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Diagnosing Invasive Candidiasis

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Abstract.

Cultures are negative in ~50% of invasive candidiasis. Data are emerging for the performance of non-culture tests like mannan/anti-mannan, *Candida albicans* germ tube antibody, 1,3-β-D-glucan, polymerase chain reaction, and the T2Candida panel in diagnosing both candidemia and deep-seated candidiasis. In most settings, positive predictive values of non-culture test are low, and negative predictive values are high. For tests to be useful, clinicians must understand the pre-test likelihood of invasive candidiasis and test performance for the most common disease manifestation in a given patient. This paper reviews non-culture *Candida* diagnostics, and discusses how they might be used effectively in patient care.

Introduction.

There is an urgent need to develop and validate non-culture diagnostic tests for candidemia and other types of invasive candidiasis. *Candida* species are among the most common causes of nosocomial bloodstream infections, and of invasive infections in intensive care units (ICUs). Timely antifungal therapy and source control are crucial determinants of survival in patients with invasive candidiasis.(1, 2) However, definitive treatment often is delayed due to the insensitivity of microbiologic cultures, the gold standard diagnostic.(3) Several non-culture diagnostics are now available for use as adjuncts to cultures, but there is widespread uncertainty about their utility in clinical practice. The objectives of this paper are to review the performance of cultures and non-culture tests in diagnosing invasive candidiasis, and to consider how the latter might be used effectively.

Cultures and diagnosing the spectrum of invasive candidiasis.

It is impossible to interpret diagnostic test results for invasive candidiasis without understanding the spectrum of disease. Invasive candidiasis comprises candidemia and deep-seated candidiasis, which may occur concurrently or independently.(3) Primary candidemia stems most often from gastrointestinal (GI) tract translocation of commensal *Candida* or contamination/colonization of an intravenous catheter. Approximately 50% of primary candidemia causes secondary deep-seated candidiasis due to hematogenous seeding. Deep-seated candidiasis may also result from non-hematogenous introduction of *Candida* into sterile sites, most commonly the abdominal cavity following GI tract disruption or via an infected peritoneal catheter. Only 5-20% of

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such primary deep-seated candidiasis leads to candidemia (secondary candidemia). Therefore, diagnostic tests must identify three entities: 1) candidemia in the absence of deep-seated candidiasis; 2) candidemia associated with deep-seated candidiasis; and 3) deep-seated candidiasis in the absence of candidemia.

Cultures are sensitive at detecting viable *Candida*. At the time of first positive blood culture, the median *Candida* concentration is 1 colony forming unit (CFU)/mL.(4) The limit of detection of viable *Candida* by blood cultures is equivalent or superior to that for methods such as polymerase chain reaction (PCR). Blood cultures are positive in most patients if collected during active candidemia. However, they are positive in only ~40% of patients with candidemia complicated by deep-seated infection, which persists after *Candida* have been cleared from the bloodstream, and they are negative during deep-seated candidiasis that is not associated with candidemia. Across the spectrum of invasive candidiasis, the sensitivity of blood cultures is ~50%. Other limitations of blood cultures include slow turn-around and the fact that they may not turn positive until late in the disease course. Fungal selective media can improve blood culture sensitivity and shorten the time to positivity.(5) However, the clinical impact of selective media on identifying patients with candidemia or deep-seated candidiasis is unknown. Cultures of material collected from deep sites of infection are also only ~50% sensitive, likely reflecting small sample volumes, and uneven distribution and low burdens of *Candida* cells.(3) Moreover, the collection of deep tissue cultures requires invasive procedures that may be risky or contra-indicated in patients at-risk for *Candida* infections.

Non-culture tests for invasive candidiasis.

Mannan, anti-mannan antibody, and C. albicans germ tube antibody (CATGA).

