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Fungal Infections (S Bernard, Section Editor)

Utilizing Rapid Diagnostics for Detection of *Candida* Species

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Opinion statement

Rapid diagnostic tests are now available to aid in the detection of invasive candidiasis (IC) and promise significant advantages to conventional detection and identification methods. The most progress has been made on rapid species identification when blood culture bottles turn positive and/or when isolated pure colonies are available, while the diagnosis of IC directly from a clinical specimen (e.g., the patient's blood) remains problematic. For the latter, nucleic acid-based tests (PCR, PCR/electrospray ionization-mass spectrometry (PCR/ESI-MS), and T2 magnetic resonance) and immunologic tests [1,3-β-D-glucan (BG) and *Candida* antigen and antibody tests] are promising but each contains certain limitations which have restricted their impact on clinical practice. In-house PCR assays are not standardized, and commercially available PCR tests are limited by minimal clinical data and a long turnaround time. The PCR/ESI-MS and T2 magnetic resonance platforms both require substantial up-front resources, and further clinical experience is required to understand their role in the clinical laboratory. Also, the BG and *Candida* antigen and antibody tests may be useful in certain scenarios (e.g., a negative BG may help rule out IC) but require significant clinical correlation and interpretation of results. On the other hand, PNA FISH and FilmArray Blood Culture ID Panel can substantially reduce the time-to-identification from a positive blood culture, impact clinical practice, and are quite reasonable to implement in the clinical laboratory. While the clear advantages for MALDI-TOF MS are speed, versatility, and excellent accuracy for identification of organisms

from culture plates (which is its primary use in the clinical laboratory), further optimization is required before it can reliably be used for organism identification directly from blood culture bottles and its cost is currently prohibitively expensive for a small clinical laboratory. MALDI-TOF MS is very appealing, however, for rapid identification of organisms in a large clinical laboratory. All of these rapid diagnostic methods complement but do not replace the culture, however, as having the organism itself is still critical for susceptibility testing and strain typing

Introduction

Invasive candidiasis (IC) is a persistent, important public health problem and is currently the fourth most common cause of hospital-acquired bloodstream infections in the USA [1, 2]. The diagnostic gold standard is fungal culture, a method which is hampered by the time required for organism cultivation and identification.

The lack of sensitivity of the blood culture to detect IC is troubling; in a classic case series using autopsy confirmed disease as the gold standard, the blood culture detected only 11/19 (58 %) and 5/18 (28 %) of patients with disseminated and single-organ candidiasis, respectively [3]. A recent review summarized the performance of blood cultures in 13 autopsy studies (including the aforementioned study) of IC and found an overall antemortem blood culture sensitivity of 38 % (156/415 patients) with a range of 21–71 % between studies [4]. The authors of this review defined three distinct clinical scenarios to consider for the diagnosis of candidiasis: (1) patients with candidemia in the absence of deep-seated candidiasis, (2) patients with both candidemia and deep-seated candidiasis, and (3) patients with deep-seated candidiasis in the absence of candidemia, and estimated the sensitivity of blood culture as 75, 75, and 0 % in each scenario, respectively.

Because of the poor sensitivity of blood culture for IC and the lack of modern-day autopsy studies, creating a “reference standard” on which to base evaluation of novel non-culture-based tests is difficult; clinical investigators must make concerted efforts to delineate true from false positive tests. One recent study nicely addressed this issue by defining a group of patients with culture-positive deep-seated candidiasis as the reference standard. In those 24 patients, the sensitivities of blood culture, PCR (Viracor-IBT), and the 1,3-β-D-glucan (BG) test were 17, 88, and 62 %, respectively [5]. In that report, combining culture with non-culture-based tests improved overall sensitivity for IC; a multi-dimensional diagnostic approach may be required to optimize early diagnosis.

For clinicians, it has become increasingly apparent that an early diagnosis is critical so that timely antifungal therapy can be instituted; several reports have demonstrated that early effective therapy improves patient outcomes [6, 7••]. Because of the difficulty in diagnosis, empiric therapy for IC is commonly employed. However, prudent use of antifungal agents in the era of increasing *Candida* spp. resistance is increasingly important [8]. Thus, there is a clear unmet need for rapid, accurate non-culture-based tests for IC.

