MAJOR ARTICLE

Performance of *Candida* Real-time Polymerase Chain Reaction, β-D-Glucan Assay, and Blood Cultures in the Diagnosis of Invasive Candidiasis

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Background. The sensitivity of blood cultures for diagnosing invasive candidiasis (IC) is poor.

Methods. We performed a validated *Candida* real-time polymerase chain reaction (PCR) and the Fungitell 1,3-β-D-glucan (BDG) assay on blood samples collected from prospectively identified patients with IC (n = 55) and hospitalized controls (n = 73). Patients with IC had candidemia (n = 17), deep-seated candidiasis (n = 33), or both (n = 5). Controls had mucosal candidiasis (n = 5), *Candida* colonization (n = 48), or no known *Candida* colonization (n = 20).

Results. PCR using plasma or sera was more sensitive than whole blood for diagnosing IC ($P = .008$). Plasma or sera PCR was more sensitive than BDG in diagnosing IC (80% vs 56%; $P = .03$), with comparable specificity (70% vs 73%; $P = .31$). The tests were similar in diagnosing candidemia (59% vs 68%; $P = .77$), but PCR was more sensitive for deep-seated candidiasis (89% vs 53%; $P = .004$). PCR and BDG were more sensitive than blood cultures among patients with deep-seated candidiasis (88% and 62% vs 17%; $P = .0005$ and .003, respectively). PCR and culture identified the same *Candida* species in 82% of patients. The sensitivity of blood cultures combined with PCR or BDG among patients with IC was 98% and 79%, respectively.

Conclusions. *Candida* PCR and, to a lesser extent, BDG testing significantly enhanced the ability of blood cultures to diagnose IC.

Invasive candidiasis (IC) carries significant morbidity and mortality. In part, poor outcomes stem from delayed or missed diagnoses using blood and sterile-site cultures, the current gold standard tests. Indeed, blood cultures are negative for *Candida* species in approximately 50% of autopsy-proven cases of disseminated candidiasis [1–3]; moreover, they often become positive late in the disease course. Sterile-site cultures are further limited by the need for invasive sampling. As such, there is much interest in developing rapid and more sensitive diagnostic assays. Two approaches that have gained particular attention are detection of 1,3-β-D-glucan (BDG), a major constituent of fungal cell walls, and polymerase chain reaction (PCR) amplification of *Candida* DNA. Despite their promise, neither test is a standard of clinical practice. The sensitivity of a commercial assay for BDG quantitation (Fungitell, Associates of Cape Cod) has varied from 64% to 100% [4–6], and *Candida* PCR methods are not validated.

In this study, we compared the performance of a *Candida* real-time PCR assay, which was validated at Viracor-IBT Laboratories according to relevant clinical laboratory guidelines [7], with the Fungitell BDG assay and blood cultures for diagnosing IC. We included samples from a wide range of patients with IC, including those with candidemia, catheter-related candidemia, and deep-seated candidiasis with or without positive blood cultures. To rigorously evaluate the specificity of

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the assays, our control group was comprised largely of patients with mucosal candidiasis or who were colonized with *Candida* at nonsterile sites.

METHODS

We conducted a prospective study of patients at the University of Pittsburgh Medical Center between April 2009 and April 2011. Blood was collected from consenting controls and patients with IC, and serum or plasma samples were stored at −80°C. Whole blood testing was performed on fresh samples. The study was approved by our institutional review board.

Definitions

Invasive candidiasis included candidemia and deep-seated candidiasis [8], which were defined as the recovery of *Candida* species from blood or a sterile site, respectively. Controls were defined as hospitalized patients who did not have clinical or microbiological evidence of IC. Patients with mucosal candidiasis or colonized with *Candida* were also included as controls. Colonization was defined as the recovery of *Candida* species from nonsterile sites in patients without symptoms or signs of a systemic disease that were attributable to candidiasis. Blood and deep-seated cultures were considered to be concurrent if performed within 5 days of each other. Positive, indeterminate, and negative BDG results were defined as ≥80, 60 to <80, and <60 pmol/mL, respectively. *Candida* were speciated using standard mycological methods of carbohydrate assimilation using the API 20C kit (bioMérieux, Hazelwood, Missouri) and morphology on cornmeal agar. CHROMagar (BD Diagnostics) or other differential media to speciate *Candida* were not used.