The earliest non-culture diagnostics for invasive candidiasis were serum assays for *Candida* antigens and anti-*Candida* antibodies.(3) Most *Candida* antigens are limited as diagnostics by low serum concentrations and rapid clearance from the bloodstream.(6) The most successful targets are abundant constituents of the cell wall, such as mannan and 1,3-β-D-glucan (BDG). The major concerns about anti-*Candida* antibody assays are that sensitivity may be diminished among immunosuppressed hosts, time is needed to mount detectable responses, and positive results may not distinguish acute from past infections. Despite these concerns, various antibody assays have performed well in studies, including in patients with neutrophil and cell-mediated immune defects.(6) Assays measuring serum immunoglobulin G (IgG) responses, in general, have performed better than assays measuring IgM, suggesting that many patients mount rapid amnestic responses.(3, 6) Patients infected with non-*C. albicans* species can be identified by responses to *C. albicans* antigens.

Mannan and anti-mannan IgG tests (Platelia Candida Ag-Plus and Ab-Plus, Bio-Rad, Marnes-la-Coquette, France; Serion Mannan Kit, Serio GmbH, Wurzburg, Germany), and *C. albicans* germ tube antibody assays (CAGTA; Vircell Kit and VirClia IgG Monotest, Grenada, Spain) are employed at many European centers. These tests are not widely used in North America, nor are they cleared by the United States Food and Drug Administration (FDA). In a meta-analysis of 14 studies, the sensitivity and specificity of mannan and anti-mannan for invasive candidiasis were 58% and 93%, and 59% and 86%, respectively.(7) Sensitivity and specificity for a combined mannan/anti-

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mannan assay were 83% and 86%, respectively, with best performance in patients with *C. albicans*, *C. glabrata* or *C. tropicalis* infections. Data are less extensive for CAGTA, which detects responses against a hyphal protein (Hwp1) expressed during tissue invasion and biofilm formation.(8) The sensitivity and specificity of CAGTA for invasive candidiasis have ranged from 42%-96% and 54%-100%, respectively, in different reports.(8-11) In one study, CAGTA sensitivity was 69% for candidemia complicated by deep-seated candidiasis, compared to only 5% for candidemia in the absence of deep-seated candidiasis.(8) Sensitivity may be lower for infections caused by *C. tropicalis* than other *Candida* species.

BDG.

BDG is a major cell wall constituent of *Candida* and most pathogenic fungi excluding *Cryptococcus* species, *Blastomyces* species and *Mucorales*. Several commercial assays have been developed, of which the Fungitell test (Associates of Cape Cod, East Falmouth, MA) has been studied most extensively. Fungitell is FDA-cleared as a serum assay for the diagnosis of invasive fungal infections. Fungitell and other assays do not directly measure BDG concentrations, but rather use colorimetric or turbidimetric methods to quantify the activation rate of a horseshoe crab coagulation cascade that is triggered by binding BDG. Commercial kits employ reagents derived from different horseshoe crab species, and cut-off values for positive results vary. Data from comparative studies are insufficient to determine if there are clinically significant differences in performance between assays. BDG, mannan, anti-mannan, and CATGA assays do not identify *Candida* species. BDG cannot distinguish between *Candida* and other fungi.

In meta-analyses of serum BDG studies, pooled sensitivity and specificity for invasive candidiasis were ~75%–80% and ~80%, respectively (95% confidence intervals (CI): ~65%-85% and ~75%-85%, respectively).(12, 13) Performance is better if positivity is defined by two consecutive results, rather than a single result.(14) BDG sensitivity may be reduced for *C. parapsilosis* infections. Interpretation of BDG studies is complicated by heterogeneity in patient and control populations, types of *Candida* (or other fungi), testing schedules, specific assays and definitions of positive results, prior antifungal therapy, and other aspects of research design and data analysis. Most studies have employed cohort and case-control designs, in which cases were proven or probable infections and controls were patients without invasive fungal infections. Such studies may overstate performance by excluding possible disease or difficult-to-interpret cases that are encountered commonly in practice. The major concern over BDG testing is false-positivity. As discussed in detail below, the low prevalence of invasive candidiasis in most clinical settings assures that any non-culture diagnostic will generate false-positive results. At the same time, various factors associated with BDG false-positivity are common among hospitalized patients, including *Candida* or mould colonization, human blood products, hemodialysis or hemofiltration, some Gram-positive bacteria, certain β-lactam antibiotics, cellulose dressings, enteral nutrition, mucositis, and disruptions of GI tract integrity. In some populations in which several of these factors are often present, such as early lung transplant recipients, false-positive results may be particularly common.(15) Studies of BDG testing of cerebrospinal fluid and other sample types report promising results.(16)