Several new, rapid, non-culture-based diagnostic tests have been employed to aid in the diagnosis of IC. These tests target different components of the diagnostic cycle including the detection of *Candida* directly from clinical specimens (e.g., blood), from positive blood culture bottles, and specific identification from isolated colonies. The tests are also classified into groups according to what component of the invading pathogen or host immune response they target and include detection of host antibody, fungal antigen, or fungal nucleic acid (Tables 1 and 2).

This review outlines the most promising available non-culture-based rapid diagnostic tests according to which clinical specimens are targeted and provides discussion of each test’s strengths and weaknesses.

Table 1. Characteristics of the major commercially available tests for detection of *Candida* spp. directly from blood

Test name	Test description	Sensitivity (%)	Specificity (%)	Comments
Candida PCR, Quest Diagnostics	Multiplex PCR for *Candida* spp. LOD: 1–350 CFU/mL	–[a]	–[a]	Available in USA No clinical data available High LOD for certain species
Candida PCR, Viracor-IBT	Multiplex PCR for *Candida* spp.	80	70	Available in USA Lower specificity compared to other similar assays
LightCycler SeptiFast Test, Roche	Multiplex PCR for bacteria and fungi. LOD: 30–100 CFU/mL	61	99	Available in Europe; not in USA Possibly lower sensitivity for *Candida* spp. than similar tests
PCR/ESI-MS, Iridica-Abbott	PCR followed by electrospray ionization-mass spectrometry (PCR/ESI-MS) of the amplicon	83[b]	94[b]	Available in Europe; not in USA High sensitivity and specificity Expensive/requires specialized laboratory equipment
T2Candida test, T2Biosyste-ms	NAAT followed by hybridization and analysis via T2 magnetic resonance LOD: 1–3 CFU/mL	91	98	Available in USA Minimal sample preparation Low limit of detection Expensive/required specialized laboratory equipment
1,3-β-D-glucan	EIA to detect 1,3-β-D-glucan, a pan-fungal component of the cell wall	77–80	82–85	Fungitell available in USA Pan-fungal; not *Candida* specific Variable diagnostic utility dependent upon the patient population studied
Platelia *Candida* antibody/ antigen tests	EIA to detect *Candida* mannan antigen and antibodies	89[c]	64[c]	Available in Europe; not in USA High sensitivity for most species but may not reliably detect *C. parapsilosis* or *C. guilliermondii* Newer versions available (“Plus” tests) which require more study

Table references include: [13•, 14, 20, 23•, 25–27, 38]
LOD limit of detection, *EIA* enzyme immunoassay, *ESI-MS* electrospray ionization and mass spectrometry, *NAAT* nucleic acid amplification test
[a]Data are not available
[b]Among both bacterial and fungal isolates combined (*Candida*-specific data are not available)
[c]Antibody and antigen tests combined

Table 2. Characteristics of the major commercially available tests for detection of *Candida* spp. from positive blood culture bottles

Test name	Test description	Sensitivity (%)	Specificity (%)	Comments
Yeast Traffic Light PNA FISH	Probes nucleic acid sequences to detect *C. albicans/C. parapsilosis*, *C. glabrata/C. krusei*, or *C. tropicalis*	92–100	95–100	Very sensitive and specific Quick turnaround time
Candida albicans/ glabrata PNA FISH	Probes nucleic acid sequences to detect *C. albicans* or *C. glabrata*	99	100	Very sensitive and specific Quick turnaround time Does not detect *C. parapsilosis*, *C. krusei*, or *C. tropicalis*
Candida QuickFIS-H	Probes nucleic acid sequences to detect *C. albicans*, *C. glabrata*, or *C. parapsilosis*	99.7	98	Available in Europe; not in USA Very sensitive and specific Quick turnaround time
MALDI-TOF MS	Concentration of yeast pellet followed by MALDI-TOF MS analysis	0–100	?	Variable performance reported, likely due to sample preparation differences (Sepsityper vs. in-house methods) Convenient for laboratories which already use this instrument
FilmArray Blood Culture ID Panel, BioFire	Multiplex PCR	95–100	99.5–100	Minimal sample preparation Fast turnaround time Requires dedicated equipment

Table references include: [49•, 50, 55••, 56, 57]
PNA FISH peptide nucleic acid-fluorescence in situ hybridization, *MALDI-TOF MS* matrix-assisted laser desorption/ionization time of flight mass spectrometry

Diagnosis

Detecting *Candida* from blood

- The primary aim for the diagnosis of *Candida* directly from blood is to rapidly identify the presence of organism and to administer appropriate therapy rather than to rely upon blood cultures.