BDG Testing and Real-time PCR

Frozen plasma and serum samples were shipped overnight, on dry ice, in batch to Viracor-IBT Laboratories for BDG and PCR testing. Whole blood samples were sent unfrozen on ice and processed within 24 hours of venipuncture. The Fungitell BDG assay was performed according to the manufacturer's instructions. For PCR, DNA from whole blood, plasma, and serum was extracted using the DNeasy Blood and Tissue kit (Qiagen, Germantown, Maryland). After adding an internal control target (engineered bacteriophage), 500 μL of specimen was manually extracted using a 35-μL elution volume. An internal control cutoff quantification cycle of 37 was required for reporting a negative result. A positive extraction control, negative extraction control, and no template control were included in every PCR run. For the design of species-specific TaqMan real-time assays, alignments of available ITS1 and/or ITS2 sequences for 4 *Candida* species (*Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida tropicalis*) and 1 species complex (*Candida parapsilosis* complex) were created using Geneious software (Biomatters, Auckland, New Zealand.). Primers were designed to detect 2 species pairs (*C. albicans*/*C. tropicalis* and *C. glabrata*/*C. krusei*), and *C. parapsilosis*. The ABI 7500 Fast Instrument (Applied Biosystems, Carlsbad, California) was used with a final reaction volume of 30 μL utilizing 10 μL of template DNA. The amplification efficiencies, determined using 10-fold dilutions of plasmid standards ranging from 10 to 1×10^8 copies per reaction, were 99.3%, 96.5%, 102.5%, 97.4%, and 98.7% for *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* complex, and *C. tropicalis*, respectively. The analytical specificities for the *Candida* PCR assays tested with various targeted *Candida* species, nontargeted fungi, and bacteria and were 100% (7/7), 95% (37/39) and 100% (28/28), respectively (Table 1). The analytical sensitivity using targeted *Candida* species was 100% (7/7).

Statistical Analysis

Sensitivity and specificity were calculated for blood culture, BDG, and PCR. The McNemar χ^2 test was used to compare sensitivity and specificity between assays [9]. Univariate analysis of contingency data was done by χ^2 or Fisher exact test.

RESULTS

PCR Using Different Blood Components

In a preliminary study, we performed PCR on whole blood and plasma samples from 21 patients with IC and 27 controls. Polymerase chain reaction was more sensitive at detecting *Candida* in plasma than whole blood of patients with IC (57% [12 of 21] vs 14% [3 of 21]; $P = .008$), and specificity was comparable (81% [22 of 27] vs 96% [26 of 27]; $P = .22$). Next, we tested plasma and sera from 16 patients with IC and 15 controls. Plasma and serum samples did not differ in sensitivity (81% [13 of 16] vs 75% [12 of 16], respectively; $P = 1.0$) or specificity (67% [10 of 15] vs 73% [11 of 15], respectively; $P = 1.0$). For the remainder of the study, PCR was performed on plasma and/or serum samples.

Performance of PCR and BDG

Patients with IC had candidemia (n = 17), deep-seated candidiasis (n = 33), or both candidemia and deep-seated candidiasis (n = 5) (Table 2). Intra-abdominal infections accounted for 89% (34 of 38) of deep-seated candidiasis. Controls included 48 patients with *Candida* colonization (Table 3), 5 with mucosal candidiasis (esophagitis, n = 3; oropharyngeal, n = 1; vaginitis, n = 1), and 20 with no known *Candida* colonization.