BDG assays are relatively laborious, and kits include a single-use plate that holds >20 samples. Many hospital labs batch tests or send samples to a reference lab, which may eliminate advantages in turn-around compared to culture.

PCR.

There are no FDA-cleared PCR assays for *Candida*, but commercial and in-house tests are widely available. The vast majority of *Candida* PCR data is for whole blood or blood fractions. Even more so than for BDG, interpretation of PCR data is complicated by heterogeneity of assays and study design. Multiple commercial and in-house tests, including multiplex formats capable of detecting other fungi and/or bacteria, have been investigated. In a meta-analysis of 54 studies that included almost 5000 patients tested by blood-based PCR, pooled sensitivity and specificity for proven or probable invasive candidiasis *vs.* at-risk controls were 95% (95% CI: 82-98%) and 92% (95% CI: 87-98%), respectively.(17) Pooled sensitivity and specificity for proven, probable or possible invasive candidiasis *vs.* at-risk controls were 73% (95% CI: 58-83%) and 95% (95% CI: 92-97%), respectively. 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. There was a trend toward lower specificity among *Candida*-colonized controls.

Commercial 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 hospitals.(18) Since

microbiology can differ by center, clinicians and labs must be aware of local data.(19) 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. PCR offers potential advantages over the tests above by providing species identification.

T2Candida panel.

The T2Candida nanodiagnostic panel is FDA-cleared for the diagnosis of candidemia. T2Candida detects *Candida* directly within whole blood, in an automated process that uses K_2 EDTA vacutainer collection tubes and a dedicated instrument platform (T2Dx). 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 primers for ribosomal DNA intervening transcribed spacer region 2. Amplified product is detected by amplicon-induced agglomeration of supermagnetic particles and T2 magnetic resonance. T2Candida will not amplify freely-circulating, non-cell-associated DNA. 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. The limit of detection is 1-3 CFU/mL.(18)

FDA clearance of T2Candida was based on data from the multi-center DIRECT trial, which included >1,500 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 1-100 CFU/mL.(20) Per-patient sensitivity and specificity were 91% and 98%, respectively. The mean time to *Candida* detection and species

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identification was 4.4±1.0 hours. In the follow-up, multi-center DIRECT2 trial, T2Candida sensitivity was 89% in 36 patients at the time of positive blood cultures for *Candida*.(21) Among 152 patients with prior candidemia (i.e., within 1-6 days), T2Candida was significantly more likely to remain positive than concurrently collected blood cultures (45% *vs.* 24%). The higher positivity for T2Candida was driven by performance among patients receiving antifungal therapy.

Invalid T2Candida results were obtained for 7-9% of whole blood samples in DIRECT and DIRECT2. T2Candida performance in testing samples re-collected from patients with invalid results is undefined. More data are needed on T2Candida in routine practice, outside of large clinical trials. As for other non-culture diagnostics, uncertainties surround the clinical significance of discrepant T2-positive/culture-negative results, the precise effects of antifungal treatment on assay performance, the kinetics and prognostic value of serial test results, and the test's role in guiding patient care and limiting antifungal usage.

Recent data on diagnosing intra-abdominal candidiasis.