Nucleic acid amplification tests

Multiplex polymerase chain reaction (PCR) platforms have been studied for the detection of *Candida* spp. in whole blood specimens with sensitivity and specificity results differing widely between individual assays. Amplification of relatively minute amounts of fungal DNA is limited by the abundance of human DNA and hemoglobin in whole blood specimens which reduces PCR

yield and decreases sensitivity [9]. A systematic review and meta-analysis analyzed 54 studies which contained 4694 patients, 963 of whom had proven/probable or possible IC. When considering patients with proven/probable IC only vs. at-risk controls, the pooled sensitivity and specificity for PCR was 95 and 92 %, respectively. As expected, when the analysis included patients with both proven/probable and possible IC, the sensitivity dropped to 73 % while specificity remained high at 91 % [10••]. In a much more limited dataset including commercial PCR assays, reported sensitivities to detect candidemia have ranged from 59–61 % [5, 11]. While these tests appear to be more sensitive than blood culture to detect IC, on-site implementation of standardized, optimized PCR assays will be important for widespread adoption as adjunctive tests to the traditional culture.

Commercially available PCR assay in the USA

Quest Diagnostics (Madison, NJ) offers a qualitative real-time *Candida* PCR assay to detect *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *and C. krusei* from whole blood specimens. This assay has lower limits of detection of 1 CFU/mL for *C. parapsilosis*, 18 CFU/mL for *C.* krusei, 50 CFU/mL for *C. albicans*, 100 CFU/mL for *C. glabrata*, and 350 CFU/mL for *C. tropicalis* [12]. As there is no published clinical data for this test, its applicability to clinical practice is yet to be determined.

Viracor-IBT Laboratories (Lee's Summit, MO) uses a real-time *Candida* multiplex PCR to identify *Candida* spp. in whole blood samples using primers designed to detect either *C. albicans*/*C. tropicalis*, *C. glabrata*/*C. krusei*, or *C. parapsilosis* complex organisms. In the only published report using this assay, this test had an overall sensitivity of 80 % to detect IC (including both candidemia and deep-seated candidiasis). While the sensitivity for deep-seated disease was a robust 89 %, the test was only 59 % sensitive for candidemia. The authors theorized that these paradoxical results may be related in part to study design and to the transient nature of certain candidemia cases (e.g., line-associated); by the time of study entry, patients with transient candidemia may have been more likely to already have cleared the disease (e.g., catheters had been removed) whereas in deep-seated IC, *Candida* DNA may be continually released into the bloodstream. When PCR and blood culture results were paired in patients with IC, the combined sensitivity and specificity were 98 and 79 %, respectively [13•]. In summary, the Viracor-IBT PCR may have a role in the detection of additional cases of IC, and deep-seated disease in particular, when used as an adjunctive test to the standard blood culture.

Commercial PCR assays approved in Europe (CE Marked) but not available in USA

The LightCycler SeptiFast Test (Roche Diagnostics, Mannheim, Germany) probes for both bacterial and fungal ribosomal RNA sequences of common bloodstream pathogens, with results available in less than 6 h [11]. Fungal cells are lysed using ceramic beads and manual nucleic acid purification amplifies

ribosomal RNA targets of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*, in addition to *Aspergillus fumigatus* and 16 other bacterial species. A meta-analysis of 34 studies which enrolled 6012 patients with suspected sepsis found that SeptiFast had a sensitivity of 61 % and specificity of 99 % when used to detect fungemia [14]. This test has a higher limit of detection for *C. glabrata* (100 CFU/mL) as compared to the other *Candida* species (30 CFU/mL each). The need for manual nucleic acid preparation limits this test to laboratories with more experienced personnel, and again, this is most useful when used in conjunction with standard blood culture.