The performance of PCR and BDG is summarized in Table 4. The sensitivity of the tests was not affected by antifungal therapy (Table 5). Using the standard BDG cutoff for positivity (≥80 pmol/mL; indeterminate result = negative), both

Table 1. Fungi and Bacteria Used to Test the *Candida* Polymerase Chain Reaction Assays

Candida Target Species	Fungal (Non-*Candida*)	Bacteria
C. albicans (11) [CA/CT]	*Absidia corymbifera*	*Acinetobacter baumannii*
C. glabrata (6) [CG/CK]	*Alternaria alternata*	*Acinetobacter lwoffii*
C. krusei (3) [CG/CK]	*Aspergillus fumigatus*	*Acromobacter xylosoxidans*
C. metapsilosis (2) [CP]	*Aspergillus clavatus*	*Bordetella parapertussis*
C. orthopsilosis (2) [CP]	*Aspergillus flavus*	*Bordetella pertussis*
C. parapsilosis (4) [CP]	*Aspergillus nidulans*	*Burkholderia cepacia*
C. tropicalis (5) [CA/CT]	*Aspergillus niger*	*Burkholderia multivorans*
	Aspergillus terreus	*Chlamydia pneumoniae*
Candida nontarget species	*Aspergillus versicolor*	*Clostridium difficile*
C. bracariensis	*Blastomyces dermatitidis*	*Enterobacter cloacae*
C. dubliniensis	*Cryptococcus neoformans* (3)	*Enterococcus faecalis*
C. famata	*Fusarium solani*	*Escherichia coli*
C. guillermondii	*Irpex lacteus*	*Haemophilus influenzae*
C. inconspicua	*Mucor circinelloides*	*Haemophilus parainfluenzae*
C. kefyr (2)	*Mucor indicus*	*Klebsiella pneumoniae*
C. lipolytica	*Mucor ramosissimus*	*Legionella pneumophila*
C. lusitaniae (3)	*Pichia pastoris*	*Moraxella catarrhalis*
C. norvegensis	*Penicillium chrysogenum*	*Mycobacterium tuberculosis*
C. norvegica	*Penicillium marneffei*	*Neisseria meningitides*
C. pelliculosa	*Penicillium purpurogenum*	*Proteus mirabilis*
C. rugosa (3) [CG/CK, CP]	*Penicillium citrinum*	*Pseudomonas aeruginosa*
	Pseudallescheria boydii	*Serratia marcescens*
	Rhizopus microsporus	*Staphylococcus aureus*
	Rhizopus oryzae	*Staphylococcus epidermidis*
	Rhizopus pusillus	*Stenotrophomonas maltophila*
	Trichosporon cutaneum (2) [CG/CK]	*Streptococcus pneumoniae*
	Trichosporon mucoides	*Streptococcus pyogenes*
		Yersinia enterocolitica

Candida PCR assays were tested against 7 target species, 12 nontargeted *Candida* species, 27 other fungi, and 28 bacteria. Unless noted in parentheses, the number of isolates tested was 1 per organism. Target *Candida* species were detected by the indicated assays in brackets.

Abbreviations: CA, *Candida albicans*; CG, *Candida glabrata*; CK, *Candida krusei*; CP, *Candida parapsilosis* complex; CT, *Candida tropicalis*.

BDG and PCR were positive for 42% (23 of 55) of patients with IC and negative for 5% (3 of 55) (Table 6). Details of the patients with false negative results by both assays appear in Table 7. Polymerase chain reaction was positive and BDG negative for 38% (21 of 55) of patients, and BDG was positive and PCR negative for 15% (8 of 55). The sensitivity of either a positive PCR or BDG for diagnosing IC was 95% (52 of 55), and specificity was 56% (41 of 73).

Comparison of PCR and BDG With Blood Cultures

Among the 24 patients with deep-seated candidiasis in whom blood cultures were performed concurrently, both PCR (88% [21 of 24]) and BDG (62% [15 of 24]) were more sensitive than blood cultures (17% [4 of 24]; $P = .0005$ and $P = .003$, respectively) (Figure 1). If indeterminate BDG results were considered positive, sensitivity was 67% (16 of 24; $P = .002$ vs blood culture). At either BDG cutoff, sensitivity did not differ from PCR ($P = .15$ and .23, respectively).

Among the 42 patients with IC in whom blood cultures were obtained, the sensitivity of either a positive PCR or positive blood culture was 98% (41 of 42). The sensitivities of either a positive BDG or positive blood culture were 79% (33 of 42; indeterminate = negative) and 81% (34 of 42; indeterminate = positive).