T2Candida data and the vast majority of data for other assays are for the diagnosis of candidemia. Several recent studies have explored non-culture tests for the diagnosis of deep-seated infections, in particular intra-abdominal candidiasis, and included rigorous control groups that were comprised largely of at-risk ICU patients (Table 1).(9, 10, 22, 23) Blood culture sensitivity was ≤20%. Mannan and anti-mannan IgG were included in one multi-center study, which found poor sensitivity (40% and 25%, respectively). Across several studies, the performance of CATGA and BDG in

identifying deep-seated candidiasis was roughly similar. CATGA sensitivity and specificity ranged from 53%-73% and 54%-80%, respectively. For BDG, the corresponding ranges were 56%-77% and 57%-83%. The sensitivity of PCR assays ranged from 86%-91%, but specificity varied widely, from 33% to 70% to 97%. The studies with the highest and lowest specificity used the same multiplex PCR assay. Since culture is a suboptimal gold standard, specificity is a major uncertainty in any study of *Candida* diagnostics, especially if controls are at-risk for invasive candidiasis.

Interpreting non-culture test results.

No matter how sensitive or specific a non-culture assay for invasive candidiasis may be, clinicians must accept a level of uncertainty when interpreting results. Indeed, non-culture tests are Bayesian biomarkers that assign a probability of infection, rather than definitive diagnostics.(24) Positive and negative predictive values (PPVs, NPVs) are dependent upon sensitivity and specificity, and the pre-test likelihood of invasive candidiasis. Pre-test likelihoods of candidemia and intra-abdominal candidiasis can be estimated from disease prevalence in various clinical settings.

Candidemia is a low-prevalence disease among relatively large at-risk populations. Risk factors such as broad-spectrum antibiotics, intravenous access devices, total parenteral nutrition, mechanical ventilation, hemodialysis, diabetes mellitus, corticosteroids, neutropenia or neutrophil dysfunction, and *Candida* colonization are non-specific and common in hospitalized patients. The prevalence of candidemia increases from <1% to ~10% as one moves from any patient in whom blood cultures are collected, to low-risk ICU patients, to 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). In contrast, intra-abdominal candidiasis is a relatively high-prevalence disease among more narrowly-defined populations. In addition to the factors above, patients also 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).(24-28) In most patients, the predominant type of invasive candidiasis should be apparent when a test is ordered.

Using the sensitivities and specificities of different tests for candidemia and intra-abdominal candidiasis, anticipated PPVs and NPVs can be calculated (Tables 2 and 3). At low pre-test likelihoods of either disease, 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 non-culture diagnostics are exceptional (≥97%). If the combined mannan/anti-mannan and BDG assays perform as reported in meta-analyses, PPVs are anticipated to increase to ~30% for high-risk ICU patients who fulfill clinical prediction criteria for candidemia. PPVs for PCR and T2Candida are expected to be ~50% and ~80%, respectively.

The limited data to-date suggest that sensitivity and specificity of non-culture diagnostics may be lower for intra-abdominal candidiasis than candidemia. CATGA and BDG NPVs for intra-abdominal candidiasis should be strong (>98%) in patients at low-risk for intra-abdominal candidiasis, but values drop to ~80% in higher-risk settings (e.g., severe acute or necrotizing pancreatitis, high-risk GI surgery). Anticipated PPVs

of these assays rise to ~50% among the highest-risk patients. PCR performance will depend upon which of the highly disparate specificities reported thus far for intra-abdominal candidiasis is correct. If specificity is only 33%, NPVs will be similar to those for CAGTA or BDG, but PPVs will not be significantly different from the pre-test likelihood. If specificity is 70%, NPVs and PPVs should be superior and comparable, respectively, to those for CAGTA or BDG. If specificity is 97%, NPVs would be further improved; moreover, PPV would approach 50% in low-risk patients, and exceed 90% in highest-risk patients.

Incorporating non-culture tests into patient care.

