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Review article

Current nonculture-based diagnosis of candidemia

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Abstract

Candidemia is the most common healthcare-associated invasive fungal infection with high crude mortality rates. It primarily affects critically ill or severely immunocompromised patients, complicating early diagnosis and prompting the initiation of appropriate antifungal therapy. The gold standard for diagnosing candidemia is blood culture; however, the sensitivity of this test is low and requires at least two days for species identification. These limitations have led to the development of alternative diagnostic methods that are more sensitive and have shorter turnaround times. Here, we review the currently available methods for the nonculture-based diagnosis of candidemia, including (i) immunological diagnostics targeting *Candida*-related antigens, (ii) human antibodies to *Candida*-related antigens, and (iii) molecular diagnostics. The strengths, uses, and limitations of each methodology are discussed. Immunological diagnostics targeting *Candida*-related antigens and human antibodies to these antigens provide supportive evidence for diagnosing candidemia. Advances in molecular diagnostics have shown promising results in facilitating early candidemia diagnosis, potentially improving patient outcomes.

Keywords: Beta-glucans, Candidemia, Galactomannan, Immunological tests, Molecular diagnostic techniques

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Introduction

Candidemia is the most common healthcare-associated invasive fungal infection [1] and is associated with high morbidity and mortality rates [2,3]. Its increasing incidence has been observed in intensive care units (ICUs), particularly among immunocompromised patients, those treated with broad-spectrum antibiotics, and those requiring invasive procedures and devices [4]. Candidemia is the fifth leading cause of healthcare-associated bloodstream infections (BSIs) in ICUs in European countries. Furthermore, *Candida* species have been reported as the second most prevalent pathogens causing healthcare-associated BSIs in the United States and South Korea [5,6]. Blood culture is the gold standard for diagnosing candidemia [7]. However, the definitive treatment of candidemia is often delayed because of the limited sensitivity of this method. While blood cultures are essential for performing antifungal susceptibility testing, their overall sensitivity for detecting invasive candidiasis is only approximately 50%, and results may take up to five days to obtain [8]. These unmet requirements have prompted efforts to develop alternative diagnostics with higher sensitivities and faster turnaround times, including the development and validation of nonculture diagnostic tests for

candidemia [9]. Over the last 10 years, various nonculture-based methods have been devised to overcome the limitations of conventional culture-based diagnostics. These include (i) immunological diagnostics targeting *Candida*-related antigens; (ii) the use of human antibodies against *Candida*-related antigens, such as *C. albicans* germ tube antibodies and anti-mannan antibodies; and (iii) molecular diagnostics (Table 1). This review aims to provide comprehensive and up-to-date information on the currently available methods for nonculture-based diagnostics of candidemia.

Table 1. Characteristics of nonculture-based diagnostics for candidemia

Method	Target or principle of assay	Advantage	Shortcoming
Antigen detection-based immunological assay	*Candida* (1,3)-β-D-glucan	Rapid, high sensitivity, high negative predictive value	Low specificity, high false-positive
	Candida mannan	Rapid, earlier diagnosis of candidemia	Low sensitivity
Antibody-based immunological assay	Anti-mannan Ab; *C. albicans* germ tube antibody	Rapid, enhancing performance in combination with antigen testing	Low sensitivity, low specificity
Nucleic acid amplification-based molecular assay	Conventional PCR; nested PCR; multiplex PCR; real-time PCR; T2 magnetic resonance	Highly sensitivity, high specificity	Expensive, multiple step, potential false-negative out of specific targets within the panel

Detection of *Candida* (1,3)-β-D-glucan (BDG)

