Current Fungal Infection Reports (2018) 12:71–77
https://doi.org/10.1007/s12281-018-0313-1

ADVANCES IN DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS (S CHEN, SECTION EDITOR)

# PCR-Based Methods for the Diagnosis of Invasive Candidiasis: Are They Ready for Use in the Clinic?

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Published online: 3 April 2018


## Abstract

**Purpose of Review** We review the performance of Candida PCR and the T2Candida panel (T2Biosystems, Lexington, MA) in diagnosing invasive candidiasis, consider how these tests may be incorporated into patient care, and determine if they are ready to be used in the clinic.

**Recent Findings** PCR and T2Candida sensitivity/specificity for diagnosing candidemia are ~ 90%/90% and ~ 90%/98%, respectively. Limited data for intra-abdominal candidiasis suggest PCR sensitivity of ~ 85–90%, but specificity has varied from 33 to 97%. T2Candida data are lacking for infections other than candidemia.

**Summary** PCR and T2Candida will have the greatest value if their use is restricted to cases in which positive and negative predictive values differ in a clinically meaningful way from the pre-test likelihood. Studies are needed to establish that patient care and stewardship strategies incorporating Candida PCR or T2Candida improve patients' outcomes, reduce unnecessary antifungal usage, limit emergence of resistance, and are cost-effective. The development and validation of standardized PCR assays is a top priority.

**Keywords** Candidiasis · Candidemia · PCR · T2Candida · Diagnostic · Bayesian

## Introduction

The clinical entity of invasive candidiasis encompasses bloodstream and deep tissue infections by Candida species [1]. Candidemia is among the four most common bloodstream infections in hospitals of the developed world. Intra-abdominal candidiasis, the most common type of non-hematogenous, deep-seated candidiasis, manifests most frequently as peritonitis or abscesses and may occur as often as candidemia at certain centers [2]. Mortality rates among patients with candidemia or intra-abdominal candidiasis range from 20 to 40% despite antifungal treatment [2, 3]. At least in part, poor outcomes stem from delays in institution of treatment due to the insensitivity of blood cultures, the current diagnostic gold standard. Blood cultures are positive for Candida in < 50% of hematogenously disseminated candidiasis, and < 20% of intra-abdominal candidiasis [1, 2]. Moreover, blood cultures typically turn positive late in the course of invasive candidiasis, sensitivities are diminished after a single dose of an active antifungal agent, and 2 or more days of incubation usually are necessary to detect Candida growth. The development and validation of non-culture diagnostic tests for invasive candidiasis is recognized as a pressing medical priority [4].

Candida albicans germ tube antibody (CAGTA), mannan and anti-mannan IgG, and 1,3-β-D-glucan assays are employed in many parts of the world [5–7]. Meta-analyses of studies assessing mannan/anti-mannan and 1,3-β-D-glucan assays reported sensitivities/specificities of approximately 80%/80% for diagnosing invasive candidiasis [7–10]. CAGTA, mannan/anti-mannan, and 1,3-β-D-glucan may be positive prior to blood cultures, and positive despite negative blood cultures, in some patients with invasive candidiasis. Limitations of these tests include potential for false positivity in high-risk patients (1,3-β-D-glucan), rapid clearance from serum (mannan), diminished responses and delays in

Topical Collection on *Advances in Diagnosis of Invasive Fungal Infections*

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detectability among immunosuppressed hosts (CAGTA and anti-mannan IgG), and an inability to identify Candida species (all assays). Serum 1,3-β-D-glucan testing is studied most extensively, and an assay (Fungitell, Associates of Cape Cod, East Falmouth, MA) is cleared by the US Food and Drug Administration (FDA) for the diagnosis of invasive fungal infections. BDG assays are cumbersome to perform, testing kits not available in many parts of the world, and results do not distinguish between candidiasis and other invasive fungal infections [11].

At least in principle, polymerase chain reaction (PCR)-based methods have the potential to serve as ideal diagnostics for invasive candidiasis (Table 1) [1]. At present, there is no Candida PCR assay that is cleared by FDA. Nevertheless, commercial and in-house tests are widely available. Recently, the T2Candida nanodiagnostic panel (T2Biosystems, Lexington, MA) was FDA-cleared for the diagnosis of candidemia. T2Candida uses a self-contained, automated instrument platform (T2Dx) to detect Candida directly within whole blood in $K_2$ EDTA vacutainer collection tubes, without the need for sample preparation or target extraction steps [12••, 13•]. T2Dx lyses red blood cells, concentrates Candida cells and cellular debris, lyses cells by mechanical bead-beating, and amplifies DNA using a thermostable polymerase and species-specific primers for ribosomal DNA intervening transcribed spacer region 2. Amplified product is detected by amplicon-induced agglomeration of supermagnetic particles and T2 magnetic resonance.

