 1999). Nonetheless, natural host serum arabinitol accumulates during renal insufficiency so that D-arabinitol levels need to be reported as a D-arabinitol/creatinine ratio to compensate for this occurrence (Wong *et al*., 1982).

Switchenko *et al.* (1994) used a semi-automatic enzymatic-colorimetric assay in which NADH-dependent end-products were measured. In an effort to reduce cross-reactions with D-mannitol, these researchers employed a more highly purified arabinitol dehydrogenase enzyme than that used previously. Whereas previous enzymes were derived from bacteria, this enzyme was derived from *C. tropicalis* and was cloned and expressed in *Escherichia coli* (Walsh *et al*., 1994). In this assay, the D-arabinitol is converted to D-ribulose by the recombinant arabinitol dehydrogenase in the presence of NAD. The resultant NADH reduces iodonitrotetrazolium in the presence of diaphorase to form a blue black formazan dye. This dye is detected spectrophotometrically at 500 nm. The authors automated the detection of D-arabinitol by using a conventional COBAS MIRA-S clinical chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). This system simplified the detection method and also allowed many samples to be processed rapidly. The same instrument could also be employed to detect serum creatinine levels which could then be used to normalize readings to compensate for the increased levels of serum arabinitol observed during renal dysfunction.

Walsh *et al.* (1995b) used the enzymatic-colorimetric method in a study of 3223 serum samples from 274 patients with cancer. They found that among patients with candidemia, the mean maximum serum D-arabinitol/creatinine ratio was 11 times greater than that of normal blood bank donors and 4 times greater than that of all patient controls. Patients with persistent candidemia had the highest D-arabinitol/creatinine levels: 83% of 30 patients with persistent candidemia had elevated levels compared to 74% of 42 cases of non-persistent candidemia. Elevated ratios preceded positive blood cultures in up to 50% of the cases. Moreover, serial D-arabinitol/creatinine ratios correlated with therapeutic response in 85% of 34 patients with evaluable cases of candidemia, decreasing in 89% of 9 patients with clearance of candidemia and increasing in 84% of 25 patients with persistent candidemia (Walsh *et al*., 1995b).

More recently, a recombinant *C. albicans* D-arabinitol dehydrogenase has been produced in *E. coli* and purified by dye-ligand affinity chromatography. Whereas the previous enzyme preparations metabolized host D-mannitol as well as arabinitol, the purified *C. albicans* recombinant enzyme only cross-reacts with xylitol (4.9%) among all polyols tested (Yeo *et al*., 2000). The system using the recombinant *C. albicans* enzyme could be automated to measure the initial production rate of NADH spectrofluorometrically in a COBAS FARA II centrifugal autoanalyzer (Roche Diagnostic Systems) and creatinine levels can be measured simultaneously. For 11 patients with invasive candidiasis, the mean D-arabinitol/creatinine ratio was 2.74 μM/mg/dl whereas for healthy controls it was only 1.14 μM/mg/dl (Yeo *et al*., 2000). Hence, the higher throughput and ease of use of the newer methods to detect D-arabinitol in body fluids may allow for more clinical utility of this test. Currently, however, D-arabinitol detection is only conducted in a limited number of research and specialty laboratories.

Detection of (1→3)-β-D-Glucan

The cell walls of *Candida* spp contain (1→3)-β-D-glucan (BDG) as a structural component. As this polysaccharide is not found in bacteria, viruses, or mammals, its presence in the circulation of patients has been used as an indicator of invasive disease. An assay to detect BDG has been developed which utilizes the activation of a clotting pathway found in amoebocyte lysates of the Japanese horseshoe crab, *Tachypleus tridentalis*. This clotting mechanism can be activated by two pathways: one by bacterial endotoxin and the other by fungal cell wall components. Different activating factors can be removed from

the system to make the reaction specific for either pathway. Removal of Factor G from the lysate permits activation only by endotoxin whereas removal of factors B and C permit activation only by BDG.

A test to directly measure plasma BDG was developed using isolated Factor G, the horseshoe crab coagulation factor that is highly sensitive to activation by BDG (Obayashi *et al*., 1992). It is called the Fungitec-G test and is commercially available (Seikagaku Corporation, Tokyo, Japan). The BDG detection kit contains reagents consisting of lyophilized horseshoe crab coagulation Factor G, proclotting enzyme, and the chromogenic substrate, *t*-butyloxycarbonyl-Leu-Gly-Arg-*p*-nitroanilide. This substrate is cleaved by the last step in the proteolytic cascade and can be detected colorimetrically (Obayashi *et al*., 1992; 1995). Patient plasma, derived from heparinized blood, must initially be treated with perchloric acid to precipitate interfering factors before application of the kit reagents (Obayashi *et al*., 1995).

Obayashi *et al.* (1995), in a multi-center study conducted in Japan, used this method to measure the plasma concentration of BDG at the time of routine blood culture performed for 202 febrile episodes. Forty-one febrile episodes were attributed to infections by fungal species including *Candida* spp.. An additional 59 episodes were attributed to Gram-negative or Gram-positive bacterial infections or febrile responses due to drug therapy, and 102 were of unknown origin. Normal plasma concentrations of BDG never exceeded 10 pg/ml and fungal febrile episodes could be differentiated from non-fungal episodes using a cut-off value of 20 pg/ml. Therefore, using a 20 pg/ml cut-off value for positively, 90% of 41 episodes associated with culture or autopsy-confirmed fungal infection were positive. One hundred percent of the episodes associated with non-fungal infection had BDG levels below 20 pg/ml. Of 102 episodes of fever of unknown origin, 25.5% demonstrated elevated BDG levels. If these episodes of fever of unknown origin were to be taken as non-fungal in etiology, the tests had a positive and negative predictive value of 59% and 97%, respectively (Obayashi *et al*., 1995). The highest BDG levels were observed in patients with proven deep mycoses or fungemia. On the other hand, it was speculated that the patients with fever of unknown origin who had elevated BDG levels may, in fact, have had occult deep mycoses. Therefore, 45 patients with neutropenia and fever who were unresponsive to antibacterial therapy were analyzed to determine responsiveness to antifungal therapy. Plasma BDG levels were $\geq$10 pg/ml in 49% and below 10 pg/ml in 51% of the 45 patients. The efficacy of intravenous fluconazole or miconazole therapy was significantly greater in the high BDG group (81.8%) compared with the low BDG group (43.5%) as measured by resolution of fever after 2 weeks of antifungal drug therapy. Therefore BDG levels may be helpful in the discrimination of fungal from non-fungal fevers of unknown origin.

A recent study investigated plasma BDG levels as a means for the selection of surgical patients with *Candida* colonization who might benefit from empiric antifungal therapy (Takesue *et al*., 2004). Fluconazole was administered to postoperative patients demonstrating *Candida* colonization who had risk factors for candidemia and who presented with persistent fever despite prolonged antibacterial therapy. Plasma BDG levels were analyzed to determine if a correlation existed between BDG levels and clinical outcome. Of the 32 patients who were positive for BDG, 47% responded to empiric therapy. In multiple logistic regression analysis, being positive for BDG was a significant factor predicting response to empiric therapy (Takesue *et al*., 2004). Therefore, in surgical patients with *Candida* colonization, assessment of BDG levels may be useful as a guide for the initiation of empiric therapy for suspected candidiasis.

Although the BDG test can not identify which fungus is specifically causing an infection, results can be obtained within 2 h. Such rapidity makes it very attractive as a screening test for invasive infection by common as well as less common fungi, including those for which no other serological test is available (Yoshida *et al*., 1997). Automation of this assay (Tamura *et al*., 1994) will make it more attractive for evaluation in prospective studies and for use in the clinical laboratory.

Patients undergoing hemodialysis with cellulosic membranes, such as cuprammonium rayon, which contain polysaccharides that are shed into the bloodstream during dialysis and patients receiving parenteral infusions of plasma components, such as γ-globulin, which is filtered through cellulose membranes during manufacture may give false positive results by the BDG test (Obayashi *et al*., 1992).