Identification of *Candida* Species by PCR and Culture

In 82% (36 of 44) of patients with a positive PCR result, PCR and culture identified ≥1 *Candida* species in common (Table 8). There was complete agreement in speciation between PCR and culture for 45% (20 of 44) of patients.

DISCUSSION

This study was designed as a head-to-head comparison of PCR and BDG in diagnosing IC. We prospectively enrolled and collected blood from hospitalized patients in 3 well-defined

Table 2. Patient Demographics

Characteristic	Patients With IC (n = 55)	Controls (n = 73)
Underlying gastrointestinal disorder	36% (20)	14% (10)
Short-gut syndrome	7% (4)	3% (2)
Liver or biliary disease[a]	5% (3)	1% (1)
Small bowel obstruction[a]	5% (3)	4% (3)
Pancreatitis	11% (6)	4% (3)
Underlying genitourinary disorder	4% (2)	1% (1)
Trauma/motor vehicle accident	9% (5)	14% (10)
Immunosuppressed	22% (12)	36% (26)
Organ transplantation	20% (11)	33% (24)
Malignancy	5% (3)	12% (9)
Abdominal surgery within 1 month of enrollment	24% (13)	8% (6)
Extra-abdominal surgery within 1 month of enrollment	5% (4)	7% (5)

None of the patients had hematologic malignancy or neutropenia at the time of invasive candidiasis. Fifty-four percent (30 of 55) of patients with invasive candidiasis (IC) were receiving an antifungal agent at the time blood was collected (fluconazole, n = 20; echinocandin, n = 9; voriconazole, n = 1). The median time from the start of antifungal therapy to sample collection was 5 days (range, 1–39).

[a] One patient with IC had both liver disease and small bowel obstruction.

groups: candidemia, deep-seated candidiasis, and controls without IC. To maximize the rigor of the study, the latter group was overwhelmingly composed of patients who were colonized with *Candida* species or diagnosed with mucosal

Table 3. Sites of *Candida* Colonization

Sites of Colonization	Description	Number
Wound (n = 14)	Extremity wound	7
	Abdominal wound	6
	Cheek wound	1
Respiratory tract (n = 14)	Bronchoalveolar lavage	8
	Sputum	6[a]
Indwelling surgical drain (n = 10)	Jackson Pratt	4
	Biliary drain	4
	Other	2
Urine (n = 9)	Urine	6[a]
	Urine distal to pelvic stone	2
	Urine from a patient with hematuria	1
Other (n = 2)	Catheter tip	1
	Sinus tract	1

[a] One patient was colonized in both sputum and urine.

candidiasis. Our data yielded 2 particularly important findings. First, PCR was superior to BDG for diagnosing deep-seated candidiasis (sensitivity: 89% vs 53% or 66%, depending upon the BDG interpretive cutoff; $P = .004$ and .04, respectively). Polymerase chain reaction also was significantly more sensitive for diagnosing all cases of IC at the standard cutoff for BDG positivity (80% vs 56%; $P = .03$). Second, both PCR and BDG were markedly superior to blood cultures among patients with deep-seated candidiasis (88% and 62% vs 17%; $P = .0005$ and $P = .003$, respectively). Taken together, the results indicate that PCR may join BDG as a valuable adjunctive tool for diagnosing IC. Moreover, these assays are likely to identify a significant percentage of those patients with IC who are missed by blood culture, the current gold standard diagnostic test.