BDG is a cell wall polysaccharide in various medically necessary fungi, including *Candida* species, but not in *Cryptococcus* and *Mucorales*. Several commercial assays capable of detecting circulating *Candida* BDG have been developed. These include the Fungitell® assay (Associates of Cape Cod), Fungitec-G assay (Seikagaku Kogyo Corporation), Wako test (Wako Pure Chemical Industries), and Dynamiker Fungus assay (Dynamiker Biotechnology Ltd.) (Table 2). These assays are based on the ability of BDG to activate the horseshoe crab proteolytic coagulation cascade [10]. Each test has different interpretive cutoff values: > 80 pg/mL for the Fungitell and Glucatell assays and > 20 pg/mL for the Fungitec-G assay; values > 20 pg/mL are considered positive. For the Wako assay and Dynamiker Fungus test, the cutoff values are 11 and > 95 pg/mL, respectively. This may have resulted from the reagents being sourced from different horseshoe crab species, with Fungitell reagents derived from *Limulus polyphemus,* and Fungitec and Wako reagents obtained from *Tachypleus tridentatus*. A meta-analysis reported pooled sensitivity and specificity values of BDG of 0.81 (0.75–0.86) and 0.64 (0.55–0.71), respectively (Table 2) [11]. These widely dispersed values can mainly be attributed to the heterogeneity within and between evaluations, which differs for the various BDG assays, the positive cutoff criteria used, the patient and control populations tested, and the number of BDG tests performed per individual. Several studies have explored diagnosis-driven therapies based on BDG detection. However, the diagnostic accuracy of BDG testing appears to be insufficient to inform treatment decisions [12-14] reliably. Nevertheless, the guidelines of the 3rd European Conference on Infections in Leukemia held in 2009 indicate that repeated positive BDG results may be used as supportive evidence for the presence of invasive fungal infections among patients with prolonged neutropenia who present with symptoms consistent with the infection [15]. Several factors should be considered when interpreting the

BDG results. The existing assays can produce false-positive results in high-risk populations due to various potential sources of contamination, including human blood products (coagulation factors, immunoglobulins, albumin, and plasma protein fractions), surgical gauzes or other glucan-containing materials, hemodialysis, high levels of triglycerides, certain antibiotics that include intravenous amoxicillin-clavulanic acid or piperacillin-tazobactam, and systemic bacterial infections [16-20]. BDG test performance aspects include high sensitivity, high false-positive rate, low specificity, and high negative predictive value (Table 1). Thus, it is recommended that this test be used to rule out the disease and discontinue empirical antifungal treatment in ICU patients with suspected invasive candidiasis.

Table 2. Characteristics of several immunological diagnostics for candidemia

General target	Function	Available commercial kit	Overall sensitivity (95% CI)	Overall specificity (95% CI)	Reference
Candida (1,3)- β-D-glucan	*Candida* Cell wall component	Fungitell; Wako; Fungitec-G; Dynamiker Fungus	0.81 (0.75-0.86)	0.64 (0.55-0.71)	[11]
Candida mannan	*Candida* Cell wall component	Platelia Candida Ag-Plus and Ab-Plus; Serion Mannan kit	0.58 (0.53-0.62)	0.93 (0.91-0.94)	[22]
Anti-mannan antibody	Antibodies to *Candida* polysaccharides	Platelia *Candida* Ab-Plus	0.59 (0.54-0.65)	0.83 (0.79-0.87)	[22]
C. albicans germ tube antibody (CAGTA)	Antibodies to *Candida* protein extract	Invasive Candidasis (CAGTA) IFA IgG assays; VirClia IgG Monotest	0.66 (0.59-0.73)	0.76 (0.58-0.88)	[32]

Abbreviation: CI, confidence interval.

Detection of *Candida* mannan or anti-mannan antibody

Mannans are key cell wall components of *Candida* spp. They participate in innate and acquired immunity, and are used as biomarkers [21]. *Candida* mannan can be measured in the serum or plasma of patients with candidemia using a latex agglutination test or sandwich enzyme-linked immunosorbent assay. Commercial assays, such as Platelia *Candida* Ag-Plus and Ab-Plus (Bio-Rad) and Serion Mannan kits (Serio GmbH) are available. The pooled sensitivity and specificity of mannan tests are reportedly 0.58 (0.53–0.62) and 0.93 (0.91–0.94), respectively (Table 2) [22]. Similar to protein antigen detection assays, mannan assays have low diagnostic sensitivity for candidemia (Table 1) [23]. This is mainly because of the high immunogenicity of mannan, which is rapidly cleared from circulation. They may also form immune complexes with circulating anti-mannan antibodies, which complicates their assessment [24]. The sensitivity and specificity of the mannan assay vary [22]. However, almost all the relevant studies have reported mannan-positive blood cultures, thus allowing for an earlier diagnosis of candidemia. The sensitivity of the mannan assay varies according to different *Candida* spp. and is highest for *C. albicans*, followed by *C. glabrata* and *C. tropicalis* [25,26]. It was lower in cases of *C. parapsilosis* and *Pichia kudriavzevii*, probably because of the lower amount of the mannan produced and released by these species [27,28]. Current guidelines of the European Society of Clinical Microbiology and Infectious Diseases recommend the combined use of mannan antigen and anti-mannan antibody quantification assays and serial determinations for both assays for diagnosing