Another more newly created test, the Glucatell test, is available for research purposes only in the U.S. (Associates of Cape Cod, Falmouth, Mass.) and also detects BDG. In this case, amebocytes collected from the hemolymph of *Limulus polyphemus* are washed and lysed. The lysate is processed to remove Factor C making the lysate relatively specific for activation by the fungal glucan pathway. As in the Fungi-Tec test, BDG in the sample activates Factor G, which then activates the proclotting enzyme. The clotting enzyme cleaves p-nitroaniline (pNA) from the chromogenic peptide substrate (Boc-Leu-Gly-Arg-pNA). The free pNA is measured at 405 nm (kinetic assay) or, alternatively, the pNA is diazotized to form a compound that absorbs at 540-550 nm (end-point assay). The Glucatell test detected BDG in the sera of patients with positive blood cultures for *Candida* species with a sensitivity and specificity of more than 93% (Saeki *et al*., 2001). In a study by Odabasi *et al*. (2002), BDG was detected by the Glucatell test in the serum of 100 leukemic patients undergoing chemotherapy

for invasive fungal infections, including candidiasis. Circulating BDG levels were found to correlate with invasive fungal infection in these patients. Additional large scale studies are required to fully evaluate the utility of the Glucatell test for the diagnosis of invasive candidiasis and to determine its susceptibility to non-specific activation by environmental contaminants.

Molecular biological identification using clinical material

Identification of *Candida* spp. by culture requires the presence of viable organisms in blood or body fluids. In addition, several days may be required for blood cultures to become positive and, for non-*albicans* spp. of *Candida*, additional subculturing is required to obtain pure cultures for use in subsequent phenotypic identification systems. Molecular methods have been applied directly to clinical materials or the contents of blood culture bottles to circumvent the time required for culture and subculture and to increase the sensitivity of detection. Many of the methods described for the molecular identification of *Candida* species in pure culture have also been applied to the direct detection of organisms from clinical materials but with the required addition of more sophisticated DNA extraction methods to remove PCR inhibitors often present in blood culture media or in host tissues or body fluids. Clinical materials such as whole blood, blood culture bottles, spiked whole blood, sera, urine, pus, and tissue samples, to name a few, have been used for the molecular identification of infecting *Candida* species.

In addition to reducing the time required for *Candida* species identification, the detection of fungal DNA directly from clinical materials offers several other advantages compared to conventional methods. First, fungal DNA can be amplified a million-fold or more using PCR methodology. In addition, DNA derived from dead as well as from living fungal cells can be amplified in this system. Therefore, for both of these reasons, a PCR-based assay should be more sensitive than culture. Not only should such amplification be more sensitive than culture, it should be more rapid than culture or even antibody detection assays (i.e., requiring only a few hours compared to days for conventional culture or weeks for antibody production and detection). Use of specific oligonucleotide probes can provide identification of an organism to the species level and can theoretically detect mixed fungal infections accurately.

The earliest reports describing PCR amplification of *Candida* spp. DNA derived from blood or clinical specimens used elaborate multi-step processes for DNA isolation and purification including lysis of host erythrocytes and leukocytes with salts, detergents and/or proteinase K, or pre-treatment with DNAses to remove host DNA, spheroplast production with zymolyase, RNase digestion, precipitation of proteins with sodium or potassium acetate, and multiple purification steps using hazardous chemicals such as phenol and/or chloroform before precipitation of target DNA with ethanol (Buchman *et al*., 1990; Fujita *et al*., 1995). Such methods were quite effective in recovering sufficiently pure DNA for amplification and detection. For example, Fujita *et al*. (1995) could detect as few as 2 *C. albicans* cells per 200 μl of spiked whole blood. Einsele *et al*. (1997) added an incubation with 50 mM NaOH at 95°C before zymolyase treatment and subsequently did not require the use of phenol-chloroform extraction of DNA before PCR amplification. Using this method, a detection limit of 1 to 10 fg of fungal DNA (1 cfu per ml of blood) with a sensitivity of 100% was reported for patients with invasive infections. Positive PCR preceded radiological signs by a median of 4 days for 12 of 17 patients with hepatosplenic candidiasis. Flahaut *et al*. (1998) further simplified the isolation and purification of *C. albicans* DNA from whole blood by modifying the use of a commercial kit (QIAmp Tissue Kit, Qiagen AG, Basel, Switzerland) for purifying DNA after the lysis of erythrocytes. Proteinase K and lysis buffers from the kit were applied and DNA was purified using silica-based spin columns. The limit of sensitivity from clinical specimens was 20 cfu/ml after amplification using a single copy *SAP* gene target. Colorimetric detection in an ELISA format was as sensitive as Southern blotting and detected 31 of 31 positive blood cultures (100% sensitive compared to culture) after DNA purification and amplification by this method.

Going one step further, Shin *et al*. (1997) isolated *Candida* species DNA directly from blood culture bottles that had become positive for growth in a BacT/ALERT blood culturing system. A natural amplification of target DNA occurred as yeasts were allowed to grow in blood culture bottles so that a very rapid mechanical disruption method, which did not require expensive enzymes or phenol-chloroform extraction, could be used to obtain *Candida* spp. DNA. Universal fungal PCR primers were used to amplify the DNA and species-specific oligonucleotide probes were then used to identify and differentiate *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* in less than one day in a PCR-EIA format. The limit of sensitivity for this system was 500 cfu per ml which was sufficient to detect 100% of the positive blood cultures.

Iwen *et al*. (2004) used a similar approach to compare a PCR assay with the capacity of the ESP blood culture bottle system (ESP system; TREK Diagnostic Systems, Inc., USA) to detect fungi including *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. lusitaniae* in positive blood culture bottles. The PCR assay was performed using fungus-specific primers that amplified the complete ITS1 and ITS2 regions of the rDNA complex (ITS-PCR). Prior to amplification, the extracted DNA was diluted to prevent inhibitors in the blood-broth samples from interfering with the PCR assay.

ITS-PCR was negative for all 183 samples that were negative by culture while it was positive for all 27 samples from blood culture-positive bottles giving a sensitivity and specificity of 100% compared to culture. Although the sensitivity limit was no higher than that for culture in blood bottles alone, the time to specific species identification was significantly reduced.

Newer real time PCR assays such as TaqMan (Perkin Elmer) and the Light Cycler (Roche Molecular Systems) require no post-amplification manipulations and can potentially be automated for all steps from DNA extraction to final PCR amplicon detection and quantitation. The TaqMan system was used by Shin *et al.* (1999) to identify *Candida* species from positive blood culture bottles. Universal fungal primers were used to amplify the ITS2 rDNA from all *Candida* species and then species-specific probes to this region, labeled with one of three fluorescent reporter dyes, were used to differentiate among *Candida* species. Each dye emitted a characteristic wavelength allowing up to three *Candida* species to be detected in a single reaction tube. Probes correctly detected and identified 95.1% of 61 *Candida* species recovered from blood culture bottles, including those culture positive for *C. albicans, C. parapsilosis, C. glabrata, C. krusei,* and *C. tropicalis*. No false positive results were obtained from bottles with no growth or from patients with bacteremias. This assay detected and identified *Candida* to the species level in less than one day and did not require post-PCR hybridization and incubation steps. It was specific for the detection and identification of *Candida* species from blood culture bottles, including those containing mixtures of *Candida* species (*C. albicans* and *C. glabrata*) that were overlooked by conventional culture methods due to overgrowth of culture plates with coagulase-negative *Staphylococcus*.

Another "real time" PCR system is the LightCycler (Roche Molecular Systems) which allows rapid amplification of DNA in glass capillaries and simultaneous fluorescent detection of amplicons using fluorescence energy transfer or "FRET" (Loeffler *et al*., 2000b; Schmidt *et al*., 2001). Addition of a commercial DNA extraction system to the Light Cycler detection format (MagNA Pure, Roche Molecular Biochemicals, Indianapolis, Ind.), applied after lysis and removal of erythrocytes and disruption of *C. albicans* cells with glass beads, allowed a sensitivity limit of 1 CFU per ml of whole blood (Schmidt *et al*., 2001). A recent study by Selvarangan *et al.* (2003) reported the use of the Light Cycler format to identify DNA from six *Candida* species. Target sequences in the ITS2 regions of the rRNA gene were simultaneously amplified and labeled with fluorescent dyes to identify *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. lusitaniae*. The first four species were identified in a single reaction in glass capillaries using two fluorescent hybridization probe sets whereas *C. krusei* and *C. lusitaniae* were detected in a second reaction, also using two probe sets. The assay was validated with DNA extracted from 62 BACTEC blood culture bottles positive for yeasts and was 100% concordant with culture identification results based on biochemical and morphological features.