In this paper, we will review the performance of Candida PCR and the T2Candida panel, consider how these tests may be incorporated rationally into patient management strategies, and determine if they are ready to be used in the clinic.

## Clinical Performance of Candida PCR

There is a large body of literature on PCR-based methods for diagnosing invasive candidiasis. The interpretation of PCR data is complicated by heterogeneity of assays and study designs. Multiple methodologies, including multiplex formats capable of detecting other fungi and/or bacteria, have been investigated. In a meta-analysis of 54 studies that included approximately 5000 patients tested by PCR on blood-based samples, pooled sensitivity and specificity for proven or probable invasive candidiasis (candidemia predominantly) vs at-risk controls were 95 and 92%, respectively [14]. Pooled sensitivity and specificity for proven, probable, or possible invasive candidiasis vs at-risk controls were 73 and 95%, respectively. Data for types of invasive candidiasis other than candidemia are limited. In several recent studies, the sensitivity of PCR assays for intra-abdominal candidiasis ranged from 86 to 91%, but specificity varied widely, from 33 to 70 to 97% [5, 6, 15]. Moreover, specificities of 33 and 97% were reported in different studies using the same PCR assay [5, 6]. In the PCR meta-analysis, higher sensitivity was observed with whole blood rather than serum, panfungal rRNA or P450 genes as targets, Candida- or fungal-specific assays rather than broader multiplex assays, and in vitro detection limits ≤ 10 CFU/mL [14]. There was a trend toward lower specificity among controls who were colonized by Candida.

Multiplex PCR tests generally target the five most common pathogenic Candida species (C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei), which account for > 95% of invasive candidiasis at most centers [16]. Other Candida species may be more prevalent at certain centers [17]; therefore, it is imperative that clinicians and laboratories understand their local epidemiology. No PCR assay has been validated for diagnosing invasive candidiasis in multi-center studies, and there is no conclusive evidence that any commercial test is superior. Commercial and in-house PCR assays have been validated internally at many centers. It is reasonable to assume that such assays, if undertaken with adequate quality control measures, will perform as described above in diagnosing candidemia.

**Table 1** Performance characteristics of an ideal diagnostic test for invasive candidiasis

- Blood-based assay
- Requires low-volume samples
- Rapid turn-around
- Minimal labor and laboratory technician time
- Cost-effective
- Sensitive and specific for both bloodstream and deep-seated infections
- Provides species identification
- Multiplex capabilities
- Capacity for detection of antifungal resistance
- Provides diagnostic and prognostic information (e.g., predicts outcomes of infection)

## Clinical Performance of the T2Candida Panel

T2Candida results are reported as positive or negative for C. albicans/C. tropicalis, C. glabrata/C. krusei, and C. parapsilosis, groupings that are based on typical antifungal susceptibility patterns. In the absence of prolonged prior exposure, C. albicans and C. tropicalis generally remain susceptible to antifungal agents. C. glabrata and C. krusei are notable for emergence of resistance to echinocandins and azoles, and intrinsic resistance to fluconazole, respectively. C. parapsilosis is characterized by elevated echinocandin minimum inhibitory concentrations, which are of unclear significance during the treatment of infected patients. Just as Candida species distributions may differ at certain centers, so too may antifungal

susceptibility patterns [17]. The limit of detection for T2Candida depending on species is 1–3 CFU/mL, which is superior to that generally reported for PCR assays [14].