The limitations of blood cultures for diagnosing IC are well recognized [1–3]. Indeed, blood cultures are positive in <50% of patients with hepatosplenic candidiasis or autopsy-proven IC [1, 3]. The use of antifungals for prophylaxis or empiric therapy may further reduce the sensitivity of blood cultures [10]. Although advances in microbiology techniques have improved the recovery of *Candida* species, the low magnitude and short duration of candidemia suggest that the sensitivity of blood cultures will remain inadequate [11–14]. Our results are consistent with previous reports that PCR and BDG were more sensitive than blood culture for diagnosing IC [4, 15–17]. There are several potential reasons for the heightened sensitivities of these assays. In the case of PCR, the amplification of a high-copy DNA target facilitates detection of lower inocula of *Candida* in the blood. In fact, the lower limit of detection for the assay used in this study is 80 *Candida* copies/mL of serum or plasma, which corresponds to approximately 0.4 colony-forming units/mL (<1 genome). At the same time, PCR amplifies DNA from both dead and viable *Candida* cells as well as freely circulating DNA [13, 18]. Likewise, BDG detection is not dependent on viable organisms [5, 19]. The reasons that PCR was more sensitive than BDG in diagnosing deep-seated candidiasis are not apparent, which reflects our limited understanding of the bloodstream kinetics of *Candida* DNA and BDG. In animal models of IC, serum PCR remained positive after sterilization of blood, suggesting that free DNA is eliminated slowly [13, 18].

It is notable that the sensitivity of PCR for diagnosing candidemia was significantly lower than deep-seated candidiasis ($P = .009$). Similar results were reported previously [4]. A potential explanation for our findings is that patients were enrolled at the time of positive cultures. As such, transient or catheter-associated candidemias already may have resolved spontaneously or as a result of catheter removal. During deep-seated candidiasis, *Candida* DNA may be continuously

Table 4. Performance of Polymerase Chain Reaction and 1,3-β-D-Glucan Assays

Assay	Invasive Candidiasis (n = 55)	Candidemia[a] (n = 22)	Deep-Seated Candidiasis[a,b] (n = 38)	Intra-abdominal Candidiasis (n = 34)
PCR[c]				
Sensitivity	80% (44/55)	59% (13/22)	89% (34/38)	88% (30/34)
Specificity	70% (51/73)			
BDG (positive ≥80 pmol/mL)				
Sensitivity	56% (31/55)	68% (15/22)	53% (20/38)	56% (19/34)
Specificity	73% (53/73)			
BDG (positive ≥60 pmol/mL)				
Sensitivity	69% (38/55)	81% (18/22)	66% (25/38)	65% (22/34)
Specificity	63% (46/73)			
P value[d]				
PCR vs BDG (positive ≥80 pmol/mL)	.03	.77	.004	.0015
PCR vs BDG (positive ≥60 pmol/mL)	.31	.23	.04	.06

Internal control detection was positive for all samples that were negative by PCR. The median time from diagnostic cultures for Candida to collection of samples for PCR and BDG was 4 days (interquartile range: 1-6 days).

Abbreviations: BDG, 1,3-β-D-glucan; PCR, polymerase chain reaction.

[a] Candidemia and deep-seated candidiasis groups included 5 patients who had both conditions.

[b] Deep-seated candidiasis included patients with intra-abdominal infections and infections of other sites (bone and devitalized surrounding tissue, n = 2; lumbar spine device, n = 1; cranial abscess, n = 1).

[c] PCR was positive if positive result was obtained on plasma and/or sera.

[d] *P* values are for sensitivities of the respective assays, as determined by McNemar test.

released into the bloodstream, which could also explain the persistence of serum PCR positivity after the clearance of blood cultures in animal models of IC [13, 18]. Our results call to mind previous studies in which mannoproteinemia was detected in only 7% of patients with transient or catheter-associated candidemia compared with 76% with persistent candidemia or IC [20, 21]. On the whole, the data highlight that PCR and BDG will be most useful as diagnostic adjuncts to blood cultures rather than as replacements. Indeed, the sensitivity of blood culture combined with PCR or BDG among patients with IC was 98% and 79%–81%, respectively, which was better than any of the tests alone. Since positive blood cultures by definition are diagnostic of IC, they improve the sensitivity of nonculture–based assays without impacting

Table 5. Impact of Antifungal Therapy on Polymerase Chain Reaction and 1,3-β-D-Glucan Assay Performance