How are non-culture diagnostics most likely to be useful in the clinic? Two issues confound our ability to answer this question conclusively. In many instances, test performance has not been validated for different types of candidiasis, or in different patient populations. Furthermore, threshold PPVs and NPVs that justify antifungal treatment are not firmly established. Despite these uncertainties, it is possible to propose a conceptual framework for rationally integrating non-culture tests into patient care strategies that can be investigated in future studies.(29)

For a non-culture test to be useful in clinical decision-making, it must provide sufficient value beyond simply knowing the pre-test likelihood of infection. In other words, do results change the probability of invasive candidiasis such that treatment is justified or not justified? A body of data suggests that antifungal prophylaxis is beneficial in preventing invasive fungal infections in settings with baseline rates of disease ≥15-30%.(24) Therefore, it is reasonable to hypothesize that this target

encompasses a threshold PPV for initiating empiric antifungal treatment. Along these lines, an NPV ≥~85% may justify withholding treatment. Based on these targets, non-culture tests are predicted to be most valuable in the clinical settings demarcated by the shaded boxes in Tables 2 and 3. Testing for candidemia is expected to be useful for more patients as one moves from mannan/anti-mannan, CAGTA or BDG to PCR to T2Candida. At some pre-test likelihood of candidemia, a given test adds value because a positive result increases the probability of disease above the 15-30% threshold, while a negative result virtually excludes the diagnosis. It is apparent that none of the tests is likely to have value if ordered indiscriminately each time a blood culture is collected, since anticipated PPVs are ≤15% and NPVs are not significantly lower than the pre-test probability.

Understanding where non-culture tests might fit into the management of intra-abdominal candidiasis is more uncertain since there are fewer data than for candidemia. CAGTA and BDG are most likely to be useful for patients within a window between lowest and highest risk groups. At the lowest pre-test likelihoods (e.g., <5-10%), PPVs are probably insufficient to justify treatment, and negative results minimally reduce the probability of infection. In the highest risk patients, it is not clear that PPV of ~50% would have greater practical value for decision-making than knowing a pre-test likelihood of ~30%. At the same time, the anticipated NPV of ~80% means that clinicians must be willing to forego treatment despite a ~20% chance that disease is present. PCR would have no clinical utility if specificity for intra-abdominal candidiasis is only 33%. If specificity is 70%, PCR likely would be useful for more patients than

CAGTA or BDG. If specificity is 97%, then PCR may be useful in almost any patient at-risk for intra-abdominal candidiasis.

In the end, treatment decisions based on non-culture results will depend upon clinical judgment, and must be individualized. A particularly challenging decision for clinicians is the NPV threshold at which they are comfortable withholding antifungal therapy in a given patient. Among patients at the highest risk for intra-abdominal candidiasis, for example, a negative result for an excellent PCR assay (85% sensitivity/97% specificity) would still leave a ~6% chance of infection. For an especially sick patient in whom an alternative diagnosis is not evident, treatment might be offered despite this low predictive value. In such a case, non-culture testing should not be performed, since results will not impact treatment decisions.

Tables 2 and 3 are starting points for interpreting non-culture test results. Any test is only useful in the context of all the clinical data for a patient. 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 etiologies may increase or decrease the pre-test likelihood of disease. Likewise, post-test probability may be influenced by the magnitude of results; for example, 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 invasive candidiasis, 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." Given the importance of clinical context in interpreting results and the complexity of subsequent therapeutic decisions, centers may benefit from the expertise of diagnostic stewardship teams.(30)

Conclusions.

The principles advanced here for interpreting non-culture test results will be applicable to new assays as they enter the clinic, and to existing assays as more data become available. Used and interpreted judiciously, non-culture tests have the potential to identify patients with invasive candidiasis who are currently unrecognized, and shorten the time to diagnosis. Moving forward, more data from carefully designed studies are needed for each assay, in particular for the diagnosis of culture-negative, deep-seated candidiasis. More studies that compare assays and assess the role of combination testing should be undertaken. The validation of standardized PCR assays in multi-center studies, and of T2Candida in routine use at individual centers also are priorities. For all the promise of non-culture *Candida* diagnostics, no test has been shown to reduce mortality and morbidity, shorten hospital stays, or restrain the emergence of antifungal resistance. Therefore, the most pressing task ahead is to incorporate non-culture testing into cost-effective patient management strategies that achieve these ends.