Amplification of RNA instead of DNA has also been evaluated for the identification of *Candida* spp. from clinical specimens. Nucleic Acid Sequence Based Amplification (NASBA), a non-PCR-based amplification method, was evaluated for detection of RNA from 6 different *Candida* spp. (*C. krusei, C. glabrata, C. inconspicua, C. dubliniensis, C. norvegensis*, and *C. lusitaniae*) and compared to a PCR assay which targeted the 18S rDNA region (Loeffler *et al*., 2003). Eleven patients were screened weekly after allogeneic stem cell transplantation for the presence of *Candida* spp. nucleic acid by both assays. All 5 patients culture positive for *Candida* species gave positive results by both methods. The NASBA assay detected 1 CFU using a 100 μl sample compared to 5 mL for PCR and, in contrast to PCR, NASBA is an isothermal amplification process which does not require thermal cycling for nucleic acid amplification. Therefore, the use of NASBA may have some advantages compared to PCR.

Given the limitations of some of the conventional diagnostic methods for invasive candidiasis, DNA and RNA based methods hold promise for improved sensitivity and specificity. However, most molecular tests to date have not shown improved sensitivity compared to blood culturing methods; the most valuable contribution made by molecular methods has been the significantly more rapid identification of *Candida* spp.. In addition, molecular detection methods lack standardized and commercially available DNA processing systems. Many different DNA extraction kits such as the QIAmp Tissue kit, GeneReleaser, Puregene, Dynabeads DNA, DNAzol and MagNAPure are now commercially available which may help to resolve these issues. However, in all cases, a lysis step to release fungal DNA is required before DNA purification and amplification can occur. Methods concerning the selection of the target region to be amplified, subsequent primer design, post-PCR detection methods to identify the amplified product, as well as the PCR conditions and format used (single step, multiplex, or nested PCR), all need to be standardized. Contamination can occur during PCR assays and precautions to minimize false-positive results (Kwok and Higuichi, 1989) also need to be considered before any system can be commercialized and used in a clinical laboratory.

References

Abi-Said, D., E. Anaissie, O. Uzun, I. Raad, H. Pinzcowski, and S. Vartivarian. 1997. The epidemiology of hematogenous candidiasis caused by different *Candida* species. *Clin Infect Dis*. 24, 1122-1128. Erratum in: *Clin. Infect. Dis*. 25, 352.

Al Mosaid, A., J. Sullivan, and D.C. Coleman. 2003. Differentiation of *Candida dubliniensis* from *Candida albicans* on Pal's agar. *J Clin. Microbiol*. 41, 4787-4789.

Araj, G.F., R.L. Hopfer, S. Chesnut, V. Fainstein, and G.P. Bodey GP. 1982. Diagnostic value of the enzyme-linked immunosorbent assay for detection of *Candida albicans* cytoplasmic antigen in sera of cancer patients. *J Clin. Microbiol*. 16, 46-52.

Archibald, L.K., L.C. McDonald, R.M. Addison, C. McKnight, T. Byrne, H. Dobbie, O. Nwanyanwu, P. Kazembe, L.B. Reller, and W.R. Jarvis. 2000. Comparison of BACTEC MYCO/F Lytic and WAMPOLE ISOLATOR 10 (lysis-centrifugation) systems for detection of bacteremia, mycobacteremia, and fungemia in a developing country. *J Clin. Microbiol*. 38, 2994-2997.

Bailey, J.W., E. Sada, C. Brass, and J.E. Bennett. 1985. Diagnosis of systemic candidiasis by latex agglutination for serum antigen. *J Clin. Microbiol*. 21, 749-752.

Banerjee, S.N., T.G. Emori, D.H. Culver, R.P. Gaynes, W.R. Jarvis, T. Horan, J.R. Edwards, J. Tolson, T. Henderson, and W.J. Martone. 1991. Secular trends in nosocomial primary bloodstream infections in the United States, 1980-1989. National Nosocomial Infections Surveillance System. *Am. J Med*. 91, 86S-89S.

Bar, W., and H. Hecker. 2002. Diagnosis of systemic *Candida* infections in patients of the intensive care unit. Significance of serum antigens and antibodies. *Mycoses*. 45, 22-28.

Baumgartner, C., A.M. Freydiere, and Y. Gille. 1996. Direct identification and recognition of yeast species from clinical material by using Albicans ID and CHROMagar Candida plates. *J Clin. Microbiol*. 34, 454-456.

Bautista-Munoz, C., X.M. Boldo, L. Villa-Tanaca, and C. Hernandez-Rodriguez. 2003. Identification of *Candida* spp. by randomly amplified polymorphic DNA analysis and differentiation between *Candida albicans* and *Candida dubliniensis* by direct PCR methods. *J Clin. Microbiol*. 41, 414-420.

Berenguer, J., M. Buck, F. Witebsky, F. Stock, P.A. Pizzo, and T.J. Walsh. 1993. Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis. *Diagn. Microbiol. Infect. Dis*. 17, 103-109.

Bernal, S., E. Martin Mazuelos, M. Chavez, J. Coronilla, and A. Valverde. 1998. Evaluation of the new API *Candida* system for identification of the most clinically important yeast species. *Diagn. Microbiol. Infect. Dis*. 32, 217-221.

Bikandi, J., R.S. Millan, M.D. Moragues, G. Cebas, M. Clarke, D.C. Coleman, D.J. Sullivan, G. Quindos, and J. Ponton. 1998. Rapid identification of *Candida dubliniensis* by indirect immunofluorescence based on differential localization of antigens on *C. dubliniensis* blastospores and *Candida albicans* germ tubes. *J Clin. Microbiol*. 36, 2428-2433.

Borst, A., B. Theelen, E. Reinders, T. Boekhout, A.C. Fluit, and P.H. Savelkoul. 2003. Use of amplified fragment length polymorphism analysis to identify medically important *Candida* spp., including *C. dubliniensis*. *J Clin. Microbiol*. 41, 1357-1362.

Boucher, H., S. Mercure, S. Montplaisir, and G. Lemay. 1996. A novel group I intron in *Candida dubliniensis* is homologous to a *Candida albicans* intron. *Gene*. 180, 189-196.

Bougnoux, M.E., C. Hill, D. Moissenet, M. Feuilhade de Chauvin, M. Bonnay, I. Vicens-Sprauel, F. Pietri, M. McNeil, L. Kaufman, and J. Dupouy-Camet. 1990. Comparison of antibody, antigen, and metabolite assays for hospitalized patients with disseminated or peripheral candidiasis. *J Clin. Microbiol*. 28, 905-909.

Buchman, T.G., M. Rossier, W.G. Merz, and P. Charache. 1990. Detection of surgical pathogens by *in vitro* DNA amplification. Part I. Rapid identification of *Candida albicans* by *in vitro* amplification of a fungus-specific gene. *Surgery*. 108, 338-347.

Burgener-Kairuz, P., J.P. Zuber, P. Jaunin, T.G. Buchman, J. Bille, and M. Rossier. 1994. Rapid detection and identification of *Candida albicans* and *Torulopsis* (*Candida*) *glabrata* in clinical specimens by species-specific nested PCR amplification of a cytochrome P-450 lanosterol-a-demethylase (L1A1) gene fragment. *J Clin. Microbiol*. 32, 1902-1907.

Burnie, J.P. 1985. A reverse passive latex agglutination test for the diagnosis of systemic candidiasis. *J Immunol. Methods*. 82, 267-280.

Campbell, C.K., K.G. Davey, A.D. Holmes, A. Szekely, and D.W. Warnock. 1999. Comparison of the API *Candida* system with the AUXACOLOR system for identification of common yeast pathogens. *J Clin. Microbiol*. 37, 821-823.

Centers for Disease Control and Prevention. 2000. Monitoring hospital acquired infections to promote patient safety in the United States, 1990-1999. *Morb. Mortal Wkly Rep*. 49, 149-153.

Chen, Y.C., J.D. Eisner, M.M. Kattar, S.L. Rassoulian-Barrett, K. LaFe, S.L. Yarfitz, A.P. Limaye, and B.T. Cookson. 2000. Identification of medically important yeasts using PCR-based detection of DNA sequence polymorphisms in the internal transcribed spacer 2 region of the rRNA genes. *J Clin. Microbiol*. 38, 2302-2310.