candidemia and chronic disseminated candidiasis [29]. In a meta-analysis of 14 studies [22], the sensitivity and specificity of anti-mannan IgG antibody for invasive candidiasis were 0.59 (0.54–0.65) and 0.83 (0.79–0.87), respectively (Table 2). The sensitivity and specificity for a combined mannan/anti-mannan assay were 0.83 and 0.86, respectively, with the best performance in patients with *C. albicans*, *C. glabrata*, or *C. tropicalis* infections. Several studies have shown that combining these assays with BDG or mannan detection assays may enhance diagnostic accuracy (Table 1) [30]. Because BDG is nonspecific, additional positive results for mannan or anti-mannan antibodies may indicate fungal disease due to *Candida*, whereas additional negative results for mannan or anti-mannan antibodies may indicate infection caused by other fungi.

Detection of *C. albicans* germ tube antibody (CAGTA)

The response to the hyphal wall protein 1 mycelial phase antigen of *C. albicans*, which is essential for biofilm development and tissue invasion, has been studied for the serodiagnosis of invasive candidiasis [31]. Originally termed CAGTA, reflecting the original design for *C. albicans*, subsequent research has demonstrated that other *Candida* species, including *C. tropicalis, C. parapsilosis, C. glabrata, C. dubliniensis, C. guilliermondii*, and *P. kudriavzevii*, can also produce CAGTA to a greater or lesser degree [32]. There are two commercial CAGTA assays: an indirect immunofluorescence test [IC (CAGTA) IFA IgG, Vircell Microbiologists] and an indirect chemiluminescent immunoassay [IC (CAGTA) VirClia®, Vircell Microbiologists]. The overall sensitivity and specificity of CAGTA has been reported as 0.66 (0.59–0.73) and 0.76 (0.58–0.88) (Table 2) [32]. Wei et al. [32] suggested that the accuracy of CAGTA is marginal and that the results should be used as part of a full assessment of the clinical features, imaging findings, and other laboratory results for diagnosing candidemia. Furthermore, CAGTA detection does not specify the fungal genus, limiting the use of targeted treatments in practice.

Molecular diagnostics

Molecular amplification techniques enable rapid and sensitive detection and identification by directly analyzing small amounts of fungal DNA in clinical samples, thereby eliminating the need for prior cultivation (Table 1). This direct analysis feature makes these tests particularly advantageous for early diagnosis of candidemia, especially in cases often missed by culture methods. Various polymerase chain reaction (PCR) assays have been developed to target different genetic sequences, including 18S, 28S, and 5.8S ribosomal DNA (rDNA), internal transcribed spacer regions, and mitochondrial DNA, to detect various fungi across different specimens. Numerous commercial PCR-based assays have been designed and evaluated to detect *Candida* DNA in patients at risk of candidemia. The multiplex platforms have been applied, such as the eplex® BCID FP Panel (GenMark DX), CandID (Olm Diagnostics), Fungiplex Candida (Bruker Daltonik), LightCycler® SeptiFast (Roche Diagnostics), Magicplex Sepsis (Seegene), FilmArray BCID2 Panel (bioMérieux), and T2Candida (T2 Biosystems) (Table 3).