Other assays

Several preparation techniques have been shown to be effective in reducing human DNA and increasing the yield and sensitivity of PCR from whole blood specimens when paired with multiplex PCR and sequencing assays [15•]. SepsiTest (Molzym, Bremen, Germany) uses enzymatic sample preparation followed by universal 18S ribosomal RNA PCR to detect pathogens in whole blood samples. Within 4 h, a positive or negative result is given, and species identification is then obtained by sequencing of the amplified product [8]; it is approved for use in Europe. The VYOO assay (Analytik Jena, Germany) is a platform that combines proprietary enrichment of bacterial and fungal DNA (LOOXSTER) with multiplex PCR analysis. VYOO detects *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* in addition to *Aspergillus fumigatus* and 34 other bacterial species in whole blood samples; it advertises a time-to-result of 7 h. Magicplex Sepsis Real-Time Test (Seegene, Seoul, South Korea) contains a purification step to lyse human cells in whole blood prior to amplifying target nucleic acids This assay is designed to detect *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei* in addition to *Aspergillus fumigatus* and multiple bacterial species within 6 h [16]. While the above assays are all designed to decrease amounts of interfering human DNA and increase sensitivity to detect fungal DNA, clinical data regarding their effectiveness in detecting *Candida* spp. in whole blood samples are lacking.

PCR with electrospray ionization-mass spectrometry (PCR/ESI-MS)

A PCR/ESI-MS platform, marketed currently as Iridica (Abbott, Abbott Park, Illinois; formerly PLEX-ID, Ibis Biosciences) and approved in Europe, has been developed to detect DNA of over 750 bacterial and fungal pathogens. Broad range PCR is used to amplify DNA from various clinical specimens, followed by ESI-MS to determine the nucleic acid base composition of the amplicon. The nucleic acid base composition is then compared to a database to identify the microorganism and identify certain bacterial resistance markers, giving a time-to-result of 6 h [17, 18]. While early PCR/ESI-MS protocols had sensitivities of approximately 50 %, more recent techniques have been developed to optimize the amplification of fungal DNA [19], with sensitivity of 83 % and specificity of

94 % and limits of detection of as low as 16 CFU/mL [20]. Detecting organisms using data from their nucleic acid composition without pure culture is an advantage of this technique, but more clinical experience is required before it is incorporated into regular clinical use.

T2 magnetic resonance

In September 2014, the FDA granted market authorization for the T2Candida Panel (T2Biosystems Inc., Lexington, MA), which detects *Candida* species in whole blood specimens. After nucleic acid amplification of fungal DNA, amplicons are hybridized to oligonucleotide probes bound to nanoparticles specific to *C. albicans*/*C. tropicalis*, *C. glabrata*/*C. krusei*, or *C. parapsilosis*. T2 magnetic resonance (T2 MR) is then used to measure changes in T2 relaxation times, which correspond to increasing levels of *Candida* DNA in samples [21]. Results are reported as positive for *C. albicans*/*C. tropicalis* or *C. glabrata*/*C. krusei* as these pairs of organisms have similar antifungal resistance profiles and empiric therapy may not differ between the two groups; note that these organism pairs are slightly different than those of the Yeast Traffic Light PNA FISH test (see below) [22]. This assay has been shown to have a limit of detection of 1 CFU/mL of whole blood for *C. albicans*/*C. tropicalis*, 2 CFU/mL for *C. glabrata*/*C. krusei*, and 3 CFU/mL for *C. parapsilosis*. In a clinical study by Mylonakis et al., T2Candida had a mean time-to-result of <5 h and a per-patient sensitivity of 91.0 % and specificity of 98.4 %. However, a limitation of this study was that the assay was positive in only four of the six (67 %) candidemia cases; the majority of positive samples for *Candida* spp. were detected in contrived specimens [23•]. T2Candida is appealing due to the fully automated nature of the test (no need for DNA extraction/purification) and its quick turnaround time. Further studies are needed to determine the cost-effectiveness of this technology when applied to large patient populations and to determine its accuracy in detecting candidemia and other IC in infected patients.