Christensson, B., G. Sigmundsdottir, and L. Larsson. 1999. D-arabinitol - a marker for invasive candidiasis. *Med. Mycol*. 37, 391-396.

Cirak, M.Y., A. Kalkanci, and S. Kustimur. 2003. Use of molecular methods in identification of *Candida* species and evaluation of fluconazole resistance. *Mem. Inst. Oswaldo. Cruz*. 98, 1027-1032.

Coignard, C., S.F. Hurst, L.E. Benjamin, M.E. Brandt, D.W. Warnock, and C.J. Morrison. 2004. Resolution of discrepant results for *Candida* species identification by using DNA probes. *J Clin. Microbiol*. 42, 858-861.

Crampin, A.C. and R.C. Matthews. 1993. Application of the polymerase chain reaction to the diagnosis of candidosis by amplification of an HSP 90 gene fragment. *J Med. Microbiol*. 39, 233-238.

Creger, R.J., K.E. Weeman, M.R. Jacobs, A. Morrissey, P. Parker, R.M. Fox, and H.M. Lazarus. 1998. Lack of utility of the lysis-centrifugation blood culture method for detection of fungemia in immunocompromised cancer patients. *J Clin. Microbiol*. 36, 290-293.

Davey, K.G., P.M. Chant, C.S. Downer, C.K. Campbell, and D.W. Warnock. 1995. Evaluation of the AUXACOLOR system, a new method of clinical yeast identification. *J Clin. Pathol*. 48, 807-809.

De Caux, B.S., D. Thomas, P.J. Stephens, and C. Booth. 2004. Clinical evaluation of a new selective and differential medium for the rapid isolation and presumptive identification of *Candida* species. Abstr. C-104, p. 141. Abstr. 104th Annu. Meet. Am. Soc. Microbiol.

Denning, D.W., C.C. Kibbler, and R.A. Barnes. 2003. British Society for Medical Mycology proposed standards of care for patients with invasive fungal infections. *Lancet Infect. Dis*. 3,

230-240.

De Repentigny, L., L.D. Marr, J.W. Keller, A.W. Carter, R.J. Kuykendall, L. Kaufman, and E. Reiss. 1985. Comparison of enzyme immunoassay and gas-liquid chromatography for the rapid diagnosis of invasive candidiasis in cancer patients. *J Clin. Microbiol*. 21, 972-979.

Doern, G.V., A. Barton, and S. Rao. 1998. Controlled comparative evaluation of BacT/Alert FAN and ESP 80A aerobic media as means for detecting bacteremia and fungemia. *J Clin. Microbial*. 36, 2686-2689.

Donnelly, S.M., D.J. Sullivan, D.B. Shanley, and D.C. Coleman. 1999. Phylogenetic analysis and rapid identification of *Candida dubliniensis* based on analysis of *ACT1* intron and exon sequences. *Microbiol*. 145, 1871-1882.

Edmond, M.B., S.E. Wallace, D.K. McClish, M.A. Pfaller, R.N. Jones, and R.P. Wenzel. 1999. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin. Infect. Dis*. 29, 239-244.

Edwards Jr, J.E. 1992. Should all patients with candidemia be treated with antifungal agents? *Clin. Infect. Dis*. 15, 4222-4223.

Einsele, H., H. Hebart, G. Roller, J. Loffler, I. Rothenhofer, C.A. Muller, R.A. Bowden, J. van Burik, D. Engelhard, L. Kanz, and U. Schumacher. 1997. Detection and identification of fungal pathogens in blood by using molecular probes. *J Clin. Microbiol*. 5, 1353-1360.

Elie, C.M., T.J. Lott, E. Reiss, and C.J. Morrison. 1998. Rapid identification of *Candida* species using species-specific DNA probes. *J Clin. Microbiol*. 36, 3260-3265.

Ellepola, A.N., S.F. Hurst, C.M. Elie, and C.J. Morrison. 2003. Rapid and unequivocal differentiation of *Candida dubliniensis* from other *Candida* species using species-specific DNA probes: comparison with phenotypic identification methods. *Oral. Microbiol. Immunol*. 18, 379-388.

El Moudni, B., M.H. Rodier, G. Daniault, and J.L. Jacquemin. 1998. Improved immunodiagnosis of human candidiasis by an enzyme-linked immunosorbent assay using a *Candida albicans* 52-kilodalton metallopeptidase. *Clin. Diagn. Lab. Immunol*. 5, 823-825.

Espinel-Ingroff, A., L. Stockman, G. Roberts, D. Pincus, J. Pollack, and J. Marler. 1998. Comparison of RapID Yeast Plus system with API 20C system for identification of common, new, and emerging yeast pathogens. *J Clin. Microbiol*. 36, 883-886.

Flahaut, M., D. Sanglard, M. Monod, J. Bille, and M. Rossier. 1998. Rapid detection of *Candida albicans* in clinical samples by DNA amplification of common regions from *C. albicans*-secreted aspartic proteinase genes. *J Clin. Microbiol*. 36, 395-401.

Freydiere, A.M. 1996. Evaluation of CHROMagar Candida plates. *J Clin. Microbiol*. 34, 2048.

Freydiere, A.M., L. Buchaille, and Y. Gille. 1997a. Comparison of three media for direct identification and discrimination of *Candida* species in clinical specimens. *Eur. J Clin. Microbiol. Infect. Dis*. 16, 464-467.

Freydiere, A.M., L. Buchaille, R. Guinet, and Y. Gille. 1997b. Evaluation of latex reagents for rapid identification of *Candida albicans* and *C. krusei* colonies. *J Clin. Microbiol*. 35, 877-880.

Freydiere, A.M., R. Guinet, and P. Boiron. 2001. Yeast identification in the clinical microbiology laboratory: phenotypical methods. *Med. Mycol*. 39, 9-33.

Fujita, S., F. Matsubara, and T. Matsuda. 1986. Enzyme-linked immunosorbent assay measurement of fluctuations in antibody titer and antigenemia in cancer patients with and without candidiasis. *J Clin. Microbiol*. 23, 568-575.

Fujita, S.I., B.A. Lasker, T.J. Lott, E. Reiss, and C.J. Morrison. 1995. Microtitration plate enzyme immunoassay to detect PCR-amplified DNA from *Candida* species in blood. *J Clin. Microbiol*. 33, 962-967.

Fujita, S.I. and T. Hashimoto. 1992. Detection of serum *Candida* antigens by enzyme-linked immunosorbent assay and a latex agglutination test with anti-*Candida albicans* and anti-*Candida krusei* antibodies. *J Clin. Microbiol*. 30, 3131-3137.

Fuller, D.D., T.E. Davis Jr, G.A. Denys, and M.K. York. 2001. Evaluation of BACTEC MYCO/F Lytic medium for recovery of mycobacteria, fungi, and bacteria from blood. *J Clin. Microbiol*. 39, 2933-2936.

Gentry, L.O., I.D. Wilkinson, A.S. Lea, and M.F. Price. 1983. Latex agglutination test for detection of *Candida* antigen in patients with disseminated disease. *Eur J Clin. Microbiol. Infect. Dis*. 3, 122-128.

Graf, B., A. Trost, J. Eucker, U.B. Gobel, and T. Adam. 2004. Rapid and simple differentiation of *C. dubliniensis* from *C. albicans*. *Diagn. Microbiol. Infect. Dis*. 48, 149-151.

Greenfield, R.A., M.J. Bussey, J.L Stephens, and J.M. Jones. 1983. Serial enzyme-linked immunosorbent assay for antibody to *Candida* antigens during induction chemotherapy for acute leukemia. *J Infect. Dis*. 148, 275-283.

Greenfield, R.A. and J.M. Jones. 1981. Purification and characterization of a major cytoplasmic antigen of *Candida albicans*. *Infect. Immun*. 34, 469-477.

Gudlaugsson, O., S. Gillespie, K. Lee, J. Vande Berg, J. Hu, S. Messer, L. Herwaldt, M. Pfaller, and D. Diekema. 2003. Attributable mortality of nosocomial candidemia, revisited. *Clin. Infect Dis*. 37, 1172-1177.

Guiver, M., K. Levi, and B.A. Oppenheim. 2001. Rapid identification of *Candida* species by TaqMan PCR. *J Clin. Pathol*. 54, 362-366.