FDA clearance of T2Candida was based on data from the multi-center DIRECT trial, which included > 1500 control patients with Candida-negative blood cultures, 6 patients with Candida-positive blood cultures, and 250 contrived blood specimens spiked with C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, or C. krusei at concentrations ranging from 1 to 100 CFU/mL [18]. Per-patient sensitivity and specificity were 91 and 98%, respectively; there were no significant differences in sensitivity for the five species. The mean time to Candida species identification was 4.4 ± 1.0 h, compared to 129.9 ± 26.3 h for blood cultures. In the follow-up multi-center DIRECT2 trial, T2Candida sensitivity was 89% in 36 patients at the time of positive blood cultures for Candida [12••]. Among 152 patients with prior candidemia (i.e., within 1–6 days), T2Candida was significantly more likely to be positive than concurrently collected blood cultures (45 vs 24%). The higher positivity for T2Candida compared to blood cultures was driven by performance among patients receiving antifungal therapy. Therefore, T2Candida may offer particular advantages over blood cultures for cases in which empiric or prophylactic antifungal treatment has been initiated.

At present, there are no data on T2Candida performance for types of invasive candidiasis other than candidemia. Invalid T2Candida results were obtained for 7–9% of thawed whole blood samples in DIRECT and DIRECT2; rates using fresh blood samples in routine clinical practice are undefined. Other uncertainties for T2Candida and PCR assays include the clinical significance of discrepant T2Candida-positive/culture-negative results, the precise effects of antifungal treatment on assay performance, the kinetics and prognostic value of serial test results, and the tests' roles in guiding patient care.

## Candida PCR and T2Candida as Bayesian Biomarkers

Candida PCR and T2Candida are not categorical diagnostics, but rather Bayesian biomarkers that assign a probability of infection [19, 20]. Positive and negative predictive values (PPVs, NPVs) are determined by sensitivity and specificity, and the patient's pre-test likelihood of invasive candidiasis. Management decisions based on test results will be left to the best judgment of providers.

Pre-test likelihoods of candidemia and intra-abdominal candidiasis can be estimated in patients with signs of infection from data on disease prevalence in various clinical settings. Risk factors for candidemia are relatively common in hospitalized patients, including receipt of broad-spectrum antibiotics, presence of intravenous access devices, total parenteral nutrition, mechanical ventilation, hemodialysis, diabetes mellitus, corticosteroids, neutropenia or neutrophil dysfunction, and Candida colonization. The prevalence of candidemia increases from < 1 to ~ 10% as one moves from any patient in whom blood cultures are collected, to low-risk intensive care unit (ICU) patients, to

**Table 2** Prevalence of candidemia in different populations and anticipated PPVs and NPVs of PCR and T2Candida

| Prevalence | Representative patient (Reference) | PCR[1] | | T2Candida[2] | |
|---|---|---|---|---|---|
| | | PPV | NPV | PPV | NPV |
| 0.4% | Any hospitalized patient in whom a blood culture is collected [13•] | 3% | >99.9% | 15% | >99.9% |
| 1% | Patient admitted to intensive care unit [21, 22] | 8% | 99.9% | 31% | 99.9% |
| 2% | Patient with febrile neutropenia, baseline rate of candidemia prior to empiric antifungal treatment [23-26] | 16% | 99.8% | 47% | 99.8% |
| 3% | Patient with septic shock and >3-7 day stay in intensive care unit [22, 27-29] | 22% | 99.6% | 67% | 99.7% |
| 5% | Patient with left ventricular assist device and evidence of active infection [30, 31] | 32% | 99.4% | 70% | 99.5% |
| 10% | Patient at increased risk for candidemia based on clinical prediction models [32-34] | 50% | 98.8% | 82% | 99% |

Sensitivity and specificity of each assay for candidemia are estimated from a PCR meta-analysis and T2Candida DIRECT and DIRECT2 studies [12–14]. PPVs and NPVs within the dark black lines signify patients in whom non-culture testing may have greatest clinical utility, assuming that antifungal treatment is justified at a threshold likelihood of invasive candidiasis of ≥ ~ 15–30%. For the patients indicated, a positive result is anticipated to move the likelihood of candidemia from below the threshold to above the threshold. At the same time, negative tests make candidemia extremely unlikely (≤ 3% probability). The precise borders of the box may vary somewhat, depending on where within the 15–30% range the threshold value is set. Treatment interventions based on this conceptual framework warrant validation in clinical trials

*PPV* positive predictive value, *NPV* negative predictive value

[1] Sensitivity/specificity, 90%/90%

[2] Sensitivity/specificity, 90%/98%

more moderate-risk patients who are ICU residents for ≥ 4 days or who are in septic shock, to higher-risk ICU patients identified by clinical prediction scores (Table 2) [12••]. Intra-abdominal candidiasis occurs in a subset of patients who, in addition to risk factors for candidemia, have predisposing GI tract or digestive system abnormalities. The prevalence of intra-abdominal candidiasis increases from ~ 5 to ~ 30% as one moves from low-to-moderate-risk peritoneal dialysis patients with peritonitis, to high-risk patients with severe necrotizing pancreatitis or recurrent GI tract leaks (Table 3) [20, 35–38]. In most patients in whom an infection is suspected, the predominant type of invasive candidiasis should be apparent when a test is ordered.