Immunologic tests

1,3-β-D-glucan

1,3-β-D-glucan (BG) is a major component of the cell wall of many different fungi, and "the ability of BG to activate an enzyme in the clotting cascade of the horseshoe crab led to the development of assays capable of detecting very small amounts of BG." Four separate tests have been developed, each using individualized cutoff values to define positivity; assays include Fungitell (FDA cleared in USA; formerly Glucatell; Associates of Cape Cod, East Falmouth, MA), Fungitec-G (Seikagaku, Tokyo, Japan), Wako turbidimetric assay (Wako Pure Chemical Industries, Tokyo, Japan), and Maruha colorimetric assay (Maruha-Nichiro Foods, Tokyo, Japan) [24]. Because it is a pan-fungal antigen, the test has been evaluated in studies focused on not only IC but other fungal disease; the three primary clinical scenarios for use are (a) in the high-risk hemato-oncologic population, often in conjunction with serum galactomannan aimed at the detection of molds and yeasts (invasive fungal infection or IFI), (b) high-risk intensive care unit patients aimed

primarily at the detection IC, and (c) for detection of *Pneumocystis jirovecii* pneumonia (PCP) in patients at risk of disease.

Three recently performed meta-analyses demonstrated that the diagnostic accuracy of BG was moderate for detection of IFI and IC (sensitivity 77–80 %; specificity 82–85 %) but high for the diagnosis of PCP (sensitivity 95–95 %; specificity 84–86 %) [25–27]. A separate systematic review and meta-analysis limited the analysis to six cohort studies of high-risk adult hemato-oncological patients and found that the diagnostic performance of two consecutive positive tests was superior to that of only one positive test; the sensitivity and specificity of two positive tests were 49.6 % (95 % CI, 34.0–65.3 %) and 98.9 % (95 % CI, 97.4–99.5 %), respectively [24]. A wide variety of IFI were detected in this study including invasive aspergillosis (n=90), IC (n=80), PCP (n=14), and other (n=31).

An important test limitation is the inability to distinguish among fungi (e.g., a positive test indicates presence of one of many potential fungal pathogens). Also, BG is not found on either *Cryptococcus* or the agents of mucormycosis and thus will not detect those diseases. Finally, false positive results are associated with receipt of hemodialysis, recent surgery, exposure to immunoglobulin or albumin products, receipt of certain medications (e.g., amoxicillin-clavulanic acid), and bacteremia [28–35].

Candida antigen and antibody tests

The mannan component of the *Candida* cell wall is a major antigen; however, assays targeting this antigen are limited by rapid clearance of mannan from the bloodstream. Anti-mannan antibody tests have been developed in order to overcome this weakness. Two tests based on the mannan antigen and anti-mannan antibody have been marketed in Europe as the Platelia *Candida* antibody test and the Platelia *Candida* antigen test [36]. In a large review evaluating 14 studies using the Platelia *Candida* assays, the pooled per-patient sensitivity of the Platelia *Candida* antigen, Platelia *Candida* antibody, and both tests combined was 58, 59, and 83 %, respectively. The corresponding specificities were 93, 83, and 86 %, respectively [37]. Both tests have been updated and are currently marketed as *Candida* Ag Plus and *Candida* Ab Plus. In one published study evaluating candidemic patients vs. controls using the updated tests, the sensitivity and specificity were 59 and 98 % for *Candida* Ag Plus, 63 and 65 % for *Candida* Ab Plus, and 89 and 63.0 % for the tests combined [38]. One important apparent limitation of the *Candida* antigen test is that certain *Candida* species are not well detected. In the above study of the *Candida* Ag Plus assays, *C. parapsilosis* and *C. guilliermondii* were not detected; the authors commented that this differential sensitivity by species had also been reported with the previous *Candida* Ab test.

Detecting *Candida* from positive blood culture bottles

- The primary goal for rapid detection of *Candida* once a blood culture bottle is positive (as opposed to directly from the blood of a patient) is

to obtain a species-level identification which may impact initial treatment decisions.