Assay	Invasive Candidiasis (n = 55)	Candidemia[a] (n = 22)	Deep-seated Candidiasis[a] (n = 38)
PCR sensitivity			
On antifungal therapy	77% (23/30)	62% (10/16)	83% (15/18)
Not on antifungal therapy	84% (21/25)	50% (3/6)	95% (19/20)
P value	.74	.66	.33
BDG sensitivity[b]			
On antifungal therapy	56% (17/30)	58% (11/16)	56% (10/18)
Not on antifungal therapy	73% (14/25)	67% (4/6)	50% (10/20)
P value	1.0	1.0	.76
BDG sensitivity[c]			
On antifungal therapy	67% (20/30)	81% (13/16)	61% (11/18)
Not on antifungal therapy	72% (18/25)	83% (5/6)	70% (14/20)
P value	.77	1.0	.73

Abbreviations: BDG, 1,3-β-D-glucan; PCR, polymerase chain reaction.

[a] Candidemia and deep-seated candidiasis groups included 5 patients who had both conditions.

[b] Positive BDG defined as ≥80 pmol/mL (indeterminate = negative).

[c] Positive BDG defined as ≥60 pmol/mL (indeterminate = positive).

Table 6. Agreement Between Polymerase Chain Reaction and 1,3-β-D-Glucan Assays, Stratified by Type of Invasive Candidiasis

	Invasive Candidiasis						
		Candidemia		Intra-abdominal Infections			Other Deep-seated Infections
Test Results	Total[a] (n = 55)	Blood Cultures, Catheter Only (n = 3)	Blood Cultures, Peripheral +/− Catheter (n = 14)	Blood and Intra-abdominal Cultures Positive (n = 5)	Blood Cultures Negative (n = 20)	Blood Cultures Not Drawn (n = 9)	Blood Cultures Not Drawn (n = 4)
PCR^+/BDG^{+a}	42% (23/55)	0% (0/3)	43% (6/14)	40% (2/5)	50% (10/20)	44% (4/9)	25% (1/4)
PCR^+/BDG^-	38% (21/55)	0% (0/3)	29% (4/14)[b]	20% (1/5)[b]	45% (9/20)[b]	44% (4/9)[b]	75% (3/4)[c]
PCR^-/BDG^{+a}	15% (8/55)	67% (2/3)	21% (3/14)	40% (2/5)	5% (1/20)	0% (0/9)	0% (0/4)
PCR^-/BDG^-	5% (3/55)	33% (1/3)	7% (1/14)[b]	0% (0/5)	0% (0/20)	11% (1/9)	0% (0/4)

Abbreviations: BDG, 1,3-β-D-glucan; PCR, polymerase chain reaction.

[a] Positive BDG defined as ≥80 pmol/mL (indeterminate = negative). If positive BDG was defined as ≥60 pmol/mL (indeterminate = positive), the agreement between assays among patients with invasive candidiasis was as follows: PCR^+/BDG^+ (53%, 29 of 55), PCR^-/BDG^- (4%, 2 of 55), PCR^+/BDG^- (27%, 15 of 55), and PCR^-/BGD^+ (16%, 9 of 55). The sensitivity and specificity of either a positive PCR or BDG were 96% (53 of 55) and 47% (34 of 73), respectively.

[b] One patient had BDG in the indeterminate range (60–79 pmol/mL).

[c] Two patients had BDG in the indeterminate range (60–79 pmol/mL).

their specificity. Blood cultures also permit antifungal susceptibility testing. The combination of PCR and BDG was less useful. Depending on the BDG cutoff, the sensitivity of the combined tests was 95%–96%, but specificity was only 47%–56%.

It is difficult to compare our results with previous reports because of differences in study design, definitions of IC, patient populations and negative controls, and interpretive criteria [7, 16]. Technical issues also differ, including the fraction of specimens subjected to DNA extraction, choice of primers and probes, amplification parameters in PCR studies, and the particular detection assay for BDG [6, 22]. In this regard, the use of a validated and publically available PCR assay in this study is a major advance, which should facilitate comparisons between centers and populations in the future. Along these lines, it is notable that we corroborated earlier observations that the sensitivity of PCR on plasma or sera was superior to whole blood [13, 18, 23, 24], which likely reflects an absence of PCR inhibitors, less cumbersome DNA extraction methods, and the more ready detection of cell-free fungal DNA within these compartments. On balance, the performance of PCR was in keeping with other reports, in which sensitivities ranged from 73% to 95% [7, 16]. In studies of BDG monitoring for early diagnosis of IC, sensitivity and specificity varied widely (64%–100% and 71%–98%, respectively) [4–6, 22]. The lower sensitivity of BDG in our experience may reflect the large percentage of patients with deep-seated candidiasis.