Anticipated PPVs and NPVs of Candida PCR and T2Candida for the diagnosis of candidemia in various patient populations can be calculated using published sensitivities and specificities (Table 2). However, such calculations cannot be made confidently for intra-abdominal candidiasis due to conflicting specificity data from different studies of Candida PCR, and the lack of data for T2Candida (Table 3). At low pre-test likelihoods of candidemia, PPVs and NPVs are extremely low and extremely high, respectively. As likelihoods increase, PPVs increase and NPVs decrease. For each type of patient at risk for candidemia in Table 2, NPVs of PCR and T2Candida are exceptional (> 98%). Anticipated PPVs increase to 50 and 82%, respectively, for relatively high-risk ICU patients

**Table 3** Prevalence of intra-abdominal candidiasis in different populations and impact of PCR specificity on anticipated PPVs and NPVs

| Prevalence (Reference) | Representative patient | PCR | | | | | |
|---|---|---|---|---|---|---|---|
| | | [1]Leon *et al.* [6] | | [2]Nguyen *et al.* [15] | | [3]Fortun *et al.* [5] | |
| | | PPV | NPV | PPV | NPV | PPV | NPV |
| 5% [35, 36] | - Low-to-moderate risk peritoneal dialysis patient with peritonitis | 6% | 97.7% | 13% | 98.9% | 59% | 99.2% |
| 10% [37] | - Patient with emergent surgery for intra-abdominal infection<br>- Patient with colonic perforation | 12% | 95.2% | 24% | 97.7% | 76% | 98.3% |
| 20% [35, 37] | - Patient with high-risk severe acute or necrotizing pancreatitis<br>- Patient with small bowel perforation<br>- Patient with emergent surgery for nosocomial intra-abdominal infection | 24% | 89.9% | 41% | 94.9% | 88% | 97.5% |
| 30% [38, 39] | - Patient who has undergone high-risk GI/hepatobiliary surgery<br>- Patient with a biliary leak<br>- Patient with a gastric/duodenal perforation | 35% | 83.7% | 55% | 91.6% | 93% | 93.8% |

Sensitivity and specificity of PCR are estimated from three studies of deep-seated candidiasis [5, 6, 15]. Sensitivity was rounded to 85% here for comparative purposes. There are no data on the performance of T2Candida for the diagnosis of deep-seated candidiasis, in the absence of candidemia. PPVs and NPVs within the dark black lines signify patients in whom non-culture testing may have greatest clinical utility, assuming that antifungal treatment is justified at a threshold likelihood of invasive candidiasis of ≥ ~ 15–30%. For these patients, a positive result is anticipated to move the likelihood of intra-abdominal candidiasis from below the threshold to above the threshold. At the same time, negative tests should assure that the likelihood of intra-abdominal candidiasis is less than the threshold. The precise borders of the box may vary somewhat, depending on where within the 15–30% range the threshold value is set. Taken together, the data suggest that Candida PCR would have no clinical value if specificity is only 33%. PCR would have a value in patients at moderate to high risk for intra-abdominal candidiasis if specificity is 70%. PCR would be expected to be useful in any patient at risk for intra-abdominal candidiasis if specificity is 97%. Treatment interventions based on this conceptual framework warrant validation in clinical trials

*PCR* polymerase chain reaction, *PPV* positive predictive value, *NPV* negative predictive value, *GI* gastrointestinal

[1] Sensitivity/specificity, 85%/33%

[2] Sensitivity/specificity, 85%/70%

[3] Sensitivity/specificity, 85%/97%

who fulfill criteria of clinical prediction scores for candidemia. The impact of different PCR specificities for intra-abdominal candidiasis on PPVs and NPVs is dramatic, as summarized in Table 3.