Disclosures.

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Table 1. Performance of non-culture tests for diagnosing deep-seated candidiasis.

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Test	Method	Study groups (*n*)	Sensitivity	Specificity	Study, year (Reference)
Mannan	Platelia	IAC (*n*=20) *vs*. at-risk ICU pts (*n*=202)	40%	67%	Leon *et al.*, 2016 (10)
Anti-mannan	Platelia	IAC (*n*=20) *vs*. at-risk ICU pts (*n*=202)	25%	89%	Leon *et al.*, 2016 (10)
CATGA	Vircell	IAC or urologic candidiaisis (*n*=11) *vs.* at-risk ICU pts and healthy controls (*n*=76)	73%	54%	Fortun *et al.*, 2014 (9)
	Vircell	IAC (*n*=20) *vs*. at-risk ICU pts (*n*=202)	53%	64%	Leon *et al.*, 2016 (10)
	Vircell	IAC (*n*=18) *vs*. at-risk ICU pts (*n*=18)	61%*	80%*	Parra Sanchez *et al*., 2017 (11)
BDG	Fungitell	IAC (*n*=34) *vs*. at-risk ICU pts (*n*=73)	56%**	73%	Nguyen *et al.*, 2012 (22)
	Fungitell	IAC (*n*=29) *vs*. at-risk ICU pts (*n*=60)	65%***	78%	Tissot *et al.*, 2013 (23)
	Fungitell	IAC or urologic candidiaisis (*n*=11) *vs.* at-risk ICU pts and healthy controls (*n*=76)	64%	83%	Fortun *et al.*, 2014 (9)
	Fungitell	IAC (*n*=20) *vs*. at-risk ICU pts (*n*=202)	77%	57%	Leon *et al.*, 2016 (10)
PCR	*Candida* Real-time PCR Panel[1]	IAC (*n*=34) *vs*. at-risk ICU pts (*n*=73)	88%**	70%	Nguyen *et al.*, 2012 (22)
	Multiplex *Candida* Real-time PCR[2]	IAC or urologic candidiaisis (*n*=11) *vs.* at-risk ICU pts and healthy controls (*n*=76)	91%	97%	Fortun *et al.*, 2014 (9)

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	Multiplex *Candida* Real-time PCR[2]	IAC (*n*=20) *vs*. at-risk ICU pts (*n*=202)	86%[3]	33%[3]	Leon *et al.*, 2016 (10)
T2Candida			No data		

[1] Viracor Eurofins, Lee's Summit, MO. The *Candida* Real-time PCR Panel detects *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei*. The assay is no longer offered commercially.
[2] Mycology Service of the Spanish National Microbiology Center and Ramon y Cajal Hospital, Madrid, Spain. The Multiplex *Candida* Real-time PCR detects *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei*, *C. guilliermondii*.
[3] PCR was not performed in all patients. Results were positive in 12/14 patients with IAC, and negative in 57/85 at-risk critical care patients and healthy controls.
*Sensitivity/Specificity of the VirClia CATGA assay was 67%/76%.
**Sensitivity of blood cultures for IAC: 17%.
***Sensitivity of blood cultures for IAC: 6%.
BDG: 1,3-β-D-glucan. PCR: polymerase chain reaction. IAC: intra-abdominal candidiasis. Pts: patients. At-risk ICU pts: Patients in an intensive care unit with risk factors for invasive candidiasis.

Table 2. Prevalence of candidemia in different populations, and anticipated PPVs and NPVs of non-culture tests.