Overall, PCR and BDG demonstrated adequate specificity (70% and 63%–73%, respectively, depending on BDG cutoff). In fact, the specificity of PCR and BDG in this study is likely to be lower than in most clinical practices, due to the composition

Table 7. Patients With Invasive Candidiadis and False-Negative Results by Both Polymerase Chain Reaction and 1,3-β-D-Glucan Assays

Type of Invasive Candidiasis	*Candida* Species	1,3-β-D-Glucan Result	Antifungal Therapy Prior to Sample Collection	Source of Invasive Candidiasis	Comments
Candidemia	*C. parapsilosis*	Indeterminate (62 pmol/mL)	None	Catheter and peripheral cultures positive	Catheter removed prior to sample collection
Candidemia	*C. glabrata*	Negative (36 pmol/mL)	None	Catheter-associated (only catheter cultures positive; multiple peripheral negative)	Catheter removed prior to sample collection
Deep-seated (no blood culture obtained)	*C. albicans*	Negative (<31 pmol/mL)	None	Intra-abdominal abscess from perforated peptic ulcer	Immediate surgical debridement

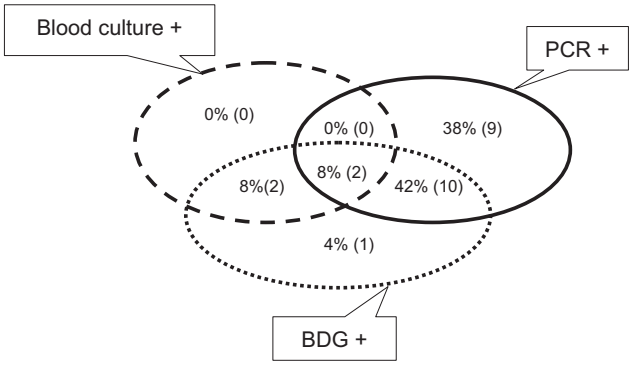

Figure 1. Venn diagram of sensitivities of blood culture, polymerase chain reaction (PCR), and 1,3-β-D-glucan (BDG) assays among patients with deep-seated candidiasis.

of our control group. Seventy-five percent of controls were colonized with *Candida* or had mucosal candidiasis, 36% were immunosuppressed, and almost half either underwent organ transplantation or had underlying gastrointestinal disease. Indeed, in several instances, our false-positive results may have represented unrecognized IC. As an example, 1 PCR-positive control was diagnosed with severe esophageal candidiasis following endoscopy for hematemesis. It is plausible that extensive mucosal disruption allowed *Candida* cells or DNA, which was not detected by blood cultures due to their poor sensitivity and/or the effect of antifungal therapy, to penetrate into the bloodstream. Colonization is generally accepted as the principal factor limiting the specificity of PCR, but relatively few studies have investigated the issue for candidiasis. In a recent meta-analysis, there was a trend toward lower specificity of *Candida* PCR among colonized patients [16]. False-positive BDG results have also been attributed to *Candida* colonization, systemic bacterial infections, antibiotics, cellulose membranes used during hemodialysis, and cotton gauze and sponges [25]. Of note, the specificity of BDG at the lower cutoff for positivity (≥60 pmol/mL) was only 50% among colonized patients, which may limit the utility of this cutoff.