PNA FISH

Fluorescence in situ hybridization (FISH) probes locate specific DNA or RNA sequences on chromosomes and are now widely used in both microbiology and histopathology. For *Candida* identification, peptide nucleic acid FISH (PNA FISH; AdvanDx, Woburn, MA) has been commercially available since 2003. Currently marketed tests include the *C. albicans*/*C. glabrata* PNA FISH test, the Yeast Traffic Light PNA FISH test, and the *Candida* QuickFISH, the latter currently approved for use only in Europe. While all three tests are extremely accurate (>98 % sensitivity and specificity), the primary inter-assay differences are the laboratory turnaround time (90 min for *C. albicans*/*C. glabrata* and Yeast Traffic Light test vs. 20–30 min for QuickFISH test) and the identified species. As the name suggests, *C. albicans*/*C. glabrata* identifies only those species, while the Yeast Traffic Light test identifies three groups of yeasts color coded corresponding to anticipated fluconazole susceptibility; these groups are *C. albicans*/*C. parapsilosis* (green), *C. tropicalis* (yellow/orange), and *C. glabrata*/*C. krusei* (red). The newer QuickFISH test identifies the three most commonly encountered species: *C. albicans*, *C. glabrata*, and *C. parapsilosis*. PNA FISH tests have demonstrated cost-savings by reducing echinocandin use for fluconazole-susceptible species in several reports [12, 39].

MALDI-TOF MS

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has been established to rapidly identify fungi from pure culture (discussed below) [40••] and is being adopted to detect *Candida* directly from positive blood culture bottles. This adaptation of MALDI-TOF MS can be performed in-house or in conjunction with a commercially available Sepsityper kit (Bruker Daltonics, Billerica, MA). Using Sepsityper, red blood cells are lysed, centrifuged, and washed to create a concentrated yeast pellet which is then analyzed using MALDI-TOF MS. An increasing number of reports on this topic have demonstrated a wide range of sensitivity (0–100 %) [41–46] to identify *Candida* to the species level. The authors of one of the largest such reports, using an in-house method to prepare positive blood culture broth for subsequent MALDI-TOF MS analyzed by the Bruker Biotyper software (version 2.0), found a sensitivity of 91 % (316/376) and attributed much of the variability reported to significant methodologic differences across studies [41].

A recently published novel approach using this technology combined the Sepsityper/MALDI-TOF MS method with simultaneous inoculation of the resuspended yeast cell pellet from the Sepsityper kit into the VITEK 2 (bioMérieux, Durham, NC) susceptibility testing device (Sepsityper/VITEK2), comparing results of direct positive blood culture bottle inoculation to those from standard subculture (24-h delayed). Direct Sepsityper/MALDI-TOF MS yeast identification was achieved in only

63 % (15/24) of samples tested vs. 100 % after subculture for 24 h. The direct Sepsityper/VITEK 2 antifungal susceptibility analysis produced results for 73 % (16/22) of samples, and, although numbers were small, 33 % (5/15) resistant isolates were interpreted as falsely susceptible (i.e., were "very major errors"). The investigators postulated that error rates were likely due to low organism numbers in the yeast cell pellets and acknowledged that further test optimization was required [47].

In summary, MALDI-TOF MS, when used to identify organisms from positive blood culture bottles, is not yet as reliable as the other technologies discussed herein and would benefit from methodologic optimization and standardization. For laboratories with MALDI-TOF MS capacity, the question of whether to implement an alternative technology vs. optimize the MALDI-TOF MS requires an informed laboratory decision based on multiple factors.

FilmArray blood culture ID panel

The FilmArray Blood Culture ID Panel (BioFire, Salt Lake City, UT) uses multiplex nucleic acid amplification to identify the five major *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*) in addition to 19 bacterial species from positive blood culture bottles [48]. This system combines extraction of nucleic acid, nested multiplex PCR, and DNA melting curve analysis to identify fungal species directly from positive blood culture bottles. In a large clinical evaluation of the panel, the overall performance of the test was excellent; for *Candida* detection, the test sensitivity was 100 % (11/11) and specificity 99.5 % (187/188) [49•]. When compared with MALDI-TOF MS using conventional identification methods as a standard, FilmArray identified 20/21 (95 %) of *Candida* species directly from positive blood culture bottles whereas MALDI-TOF MS correctly identified 8/21 species (38 %) [50]. This assay is attractive due to the minimal hands-on time required and time-to-result of about 1 h. Other panels currently available from BioFire aid in the molecular diagnosis of upper respiratory tract and gastrointestinal infections. Conveniently, these panels are all tested on the same device; however, only one sample can be run at any one time, which may increase the time-to-result depending on laboratory capacity and work flow.