Prevalence*	Representative patient*	Mannan/Anti-mannan and BDG[1]		PCR[2]		T2Candida[3]	
		PPV	NPV	PPV	NPV	PPV	NPV
0.4%	Any hospitalized patient in whom a blood culture is collected	1%	99.9%	3%	>99.9%	15%	>99.9%
1%	Patient admitted to intensive care unit	4%	99.7%	8%	99.9%	**31%**	**99.9%**
2%	Patient with febrile neutropenia, baseline rate of candidemia prior to empiric antifungal treatment	7%	99.5%	16%	99.8%	**47%**	**99.8%**
3%	Patient with sepsis, shock or >3-7 day stay in intensive care unit	11%	99.2%	**22%**	**99.6%**	**67%**	**99.7%**
10%	Patient at increased risk for candidemia based on clinical prediction models	**31%**	**97%**	**50%**	**98.8%**	**82%**	**99%**

*References for prevalence of candidemia in various patient populations are summarized in (21).
Sensitivity and specificity of each assay for candidemia are estimated from meta-analyses of combined mannan/anti-mannan, BDG and PCR assays, and the T2Candida DIRECT and DIRECT2 studies, as cited in the text of this article. Data for CAGTA are more limited, but performance for the diagnosis of candidemia complicated by deep-seated candidiasis appears to be comparable to mannan/anti-mannan and BDG.
[1]Sensitivity/specificity: 80%/80%
[2]Sensitivity/specificity: 90%/90%
[3]Sensitivity/specificity: 90%/98%
PPV: Positive predictive value. NPV: Negative predictive value.

PPVs and NPVs in bold text within more darkly shaded boxes 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. Please refer to further discussion in the section of this article subtitled *Incorporating non-culture tests into patient care.*

Table 3. Prevalence of intra-abdominal candidiasis in different populations, and anticipated PPVs and NPVs of non-culture tests.

Prevalence* (Reference)	Representative patient*	CATGA and BDG[1]		PCR*					
				Leon *et al.* [10]		Nguyen *et al.* *(22)*		Fortun *et al.* [9]	
		PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
5% (26, 28)	- Low-to-moderate risk peritoneal dialysis patient with peritonitis	12%	97.6%	6%	97.7%	13%	98.9%	**59%**	**99.2%**
10% (27)	- Patient with emergent surgery for intra-abdominal infection - Patient with colonic perforation	**22%**	**95%**	12%	95.2%	**24%**	**97.7%**	**76%**	**98.3%**
20% (26, 27)	- Patient with high-risk severe acute or necrotizing pancreatitis - Patient with small bowel perforation - Patient with emergent surgery for nosocomial intra-abdominal infection	**39%**	**89.6%**	24%	89.9%	**41%**	**94.9%**	**88%**	**97.5%**
30% (23, 25)	- Patient who has undergone high-risk GI/hepatobiliary surgery - Patient with a biliary leak - Patient with a gastric/duodenal perforation	53%	83%	35%	83.7%	**55%**	**91.6%**	**93%**	**93.8%**

*References for prevalence of intra-abdominal candidiasis in various patient populations are summarized in (24). Other references are cited in the Prevalence column of this table.
Sensitivity and specificity of CATGA and BDG are estimated from the studies of deep-seated candidiasis cited in the text and Table 1 of this article. Sensitivity and specificity of PCR are estimated from the studies of deep-seated candidiasis cited in the text and Table 1 of this article.(9, 10, 22) 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. Data on mannan and anti-mannan for deep-seated candidiasis in the absence of candidemia are too limited to estimate sensitivity and specificity.
[1]Sensitivity/specificity: 65%/75%
[2]Sensitivity/specificity: 85%/33%
[3]Sensitivity/specificity: 85%/70%
[4]Sensitivity/specificity: 85%/97%
PCR: polymerase chain reaction. BDG: 1,3-β-D-glucan. PPV: Positive predictive value. NPV: Negative predictive value. GI: gastrointestinal.

PPVs and NPVs in bold text within more darkly shaded boxes 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 intrabdominal 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. Please refer to further discussion in the section of this article subtitled *Incorporating non-culture tests into patient care.*