Table 3. Characteristics of several molecular diagnostics for candidemia approved by Conformité Européenne/ In Vitro Diagnostic or Food and Drug Administration

Commercial kit	Manufacturer	Detectable *Candida* species	Needs of nucleic acid extraction	Principle	Available specimen	Assay time	Reference
eplex® BCID FP Panel[a)]	GenMark Dx	*C. albicans, C. dubliniensis, C. famata, C. glabrata, C. guilliermondii, C. kefyr, P. kudriavzevii, C. lusitaniae, C. parapsilosis, C. tropicalis, C. auris*	yes	Multiplex PCR	Blood culture positive sample	90 min	[33]
CandID	OLM Diagnostics	*C. albicans, C. dubliniensis, C. glabrata, P. kudriavzevii, C. parapsilosis, C. tropicalis*	yes	Multiplex qPCR	Blood, synthetic bronchial aspirate lavage	4 h	[36]
Fungiplex Candida	Bruker Daltonics	*C. glabrata*, *Candida* species (*C. albicans, C. dubliniensis, C. parapsilosis, C. tropicalis), P. kudriavzevii*	yes	Multiplex real-time PCR	Blood	< 2 h	[37]
SeptiFast Test kit	Roche Diagnostics	*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, P. kudriavzevii*	yes	Multiplex real-time PCR	Blood, sterile fluid, tissue, swab	6 h	[37]
Magicplex Sepsis	Seegene	*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, P. kudriavzevii*	yes	Multiplex PCR	Blood	6 h	[47]
FilmArray BCID Panel	bioMérieux	*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, P. kudriavzevii*	no	Multiplex PCR	Blood culture positive sample	60 min	[43]
FilmArray BCID2 Panel	bioMérieux	*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, P. kudriavzevii, C. auris, Cryptococcus neoformans/C. gattii*	no	Multiplex PCR	Blood culture positive sample	60 min	[44]
T2 Candida Panel	T2 Biosystems	*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, P. kudriavzevii*	no	Multiplex PCR followed by automated T2MR-based detection	Blood	< 5 h	[45,46]

[a)]The ePlex® BCID assay was rebranded as Cobas® eplex Blood Culture Identification Panels (Roche Diagnostics, Basel, Switzerland) in 2021.
Abbreviations: qPCR, quantitative PCR; T2MR, T2 magnetic resonance.

The ePlex® BCID (GenMark DX) assay, now rebranded as Cobas® eplex Blood Culture Identification Panels (Roche Diagnostics), consists of gram-positive (BCID-GP), gram-negative (BCID-GN), and fungal (BCID FP) panels. These panels detected 56 unique bacterial and fungal targets (11 *Candida* spp., *Cryptococcus gattii, C. neoformans, Fusarium,* and *Rhodotorula*) within 90 min [33]. Zhang et al. [34] tested 866 clinical samples and found that the sensitivity and specificity for detecting *Candida* species ranged from 0.97 to 1.0 and 0.99 to 1.0, respectively. Furthermore, this panel detects *C. auris*, which has emerged globally as a multidrug-resistant yeast that causes infections and outbreaks in healthcare facilities [35]. The CandID® (Olm Diagnostics) detects *C. albicans, C. glabrata, C. parapsilosis, P. kudriavzevii, C. dubliniensis*, and *C. tropicalis.* Rapid time-to-result with nucleic acid extraction through PCR amplification and result interpretation were completed within < 4 h [36]. Price et al. [36] reported that prospective testing