Hajjeh, R.A., A.N. Sofair, L.H. Harrison, G.M. Lyon, B.A. Arthington-Skaggs, S.A. Mirza, M. Phelan, J. Morgan, W. Lee-Yang, M.A. Ciblak, L.E. Benjamin, L.T. Sanza, S. Huie, S.F. Yeo, M.E. Brandt, and D.W. Warnock. 2004. Incidence of bloodstream infections due to *Candida* species and *in vitro* susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. *J Clin. Microbiol*. 42, 1519-1527.

Hall, L., S. Wohlfiel, and G.D. Roberts. 2003. Experience with the MicroSeq D2 large-subunit ribosomal DNA sequencing kit for identification of commonly encountered, clinically important yeast species. *J Clin. Microbiol*. 41, 5099-5102.

Haynes, K.A., T.J. Westerneng, J.W. Fell, and W. Moens. 1995. Rapid detection and identification of pathogenic fungi by polymerase chain reaction amplification of large subunit ribosomal DNA. *J Med. Vet. Mycol*. 33, 319-325.

Heelan, J.S., E. Sotomayor, K. Coon, and J.B. D'Arezzo. 1998. Comparison of the rapid yeast plus panel with the API20C yeast system for the identification of clinically significant isolates of *Candida* species. *J Clin. Microbiol*. 36, 1443-1445.

Holmes, A.R., R.D. Cannon, M.G. Shepherd, and H.F. Jenkinson. 1994. Detection of *Candida albicans* and other yeasts in blood by PCR. *J Clin. Microbiol*. 32, 228-231.

Hopfer, R.L., P. Walden, S. Setterquist, and W.E. Highsmith. 1993.

Detection and differentiation of fungi in clinical specimens using polymerase chain reaction (PCR) amplification and restriction enzyme analysis. *J Med. Vet. Mycol*. 31, 65-75.

Hoppe, J.E. and P. Frey. 1999. Evaluation of six commercial tests and the germ tube test for presumptive identification of *Candida albicans*. *Eur. J Clin. Microbiol. Infect. Dis*. 18, 188-191.

Horvath, L.L., B.J. George, C.K. Murray, L.S. Harrison, and D.R. Hospenthal. 2004. Direct comparison of the BACTEC 9240 and BacT/ALERT 3D automated blood culture systems for *Candida* growth detection. *J Clin. Microbiol*. 42, 115-118.

Hsu, M.C., K.W. Chen, H.J. Lo, Y.C. Chen, M.H. Liao, Y.H. Lin, and S.Y. Li. 2003. Species identification of medically important fungi by use of real-time LightCycler PCR. *J Med. Microbiol*. 52, 1071-1076.

Hube, B. 1996. *Candida albicans* secreted aspartyl proteinases. *Curr. Top. Med. Mycol*. 7, 55-69.

Hui, M., M. Ip, P.K. Chan, M.L. Chin, and A.F. Cheng. 2000. Rapid identification of medically important *Candida* to species level by polymerase chain reaction and single-strand conformational polymorphism. *Diagn. Microbiol. Infect. Dis*. 38, 95-99.

Irobi, J., A. Schoofs, and H. Goossens. 1999. Genetic identification of *Candida* species in HIV-positive patients using the polymerase chain reaction and restriction fragment length polymorphism analysis of its DNA. *Mol. Cell. Probes*. 13, 401-406.

Iwasaki, H., H. Misaki, T. Nakamura, and T. Ueda. 2000. Surveillance of the serum *Candida* antigen titer for initiation of antifungal therapy after post-remission chemotherapy in patients with acute leukemia. *Intl. J Hematol*. 71, 266-272.

Iwen, P.C., A.G. Freifeld, T.A. Bruening, and S.H. Hinrichs. 2004. Use of a panfungal PCR assay for detection of fungal pathogens in a commercial blood culture system. *J Clin. Microbiol*. 42, 2292-2293.

Jabra-Rizk, M.A., T.M. Brenner, M. Romagnoli, A.A. Baqui, W.G. Merz, W.A. Falkler Jr, and T.F. Meiller. 2001. Evaluation of a reformulated CHROMagar Candida. *J Clin. Microbiol*. 39, 2015-2016.

Jaeger, E.E., N.M. Carroll, S. Choudhury, A.A. Dunlop, H.M. Towler, M.M. Matheson, P. Adamson, N. Okhravi, and S. Lightman. 2000. Rapid detection and identification of *Candida, Aspergillus*, and *Fusarium* species in ocular samples using nested PCR. *J Clin. Microbiol*. 38, 2902-2908.

Jones, J.M. 1980a. Kinetics of antibody responses to cell wall mannan and a major cytoplasmic antigen of *Candida albicans* in rabbits and humans. *J Lab. Clin. Med*. 96, 845-860.

Jones, J.M. 1980b. Quantitation of antibody against cell wall mannan and a major cytoplasmic antigen of *Candida* in rabbits, mice, and humans. *Infect. Immun*. 30, 78-89.

Jordan, J. 1994. PCR identification of four medically important *Candida* species by using a single primer pair. *J Clin. Microbiol*. 32, 2962-2967.

Kahn, F.W. and J.M. Jones. 1986. Latex agglutination tests for detection of *Candida* antigens in sera of patients with invasive candidiasis. *J Infect. Dis*. 153, 579-585.

Kaufman, L., J.A. Kovacs, and E. Reiss. 1997. Clinical Immunomycology, p. 585-604. *In* N.R. Rose, E.C. de Macario, J.D. Folds, H.C. Lane, and R.M. Nakamura (eds.), Manual of Clinical Laboratory Immunology. ASM Press, Washington, D.C.

Kitch, T.T., M.R. Jacobs, M.R. McGinnis, and P.C. Appelbaum. 1996. Ability of RapID Yeast Plus System to identify 304 clinically significant yeasts within 5 hours. *J Clin. Microbiol*. 34, 1069-1071.

Kurtzman, C.P. and C.J. Robnett. 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. *J Clin. Microbiol*. 35, 1216-1223.

Kurzai, O., W.J. Heinz, D.J. Sullivan, D.C. Coleman, M. Frosch, and F.A. Muhlschlegel. 1999. Rapid PCR test for discriminating between *Candida albicans* and *Candida dubliniensis* isolates using primers derived from the pH-regulated *PHR1* and *PHR2* genes of *C. albicans*. *J Clin. Microbiol*. 37, 1587-1590.

Kwok, S. and R. Higuchi. 1989. Avoiding false positives with PCR. *Nature* 339, 237-238.

Kwon-Chung, K.J., D. Lehman, C. Good, and P.T. McGee. 1985. Genetic evidence for role of extracellular proteinase in virulence of *Candida albicans*. *Infect. Immun*. 49, 571-575.

Land, G.A., I.F. Salkin, M. El-Zaatari, M.R. McGinnis, and G. Hashem. 1991. Evaluation of the Baxter MicroScan 4-hour enzyme-based yeast identification system. *J Clin. Microbiol*. 29, 718-722.

Lecciones, J.A., J.W. Lee, E.E. Navarro, F.G. Witebsky, D. Marshall, S.M. Steinberg, P.A. Pizzo, and T.J. Walsh. 1992. Vascular catheter-associated fungemia in patients with cancer: analysis of 155 episodes. *Clin. Infect. Dis*. 14, 875-883.

Lemieux, C., G. St-Germain, J. Vincelette, L. Kaufman, and L. de Repentigny. 1990. Collaborative evaluation of antigen detection by a commercial latex agglutination test and enzyme immunoassay in the diagnosis of invasive candidiasis. *J Clin. Microbiol*. 28, 249-253.

Lischewski, A., M. Kretschman, H. Hof, R. Amann, J. Hacker, and J. Morschhauser. 1997. Detection and identification of *Candida* species in experimentally infected tissue and human blood by rRNA-specific fluorescent in situ hybridization. *J Clin. Microbiol*. 35, 2943-2948.

Loeffler, J., C. Dorn, H. Hebart, P. Cox, S. Magga, and H. Einsele. 2003. Development and evaluation of the nuclisens basic kit NASBA for the detection of RNA from *Candida* species frequently resistant to antifungal drugs. *Diagn. Microbiol. Infect. Dis*. 45, 217-220.