Rapid identification of *Candida* from isolated colonies on culture plates

- The primary goal of a rapid identification of *Candida* from the culture plates is similar to that of the blood culture bottles: obtain a species-level identification which may impact initial treatment decisions.

Advances in the microbiology laboratory have included many tests to identify Candida to the species level within 24 h, including the *C. albicans* test, the rapid trehalose test (for *C. glabrata* identification), and automated detection systems (e.g., VITEK), and chromogenic agar.

Also, while PCR/ESI-MS (discussed above) can reliably determine Candida species from pure culture with accuracy comparable to MALDI-TOF MS, we will not discuss PCR/ESI-MS further in this section

as this technology is hampered by a slower time-to-result and increased cost per sample.

MALDI-TOF MS

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) can be used to rapidly identify bacteria and fungi by sample ionization and protein signature measurement using mass spectrometry. The signature of the sample is compared to a database of known microbial signatures to identify the microorganism. MALDI-TOF MS has demonstrated extremely accurate and rapid (<15 min) organism identification (bacterial and fungal) after pure culture is achieved. MALDI-TOF MS is very accurate, with this system reporting correct identification of between 94–97 % of pure culture isolates [51–53]. In addition to commercial databases, extended in-house databases have been used to expand the ability of MALDI-TOF MS to accurately identify a wider range of organisms [54]. An additional advantage of MALDI-TOF MS is that multiple bacterial and/or fungal isolates are able to be tested concurrently in a high-throughput fashion. Obtaining pure fungal culture prior to performing analysis increases the overall time-to-result and, in the case of candidemia, relies on blood cultures which have poor sensitivity. Another limitation of MALDI-TOF MS is that it does not currently detect resistance markers beyond the species identification itself. Finally, the systems are expensive. Despite these potential disadvantages, MALDI-TOF MS is an accurate way to rapidly identify *Candida* spp. from pure culture and is gaining more widespread use in clinical practice. The VITEK ® MS (bioMérieux, Durham, NC) and the MALDI *Biotyper CA System* (Bruker, Billerica, MA) were both FDA cleared in 2013.

Conclusion

Recent progress has been substantial towards improved detection of IC as new, rapid non-culture-based diagnostic tests provide significant advantages to conventional identification and detection methods. For larger laboratories, introducing MALDI-TOF MS into the work flow allows for rapid identification of bacteria and fungi including *Candida* spp. from isolated colonies; and, while MALDI-TOF MS has promise, the technology is not yet universally optimized for the detection of *Candida* spp. from positive blood culture bottles. However, two available platforms are very accurate and rapid in this regard: currently, the PNA FISH Yeast Traffic Light Test and the FilmArray Blood Culture ID Panel can directly impact patient care by substantially reducing the time-to-diagnosis and receipt of appropriate antimicrobial therapy. Progress towards improved detection of IC directly from the patient's blood has also been substantial (PCR, PCR/ESI-MS, T2MR, BG, *Candida* antigen and antibody); however, each of these platforms contains certain limitations which preclude routine use in clinical practice until more data are available. An especially difficult problem that has not yet been solved is that ~50 % of patients with autopsy-proven IC have repeatedly negative blood cultures. It then follows that newer, non-culture-based

methods that accurately predict the results of blood cultures may also fail to identify a large proportion of disseminated disease. These technologies represent an exciting time in clinical and laboratory diagnostics and have the potential to decrease inappropriate antifungal use and improve patient outcomes in invasive *Candida* infections.

Compliance with Ethics Guidelines

Conflict of Interest

Christopher Pfeiffer declares that he has no conflict of interest.
Adam Brady declares that he has no conflict of interest.
Brian Wong declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by the author.

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Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

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