A possible advantage of PCR over BDG is the ability for speciation. The PCR assay used in this study was designed to distinguish fluconazole-susceptible species (*C. albicans* and *C. tropicalis*) from intrinsically or potentially resistant species (*C. krusei* and *C. glabrata*) and *C. parapsilosis*, which often demonstrates reduced echinocandin susceptibility. Overall, speciation by culture and species-specific PCR was in agreement in 82% of patients. There are several potential explanations for disagreements in the remaining cases. First, PCR speciation may be incorrect. In most instances, we do not think this was the case because there was no misspeciation of *Candida* isolates in preliminary experiments (Table 1). Second, the speciation in our clinical lab may be incorrect. Indeed, studies of clinical labs have indicated that *Candida* species were misidentified in 8%–15% of specimens [26–29]. Third, the clinical lab may have missed cases caused by multiple species. We found that 2 species were identified in 5% of blood cultures, which is consistent with rates of at least 4% reported in the literature [30, 31]. At the same time, PCR identified at least 2 species in 36% of samples, suggesting that the clinical laboratory may not have isolated unique colonies for speciation. Fourth, time lags between culture and PCR sample collection may have contributed to disagreements, because it is possible that a particular infecting *Candida* species was no longer in the circulation. Finally, many of the cultures were performed on deep-seated

Table 8. *Candida* Speciation by Culture and Polymerase Chain Reaction.

	Candida PCR Results					
Culture Results	CA/CT	CG/CK	CA/CT and CG/CK	CA/CT and CP	CG/CK and CP	CA/CT, CG/CK and CP
Single species						
C. albicans	10	2	…	2	1	5
C. tropicalis	1	…	…	1	…	…
C. glabrata	4	7	4	…	…	1
C. parapsilosis	1	…	…	…	…	1
Multiple species						
C. albicans and *C. tropicalis*	1	…	…	…	…	…
C. albicans and *C. glabrata*	…	1	…	…	…	…
C. albicans and *C. krusei*	…	…	1	…	…	…
C. tropicalis and C. glabrata	1	…	…	…	…	…

Numbers in the table are the number(s) of *Candida* isolates showing a particular pattern of speciation by the 2 methods. There was complete agreement in speciation between polymerase chain reaction (PCR) and culture for 45% (20/44) of patients and complete disagreement for 18% (8/44) of patients. PCR identified multiple species sets in 36% (16/44) of patients, whereas culture revealed multiple species in 9% (4/44; $P = .01$, Fisher exact test).

Abbreviations: CA, *Candida albicans*; CG, *Candida glabrata*; CK, *Candida krusei*; CP, *Candida parapsilosis*; CT, *Candida tropicalis*; PCR, polymerase chain reaction.

samples, whereas PCR was performed on plasma or sera. Different species may have been present at different sites. If culture and PCR give discordant results in clinical practice, the wisest course is to treat as if the more resistant species is present. In this regard, it is notable that the most common disagreement was blood culture identifying *C. albicans* and PCR identifying *C. glabrata* or *C. krusei*.

It is important to acknowledge limitations of our study. First, PCR and BDG testing was performed in batches on frozen samples. As such, we cannot exclude that some negative results may have stemmed from sample instability. Second, antifungal use did not impact the performance of PCR or BDG, but the majority of patients received an azole. Thus, we could not assess the potential impact of BDG synthesis inhibition by the echinocandins on the performance of the BDG assay. Third, the number of controls with mucosal candidiasis was small; therefore, the specificity of PCR and BDG in this population needs further study. Fourth, blood for BDG and PCR was obtained after the diagnosis of IC was confirmed by culture and at only 1 time point, which precluded the evaluation of the tests for disease screening. Future studies should include serially collected blood samples from patients at high risk for IC.

In conclusion, we demonstrated that *Candida* PCR and, to a lesser extent, BDG testing significantly enhanced the ability of blood cultures to diagnose IC. As for all diagnostic tests, best results will be obtained if PCR and BDG are limited to situations in which there is a reasonable likelihood of IC. Employed judiciously, these assays have the potential to identify a large population of patients with deep-seated candidiasis missed by blood cultures. If our findings are validated in other studies, the results will have a major impact on the treatment of IC, our understanding of its pathogenesis, and the design of clinical trials. Follow-up studies to evaluate the impact of *Candida* PCR and BDG on the diagnosis, treatment, and outcome of IC are indicated.

Notes

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