Loeffler, J., H. Hebart, S. Magga, D. Schmidt, L. Klingspor, J. Tollemar, U. Schumacher, and H. Einsele. 2000a. Identification of rare *Candida* species and other yeasts by polymerase chain reaction and slot blot hybridization. *Diagn. Microbiol. Infect. Dis*. 38, 207-212.

Loeffler, J., H. Hebart, S. Sepe, U. Schumacher, T. Klingbiel, and J. Einsele. 1998. Detection of PCR-amplified fungal DNA by using a PCR-ELISA system. *Med. Mycol*. 36, 275-279.

Loeffler, J., N. Henke, H. Hebart, D. Schmidt, L Hagmeyer, U. Schumacher, and H. Einsele H. 2000b. Quantification of fungal DNA by using fluorescence resonance energy transfer and the light cycler system. *J Clin. Microbiol*. 38, 586-590.

MacDonald, F. and F.C. Odds. 1983. Virulence for mice of a proteinase-secreting strain of *Candida albicans* and a proteinase-deficient mutant. *J Gen. Microbiol*. 129, 431-438.

Mannarelli, B.M. and C.P. Kurtzman. 1998. Rapid identification of *Candida albicans* and other human pathogenic yeasts by using short oligonucleotides in a PCR. *J Clin. Microbiol*. 36, 1634-1641.

Marcilla, A., C. Monteagudo, S. Mormeneo, and R. Sentandreu. 1999. Monoclonal antibody 3H8: a useful tool in the diagnosis of candidiasis. *Microbiol*. 145, 695-701.

Martin. C., D. Roberts, M. van Der Weide, R. Rossau, G. Jannes, T. Smith, and M. Maher. 2000. Development of a PCR-based line probe assay for identification of fungal pathogens. *J Clin. Microbiol*. 38, 3735-3742.

Mason, A.B., M.E. Brandt, and H.R. Buckley. 1989. Enolase activity associated with a *Candida albicans* cytoplasmic antigen. *Yeast* 5, S231-239.

Massonet, C., J. Van Eldere, M. Vaneechoutte, T. De Baere, J. Verhaegen, and K. Lagrou. 2004. Comparison of VITEK 2 with ITS2-fragment length polymorphism analysis for identification of yeast species. *J Clin. Microbiol*. 42, 2209-2211.

Matthews, R.J. and J.P. Burnie. 1988. Diagnosis of systemic candidiasis by an enzyme-linked dot immunobinding assay for a circulating immunodominant 47-kilodalton antigen. *J Clin. Microbiol*. 26, 459-463.

Matthews, R.J., J.P. Burnie, and S. Tabaqchali. 1987. Isolation of immunodominant antigens from sera of patients with systemic candidiasis and characterization of serological response to *Candida albicans*. *J Clin. Microbiol*. 25, 230-237.

McDonald, L.C., M.P. Weinstein, J. Fune, S. Mirrett, L.G. Reimer, and L.B. Reller. 2001. Controlled comparison of BacT/ALERT FAN aerobic medium and BACTEC fungal blood culture medium for detection of fungemia. *J Clin. Microbiol*. 39, 622-624.

Misaki, H., H. Iwasaki, and T. Ueda. 2003. A comparison of the specificity and sensitivity of two *Candida* antigen assay systems for the diagnosis of deep candidiasis in patients with hematologic diseases. *Med. Sci. Monit*. 9, MT1-7.

Miyakawa, Y., T. Mabuchi, and Y. Fukazawa. 1993. New method for detection of *Candida albicans* in human blood by polymerase chain reaction. *J Clin. Microbiol*. 31, 3344-3347.

Moghaddas, J., A.L. Truant, C. Jordan, and H.R. Buckley. 1999. Evaluation of the RapID Yeast Plus System for the identification of yeast. *Diagn. Microbiol. Infect. Dis*. 35, 271-273.

Morace, G., M. Sanguinetti, B. Posteraro, G. LoCascio, and G. Fadda. 1997. Identification of various medically important *Candida* species in clinical specimens by PCR-restriction enzyme analysis. *J Clin. Microbiol*. 35, 667-672.

Moran, G.P., D. Sanglard, S.M. Donnelly, D.B. Shanley, D.J. Sullivan, and D.C. Coleman. 1998. Identification and expression of multidrug transporters responsible for fluconazole resistance in *Candida dubliniensis*. *Antimicrob. Agents Chemother*. 42, 1819-1830.

Moran, G.P., D.J. Sullivan, M.C. Henman, C.E. McCreary, B.J. Harrington, D.B. Shanley, and D.C. Coleman. 1997. Antifungal drug susceptibilities of oral *Candida dubliniensis* isolates from human immunodeficiency virus (HIV)-infected and non-HIV-infected subjects and generation of stable fluconazole-resistant derivatives *in vitro*. *Antimicrob. Agents Chemother*. 41, 617-623.

Morrison, C.J., S.F. Hurst, and E. Reiss. 2003. Competitive binding inhibition enzyme-linked immunosorbent assay that uses the secreted aspartyl proteinase of *Candida albicans* as an antigenic marker for diagnosis of disseminated candidiasis. *Clin. Diagn. Lab. Immunol*. 10, 835-848.

Na, B.K. and C.Y. Song. 1999. Use of monoclonal antibody in diagnosis of candidiasis caused by *Candida albicans*: detection of circulating aspartyl proteinase antigen. *Clin. Diagn. Lab. Immunol*. 6, 924-929.

Nguyen, M.H, J.E. Peacock Jr, A.J. Morris, D.C. Tanner, M.L. Nguyen, D.R. Snydman, M.M. Wagener, M.G. Rinaldi, and V.L. Yu. 1996. The changing face of candidemia: emergence of non-*Candida* albicans species and antifungal resistance. *Am J Med*. 100, 617-623.

Obayashi, T., M. Yoshida, T. Mori, H. Goto, A. Yasuoka, H. Iwasaki, H. Teshima, S. Kohno, A. Horiuchi, and A. Ito. 1995. Plasma (1→3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet*. 345, 17-20.

Obayashi, T., M. Yoshida, H. Tamura, J. Aketagawa, S. Tanaka, and Kawai T. 1992. Determination of plasma (1→3)-β-D-glucan: a new diagnostic aid to deep mycosis. *J Med. Vet. Mycol*. 30, 275-280.

Odabasi, Z., G. Mattiuzzi, L. Ostrosky-Zeichner, E. Estey, H. Kantarjian, and J.H. Rex. 2002. Detection of (1→3)-β-D-Glucan in the serum of leukemia patients with invasive fungal infections. Abstr. M-902, p. 396. Abstr. 42nd ICAAC.

Odds, F. and R. Bernaerts. 1994. CHROMagar Candida, a new differential medium for presumptive identification of clinically important *Candida* species. *J Clin. Microbiol*. 12, 1923-1929.

Oliveira, K., G. Haase, C. Kurtzman, J.J. Hyldig-Nielsen, and H. Stender. 2001. Differentiation of *Candida albicans* and *Candida dubliniensis* by fluorescent in situ hybridization with peptide nucleic acid probes. *J Clin. Microbiol*. 39, 4138-4141.

Pallavicini, F., I. Izzi, M.A. Pennisi, G. Morace, G.G. Portaccio, G. Bello, F. Iodice, D. Godino, C. Del Borgo, and R. Proietti. 1999. Evaluation of the utility of serological tests in the diagnosis of candidemia. *Minerva Anestesiologica*. 65, 637-639.

Pappas, P.G., J.H. Rex, J.D. Sobel, S.G. Filler, W.E. Dismukes, T.J. Walsh, and J.E. Edwards. 2004. Guidelines for treatment of candidiasis. *Clin. Infect. Dis*. 15, 161-189.

Patterson, T.F., S.G. Revankar, W.R. Kirkpatrick, R.K. McAtee, A.W. Fothergill, D.I. McCarthy, and M.G. Rinaldi. 1996. Simple method for detecting fluconazole-resistant yeasts with chromogenic agar. *J Clin. Microbiol*. 34, 1794-1797.

Petti, C.A., A.K. Zaidi, S. Mirrett, and L.B. Reller. 1996. Comparison of Isolator 1.5 and BACTEC NR660 aerobic 6A blood culture systems for the detection of fungemia in children. *J Clin. Microbiol*. 34, 1877-1879.