generated an overall sensitivity and specificity of 0.88 and 0.82, respectively. The Fungiplex® Candida IVD PCR Kit (Bruker Daltonik, Billerica) detects *C. glabrata, P. kudriavzevii,* and *Candida* spp. (including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. dubliniensis*) in whole blood, plasma, and serum within 2 h. Kits from Qiagen and bioMérieux are recommended for DNA extraction. The assay manuals provide instrument settings for the different thermocyclers. In a small prospective study on ICU patients, the Fungiplex® Candida revealed a sensitivity of 1.0, a specificity of 0.94, and a diagnostic accuracy of 0.94 [37]. The LightCycler® SeptiFast (Roche Diagnostics) was the first real-time PCR-based system to receive a Conformité Européenne mark for simultaneous pathogen detection and identification in suspected BSIs [38]. A meta-analysis of 54 studies comparing SeptiFast with blood culture found that this assay had an estimated summary sensitivity of 0.65 and specificity of 0.86 [39]. This suggests that a positive blood test at the genus/species level returned by SeptiFast may provide a higher diagnostic value (rule-in) than a negative test result (rule-out) when compared to blood culture [40]. The Magicplex™ Sepsis Real-time Test (Seegene Inc.) can detect 90 pathogens at the genus level and 27 pathogens, including five *Candida* spp. (*C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, P. kudriavzevii*), at the species level within 6 h of whole blood collection. In this analysis, DNA pretreatment and extraction are followed by conventional PCR for amplicon generation. If amplicons are detected, conventional PCR is followed by two real-time PCRs for screening and species level identification. Denina et al. [41,42] compared the Magicplex™ test to blood cultures of 150 samples from 89 patients. *Candida* spp. were detected by the Magicplex™ in four samples, of which only one was accompanied by a positive blood culture. Further evaluation of the clinical performance of this kit is warranted. The FilmArray BCID Panel (bioMérieux) is a nested multiplex PCR system that detects 24 pathogens, including *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *P. kudriavzevii*, within 1 h. In a study involving clinical and spiked samples, this panel demonstrated a sensitivity of 0.99 and a specificity of 0.99 for *Candida* species compared to conventional cultures [43]. In a study involving clinical and spiked samples, this panel demonstrated a sensitivity and specificity of 0.99 for *Candida* species compared to conventional cultures [43]. The recently updated FilmArray® BCID2 Panel (bioMérieux) has 43 targets, including *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, P. kudriavzevii*, *C. auris,* and *C. neoformans/C. gattii*. For *Candida* species, the BCID2 Panel demonstrated a sensitivity of 0.92 and specificity of 0.995 [44]. The T2Candida® assay (T2 Biosystems) is a miniaturized molecular method recently cleared by the Food and Drug Administration for rapid diagnosis of candidemia. It combines PCR with T2 magnetic resonance imaging. This assay reportedly enables the detection of amplified DNA from *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis*, and *P. kudriavzevii* at concentrations of 1–3 colony forming units/mL in whole blood specimens within 5 h [45]. This new method is highly sensitive and specific for diagnosing candidemia and does not require viable *Candida* cells or sample purification and preparation. The results of a multicenter study indicated that the T2Candida® assay performed in patients with proven candidemia might be a better marker of complicated infection than follow-up blood cultures or detection of BDG, which

may influence the length and type of antifungal therapy in this population as antimicrobial stewardship [46]. In the study, the T2Candida® assay demonstrated limited sensitivity (36%) and negative predictive value (80%) under empirical antifungal therapy, while its specificity/positive predictive value was excellent (100%). These findings indicate that the assay is better suited for confirming the diagnosis of persistent infections [46]. However, this promising molecular diagnostic method has several limitations. It is relatively expensive, its reagents have a short expiration date, and its sensitivity may decrease if intact *Candida* cells are absent from the whole blood samples. Recently, the detection of microbial cell-free DNA (cfDNA) using next-generation sequencing has been utilized as an accurate and precise method to identify and quantify pathogens [47]. The Karius® Test was developed based on sequencing of microbial cfDNA circulating in plasma to identify over 1,000 pathogens, including bacteria, viruses, and fungi, from 5 mL blood samples. However, this method has not yet been approved [47]. This novel diagnostic tool was validated in a study showing that it could identify 94% of the microbes detected using conventional blood culture in patients with sepsis. Overall, although several molecular diagnostic methods offer rapid identification of medically important *Candida* species, it is important to be aware of the potential false-negative results that may occur in the absence of specific targets within the diagnostic panel (Table 1).

Conclusions

Candidemia remains a critical clinical challenge due to the limitations of traditional blood culture diagnostics. Among nonculture diagnostics, tests targeting BDG and mannan provide supportive evidence for the diagnosis of candidemia despite some limitations in sensitivity and specificity. Advances in molecular techniques, including PCR and next-generation sequencing, have demonstrated promising sensitivity and specificity for detecting *Candida* DNA, facilitating early diagnosis and potentially improving patient outcomes. Understanding the advantages and disadvantages of various diagnostic methods is becoming increasingly important to select the most appropriate diagnostic method for candidemia. Combining several nonculture diagnostic methods may help address their performance limitations.

Ethics statement

Because this was not a human population study, Institutional Review Board approval and informed consent were not required.

Conflicts of interest

Eun Jeong Won has been an associate editor of the *Annals of Clinical Microbiology* since January 2024. However, she was not involved in the review of this article. No potential conflicts of interest relevant to this article have been reported.

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Data availability

None.

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