## How Candida PCR and T2Candida Might Be Utilized in the Clinic

The threshold probability of invasive candidiasis that justifies antifungal treatment is not known. A number of studies in patients with hematologic malignancies, critical illnesses, and/or multiple risk factors for invasive fungal infections suggest that antifungal prophylaxis is beneficial if the baseline rate of disease is ≥ 15–30% [19, 20]. Therefore, the target PPV and NPV for triggering empiric treatment of invasive candidiasis are likely to be in the 15–30% range and > 85%, respectively. Based on these targets, PCR and T2Candida are likely to have value in some, but not all, patients who are at risk for candidemia (Table 2). At a particular pre-test likelihood of candidemia, a test becomes useful to a provider if a positive result increases the probability of disease above the 15–30% threshold, while a negative result virtually excludes the diagnosis. Given these considerations, it is readily apparent that neither PCR nor T2Candida is likely to have value for diagnosing candidemia if ordered anytime a blood culture is collected, since anticipated PPVs are ≤ 15% and NPVs are not significantly lower than the pre-test probability. In contrast, PCR may be helpful in guiding treatment decisions for patients in septic shock, and even more so for patients identified by clinical prediction scores. If T2Candida sensitivity and specificity truly are 90 and 98%, respectively, results may be useful in patients with pre-test likelihoods of candidemia as low as 1% (e.g., ICU patients with unexplained fevers).

Tables 2 and 3 provide a conceptual framework for interpreting PCR and T2Candida results. Of course, there are multiple other factors that providers must weigh as they use results to make treatment decisions for individual patients. Considerations such as number and types of risk factors for candidiasis, severity of illness, physical findings, imaging and lab data, and the possibility of alternative diagnoses may increase or decrease the pre-test likelihood of disease. Likewise, post-test probability may be influenced by the magnitude of results; two highly positive values are more compelling than a single borderline result. It is infeasible for clinicians to calculate precise running tallies of pre- and post-test likelihoods in each patient. Nevertheless, they can conceptualize probabilities qualitatively. Examples of qualitative evaluations that can guide decision-making are “my patient is reasonably likely to have candidemia, and a positive result significantly increases that possibility”, or “my patient has some risk factors for candidemia, but a negative result makes the disease extremely unlikely.”

## Conclusions

Are Candida PCR and the T2Candida panel ready for use in the clinic? The discussion above suggests that the answer to this question is “yes, with important caveats.” Clinicians must be familiar with the Bayesian nature of PCR and T2Candida results. Testing should be directed to patients at some risk for candidemia, and clinicians should pre-determine how results will be used to guide treatment. If a positive or negative result will not impact decisions on initiating or discontinuing antifungal therapy, then, a test should not be ordered. Rational treatment decisions will depend upon the anticipated PPV and NPV in a given patient, and how these values alter the pre-test likelihood of candidemia. It is not possible to estimate predictive values without understanding the local prevalence and microbiology of candidemia. For Candida PCR, clinical laboratories should assure that assays are internally validated. Finally, PCR and T2Candida are adjuncts to blood cultures, rather than replacements for them.

Used and interpreted judiciously, Candida PCR and T2Candida promise to identify at least some patients with candidemia earlier than cultures, other patients with candidemia who are missed by cultures, and large numbers of patients in whom candidemia is extremely unlikely. T2Candida and other PCR-based methods may be especially useful in patients receiving empiric or prophylactic antifungal treatment, as they are more likely to remain positive than blood cultures. Moving forward, studies are needed to establish that patient management and stewardship strategies incorporating PCR or T2Candida improve outcomes of individuals with (or at risk for) candidemia, reduce unnecessary antifungal usage, limit emergence of resistance, and are cost-effective. For both methods, data are needed urgently on diagnosing intra-abdominal and other non-hematogenous, invasive candidiasis. In addition, standardized PCR assays must be validated in multi-center trials, and the encouraging turn-around times and performance of T2Candida in the DIRECT studies must be corroborated during routine practice, outside of clinical trial settings. The Bayesian framework described here should be useful in designing future PCR and T2Candida studies, and in investigating new non-culture diagnostics that enter the clinic.

## Compliance with Ethical Standards

**Conflict of Interest** Drs. Clancy and Nguyen have served as principal investigators for clinical trials sponsored by T2 Biosystems and ViraCor Eurofins. Dr. Clancy has spoken at symposia sponsored by T2 Biosystems.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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