Pfaller, M.A., A. Houston, and S. Coffmann. 1996. Application of CHROMagar Candida for rapid screening of clinical specimens for *Candia albicans, Candida tropicalis, Candida krusei,* and *Candida* (*Torulopsis*) *glabrata*. *J Clin. Microbiol*. 34, 58-61.

Pfaller, M.A., S.A. Messer, S. Gee, S. Joly, C. Pujol, D.J. Sullivan, D.C. Coleman, and D.R. Soll. 1999. *In vitro* susceptibilities of *Candida dubliniensis* isolates tested against the new triazole and echinocandin antifungal agents. *J Clin. Microbiol*. 37, 870-872.

Phillips, P., A. Dowd, P. Jewesson, G. Radigan, M.G. Tweeddale, A. Clarke, I. Geere, and M. Kelly. 1990. Nonvalue of antigen detection immunoassays for diagnosis of candidemia. *J Clin. Microbiol*. 28, 2320-2326.

Posteraro, B., M. Sanguinetti, L. Masucci, L. Romano, G. Morace, and G. Fadda. 2000. Reverse cross blot hybridization assay for rapid detection of PCR-amplified DNA from *Candida* speceis, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* in clinical samples. *J Clin. Microbiol*. 38, 1609-1614.

Powell, H.L., C.A. Sand, and R.P. Rennie. 1998. Evaluation of CHROMagar Candida for presumptive identification of clinically important *Candida* species. *Diagn. Microbiol. Infect Dis*.

32, 201-204.

Price, M.F, M.T. LaRocco, and L.O. Gentry. 1994. Fluconazole susceptibilities of *Candida* species and distribution of species recovered from blood cultures over a 5-year period. *Antimicrob. Agents Chemother*. 38, 1422-1424.

Procop, G.W., F.R. Cockerill 3rd, E.A. Vetter, W.S. Harmsen, J.G. Hughes, and G.D. Roberts. 2000. Performance of five agar media for recovery of fungi from isolator blood cultures. *J Clin. Microbiol*. 38, 3827-3829.

Pryce, T.M., I.D. Kay, S. Palladino, and C.H. Heath. 2003. Real-time automated polymerase chain reaction (PCR) to detect *Candida albicans* and *Aspergillus fumigatus* DNA in whole blood from high-risk patients. *Diagn. Microbiol. Infect Dis*. 47, 487-496.

Quindos, G., R. San Millan, R. Robert, C. Bernard, and J. Poton. 1997. Evaluation of Bichro-latex albicans, a new method for rapid identification of *Candida albicans*. *J Clin. Microbiol*. 35, 1263-1265.

Quindos, G., V. Lipperheide, and J. Ponton. 1993. Evaluation of two commercialized systems for the rapid identification of medically important yeasts. *Mycoses*. 36, 299-303.

Rabkin, J.M., S.L. Oroloff, C.L. Corless, K.G. Benner, K.D. Flora, H.R. Rosen, and A.J. Olyaei. 2000. Association of fungal infection and increased mortality in liver transplant recipients. *Am J Surg*. 179, 426-430.

Rand, K.H., H. Houck, and M. Wolff. 1994. Detection of candidemia by polymerase chain reaction. *Mol. Cell. Probes*. 8, 215-222.

Ray, T.L. and C.D. Payne. 1988. Scanning electron microscopy of epidermal adherence and cavitation in murine candidiasis: a role for *Candida* acid proteinase. *Infect. Immun*. 56, 1942-1949.

Ray, T.L., C.D. Payne, and B.J. Morrow. 1991. *Candida* acid proteinases: characterization and role in candidiasis. *Adv. Exp. Med. Biol*. 306, 173-183.

Rees, J.R., R.W. Pinner, R.A. Hajjeh, M.E. Brandt, and A.L. Reingold. 1998. The epidemiological features of invasive mycotic infections in the San Francisco Bay area, 1992-1993: results of population-based laboratory active surveillance. *Clin. Infect. Dis*. 27, 1138-1147.

Reimer, L.G., M.L. Wilson, and M.P. Weinstein. 1997. Update on detection of bacteremia and fungemia. *Clin. Microbiol. Rev.* 10, 444-465.

Reiss, E. and C.J. Morrison. 1993. Nonculture methods for the diagnosis of *Candida* infections. *Clin. Microbiol. Rev.* 6, 311-323.

Rex JH, Bennett JE, Sugar AM, P.G. Pappas, C.M. van der Horst, J.E. Edwards, R.G. Washburn, W.M. Scheld, A.W. Karchmer, and A.P. Dine. 1994. A randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in patients without neutropenia. *N Eng. J Med.* 331, 1325-1330.

Rigby, S., G.W. Procop, G. Haase, D. Wilson, G. Hall, C. Kurtzman, K. Oliveira, S. Von Oy, J.J. Hyldig-Nielsen, J. Coull, and H. Stender. 2002. Fluorescence in situ hybridization with peptide nucleic acid probes for rapid identification of *Candida albicans* directly from blood culture bottles. *J Clin. Microbiol*. 40, 2182-2186.

Ruchel, R. 1992. *Proteinase*, p. 17-33. *In* J.E. Bennett, R.J. Hay, and P.K. Peterson (eds.), New Strategies in Fungal Disease. Churchill-Livingstone, Edinburgh.

Ruchel, R. and B. Boning-Stutzer. 1983. Detection of *Candida* proteinase by enzyme immunoassay and interaction of the enzyme with alpha-2-macroglobulin. *J Immunol. Methods*. 61, 107-116.

Ruchel, R., B. Boning-Stutzer, and A. Mari. 1988. A synoptical approach to the diagnosis of candidosis, relying on serological antigen and antibody tests, on culture, and on evaluation of clinical data. *Mycoses*. 31, 87-106.

Saeki, F., R.J. Ridge, M.A. Finkelman, P.A. Ketchum, and J.H. Rex. 2001. Application of a glucan-specific limulus amebocyte lysate serum assay for diagnosis of invasive fungal infections. Abstr. J-841, p. 386. Abstr. 41st ICAAC.

Sandhu, G., B.C. Kline, L. Stockman, and G.D. Roberts. 1995. Molecular probes for diagnosis of fungal infections. *J Clin. Microbiol*. 33, 2913-2919.

San-Millan, R., L. Ribacoba, J. Ponton, and G. Quindos. 1996. Evaluation of a commercial medium for identification of *Candida* species. *Eur. J Clin. Microbiol. Infect Dis*. 15, 153-158.

Schmidt, D., J. Loeffler, H. Hebart, U. Schumacher, and H. Einsele. 2001. Rapid extraction and detection of DNA from *Candida* species in research samples by MagNA Pure LC and the light cycler. *Biochemica*. 3, 1-3.

Selvarangan, R., U. Bui, A.P. Limaye, and B.T. Cookson. 2003. Rapid identification of commonly encountered *Candida* species directly from blood culture bottles. *J Clin. Microbiol*. 41, 5660-5664.

Selvarangan, R., A.P. Limaye, and B.T. Cookson. 2002. Rapid identification and differentiation of *Candida albicans* and *Candida dubliniensis* by capillary-based amplification and fluorescent probe hybridization. *J Clin. Microbiol*. 40, 4308-4312.

Sendid, B., T. Jouault, R. Coudriau, D. Camus, F. Odds, M. Tabouret, and D. Poulain. 2004. Increased sensitivity of mannanemia detection tests by joint detection of α- and β-linked oligomannosides during experimental and human systemic candidiasis. *J Clin. Microbiol*. 42, 164-171.

Sendid, B., M. Tabouret, J.L. Poirot, D. Mathieu, J. Fruit, and D. Poulain. 1999. New enzyme immunoassays for sensitive detection of circulating *Candida albicans* mannan and antimannan antibodies: useful combined test for diagnosis of systemic candidiasis. *J Clin. Microbiol*. 37, 1510-1517.

Shin, J.H., F.S. Nolte, and C.J. Morrison. 1997. Rapid identification of *Candida* species in blood cultures by a clinically useful PCR method. *J Clin. Microbiol*. 35, 1454-1459.

Shin, J.H., F.S. Nolte, and C.J. Morrison. 1999. Rapid identification of up to three *Candida* species in a single reaction tube by a 5' exonuclease assay using fluorescent DNA probes. *J Clin. Microbiol*. 37, 165-170.

Staib, F. 1965. Serum-proteins as nitrogen source for yeast-like fungi. *Sabouraudia*. 4, 187-193.

Stevens, P., S. Huang, L.S. Young, and M. Berdischewsky. 1980. Detection of *Candida* antigenemia in human invasive candidiasis by a new solid phase radioimmunoassay. *Infection*. 8, S334-338.

St-Germain, G. and D. Beauchesne. 1991. Evaluation of the MicroScan rapid yeast identification panel. *J Clin. Microbiol*. 29, 2296-2299.

Strockbine, N.A., M.T. Largen, and H.R. Buckley. 1984a. Production and characterization of three monoclonal antibodies to *Candida albicans* proteins. *Infect. Immun*. 43, 1012-1018.

Strockbine, N.A., M.T. Largen, S.M. Zweibel, and H.R. Buckley. 1984b. Identification and molecular weight characterization of antigens from *Candida albicans* that are recognized by human

sera. *Infect. Immun*. 43, 715-721.

Sundstrom, P. and G.R. Aliaga. 1992. Molecular cloning of cDNA and analysis of protein secondary structure of *Candida albicans* enolase, an abundant, immunodominant glycolytic enzyme. *J Bacteriol*. 174, 6789-6799.

Switchenko, A.C., C.G. Miyada, T.C. Goodman, T.J. Walsh, B. Wong, M.J. Becker, and E.F. Ullman. 1994. An automated enzymatic method for measurement of D-arabinitol, a metabolite of pathogenic *Candida* species. *J Clin. Microbiol*. 32, 92-97.

Takesue, Y., M. Kakehashi, H. Ohge, Y. Imamura, Y. Murakami, M. Sasaki, M. Morifuji, Y. Yokoyama, M. Kouyama, T. Yokoyama, and T. Sueda. 2004. Combined Assessment of β-d-Glucan and Degree of *Candida* Colonization before Starting Empiric Therapy for Candidiasis in Surgical Patients. *World J Surg*. In press.

Tamura, H., Y. Arimoto, S. Tanaka, M. Yoshida, T. Obayashi, and T. Kawai. 1994. Automated kinetic assay for endotoxin and (1→3)-β-D-glucan in human blood. *Clin Chim Acta*. 226, 109-112.

Tiraboschi, I.N., J.E. Bennett, C.A. Kaufmann, J.H. Rex, C. Girmenia, J.D. Sobel, and F. Menichetti. 2000. Deep *Candida* infections in the neutropenic and non-neutropenic host: an ISHAM symposium. *Med. Mycol*. 38, 199-204.

Trick, W.E., S.K. Fridkin, J.R. Edwards, R.A. Hajjeh, and R.P. Gaynes. 2002. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989-1999. *Clin. Infect. Dis*. 35, 627-630.

van Deventer, A.J., W.H. Goessens, A. van Belkum, H.J. van Vliet, E.W. van Etten, and H.A. Verbrugh. 1995. Improved detection of *Candida albicans* by PCR in blood of neutropenic mice with systemic candidiasis. *J Clin. Microbiol*. 33, 625-628.

Verduyn Lunel, F.M., A. Voss, E.J. Kuijper, L.B. Gelinck, P.M. Hoogerbrugge, K.L. Liem, B.J. Kullberg, and P.E. Verweij. 2004. Detection of the *Candida* antigen mannan in cerebrospinal fluid specimens from patients suspected of having *Candida meningitis*. *J Clin. Microbiol*. 42, 867-870.

Verghese, S.L., P. Padmaja, P. Sutha, T. Mathew, and E.S. John. 2000. Rapid identification of fluconazole resistance using CHROMagar Candida. *Ind. J Pathol. Microbiol*. 43, 343-346.

Verweij, P.E., I.M. Breuker, A.J. Rijs, and J.F. Meis. 1999. Comparative study of seven commercial yeast identification systems. *J Clin. Pathol*. 52, 271-273.

Vetter, E., C. Torgerson, A. Feuker, J. Hughes, S. Harmsen, C. Schleck, C. Horstmeier, G. Roberts, and F. Cockerill 3rd. 2001. Comparison of the BACTEC MYCO/F Lytic bottle to the isolator tube, BACTEC Plus Aerobic F/bottle, and BACTEC Anaerobic Lytic/10 bottle and comparison of the BACTEC Plus Aerobic F/bottle to the Isolator tube for recovery of bacteria, mycobacteria, and fungi from blood. *J Clin. Microbiol*. 39, 4380-4386.

Wadlin, J.K., G. Hanko, R. Stewart, J. Pape, and I. Nachamkin. 1999. Comparison of three commercial systems for identification of yeasts commonly isolated in the clinical microbiology laboratory. *J Clin. Microbiol*. 37, 1967-1970.

Walsh, T.J. and S.J. Chanock. 1998. Diagnosis of invasive fungal infections: advances in nonculture systems. *Curr. Clin. Top Infect. Dis*. 18, 101-153.

Walsh, T.J., A. Francesconi, M. Kasai, and S.J. Chanock. 1995a. PCR and single-strand conformational polymorphism for recognition of medically important opportunistic fungi. *J Clin. Microbiol*. 33, 3216-3220.

Walsh, T.J., J.W. Hathorn, J.D. Sobel, W.G. Merz, V. Sanchez, S.M. Maret, H.R. Buckley, M.A. Pfaller, R. Schaufele, and C. Sliva. 1991. Detection of circulating *Candida* enolase by immunoassay in patients with cancer and invasive candidiasis. *N Eng J Med*. 324, 1026-1031.

Walsh, T.J., J.W. Lee, T. Sein, R. Schaufele, J. Bacher, A.C. Switchenko, T.C. Goodman, and P.A. Pizzo. 1994. Serum D-arabinitol measured by automated quantitative enzymatic assay for detection and therapeutic monitoring of experimental disseminated candidiasis: correlation with tissue concentrations of *Candida albicans*. *J Med. Vet. Mycol*. 32, 205-215.

Walsh, T.J., W.G. Merz, J.W. Lee, R. Schaufele, T. Sein, P.O. Whitcomb, M. Ruddel, W. Burns, J.R. Wingard, and A.C. Switchenko. 1995b. Diagnosis and therapeutic monitoring of invasive candidiasis by rapid enzymatic detection of serum D-arabinitol. *Am J Med*. 99, 164-172.

Washington, J.A. 1994. Collection, transport, and processing of blood cultures. *Clin Lab. Med*. 14, 59-68.

Wildfeuer, A., R. Schlenk, and W. Friedrich. 1996. Detection of *Candida albicans* DNA with a yeast-specific primer system by polymerase chain reaction. *Mycoses*. 39, 341-346.

Willinger, B. and M. Manafi. 1999. Evaluation of CHROMagar Candida for rapid screening of clinical specimens for *Candida* species. *Mycoses*. 42, 61-65.

Wingard, J.R. 1995. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. *Clin. Infect. Dis*. 20, 115-125.

Wong, B., E.M. Bernard, J.W. Gold, D. Fong, and D. Armstrong. 1982. The arabinitol appearance rate in laboratory animals and humans: estimation from the arabinitol/creatinine ratio and relevance to the diagnosis of candidiasis. *J Infect. Dis*. 146, 353-359.

Wong, B., K.L. Brauer, J.R. Clemons, and S. Beggs. 1990. Effects of gastrointestinal candidiasis, antibiotics, dietary arabinitol, and cortisone acetate on levels of the *Candida* metabolite D-arabinitol in rat serum and urine. *Infect. Immun*. 58, 283-288.

Yeo, S.F., Y. Zhang, D. Schafer, S. Campbell, and B. Wong. 2000. A rapid, automated enzymatic fluorometric assay for determination of D-arabinitol in serum. *J Clin. Microbiol*. 38, 1439-1443.

Yera, H., B. Sendid, N. Francois, D. Camus, and D. Poulain. 2001. Contribution of serological tests and blood culture to the early diagnosis of systemic candidiasis. *Eur. J Clin. Microbiol. Infect. Dis*. 20, 864-870.

Yoshida, M., T. Obayashi, A. Iwama, M. Ito, S. Tsunoda, T. Suzuki, K. Muroi, M. Ohta, S. Sakamoto, and Y. Miura. 1997. Detection of plasma (1→3)-β-D-glucan in patients with Fusarium, Trichosporon, Saccharomyces and Acremonium fungaemias. *J Med. Vet. Mycol*. 35, 371-374.