Current Fungal Infection Reports (2021) 15:67–80
https://doi.org/10.1007/s12281-021-00421-x

CLINICAL PATHOLOGY (S CHALLA, SECTION EDITOR)

Molecular Diagnosis of Yeast Infections

P. Lewis White[1] · Jessica S. Price[1] · Alan Cordey[1] · Matthijs Backx[1]

Accepted: 27 May 2021 / Published online: 18 June 2021

Abstract

Purpose of Review The use of molecular tests to aid the diagnosis of invasive yeast infection, in particular invasive candidosis, has been described for over two decades, yet widespread application is limited, and diagnosis remains heavily dependent on classical microbiology. This article will review developments from the past decade in attempt to build on existing knowledge. It will highlight clinical performance and limitations while reviewing developments on recognized procedures; it will also provide insight into novel approaches incorporated in response to clinical demand (e.g. *C. auris* and antifungal resistance) or technological advances (e.g. next-generation sequencing).

Recent Findings Limited methodological standardization and, until recently, unavailability of commercial options have hindered the integration of molecular diagnostics for yeasts. The development of certain, novel commercial methods has received considerable evaluation allowing a greater understanding of individual assay performance, but widespread multicentre evaluation of most commercial kits is lacking. The detection of emerging pathogens (e.g. *C. auris*) has been enhanced by the development of molecular tests. Molecular methods are providing a better understanding of the mycobiome, mechanisms of resistance and epidemiology/phylogeny.

Summary Despite over two decades of use, the incorporation of molecular methods to enhance the diagnosis of yeast infections remains limited to certain specialist centres. While the development of commercial tests will provide stimulus for broader application, further validation and reduced costs are required. Over the same period of time, *Aspergillus* PCR has become more widely accepted driven by international efforts to standardize methodology; it is critical that yeast PCR follows suit. Next-generation sequencing will provide significant information on the mycobiome, antifungal resistance mechanism and even broad-range detection directly from the specimen, which may be critical for the molecular detection of yeasts other than *Candida* species, which is currently limited.

Keywords *Candida* · Yeast · Molecular diagnosis · PCR

Introduction

Gaining widespread consensus in the diagnosis of yeast infections has long been a challenge [1]. As the mortality associated with invasive diseases remains high, it is imperative that diagnostic tests are developed and utilized to give both timely and accurate results, enabling effective therapy to be swiftly initiated [2]. Conventional methods of diagnosis, such as microscopy and culture are established essential investigations but lack sensitivity and can delay diagnosis [2, 3]. The current gold standard means of diagnosing invasive disease is blood culture or recovery of yeast from a sterile site (e.g. tissue biopsy) [3, 4]. However, culture takes time to become positive, with accurate identification and susceptibility testing resulting in further delay, which can increase mortality, should effective antifungal therapy be deferred [3, 5]. While empirical antifungal therapy in high-risk patients is frequently administered, it inevitably results in unnecessary overuse of antifungals, increasing expense, risking toxicity and adverse effects and is concerning in an era of increasing antifungal resistance and limited antifungal drug classes [6].

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) provides reliable and rapid identification of yeast isolates, but reliance on culture limits clinical utility, preventing a shift from historical therapeutic strategies [7]. Non-culture–based methods, such as PCR,

This article is part of the Topical Collection on *Clinical Pathology*

✉ P. Lewis White
lewis.white@wales.nhs.uk

[1] Mycology Reference Laboratory, Public Health Wales, Microbiology Cardiff, UHW, Heath Park, Cardiff CF14 4XW, UK

allow the rapid detection and identification of yeast infections, providing the potential to administer early, effective species-oriented therapy and identify genetic markers associated with antifungal resistance [8]. However, PCR management of yeast infections has had relatively limited clinical incorporation to date [3]. Limited standardization of methods, until recently the lack of commercially available options, and the absence of large-scale clinical trials have likely undermined confidence in appropriateness of non-culture approaches for the diagnosis of invasive yeast infections. Hopefully, clinical trials such as the A-STOP study (ISRCTN43895480) will provide insight in to the applicability of molecular tests for the diagnosis of invasive candidosis (IC) and provide data optimal diagnostic strategies, to improve detection of the 50% of cases of IC that are currently missed [9].

This article will review developments from the past decade, in attempt to build on existing knowledge for an approach that has been in use for over 20 years, but is to gain widespread acceptance. While reviewing developments on recognized procedures, it will provide insight into novel approaches incorporated in response to clinical demand (e.g. *C. auris* and antifungal resistance) or technological advances (e.g. next-generation sequencing). For a broader review of the subject, please refer to manuscript by the Consortium OPATHY [10••].

Clinical Performance of *Candida* PCR

The molecular diagnosis of yeast infection has almost entirely focussed on IC, which remains a major cause of sepsis [11]. In 2011, a meta-analysis and systematic review of *Candida* PCR testing of blood was performed, involving 963 cases of IC in 4694 patients and generating a pooled sensitivity and specificity for the diagnosis of candidaemia of 95% (95% CI: 88–98) and 92% (95% CI 88–95), respectively [3]. It demonstrated that PCR was superior to culture for the detection of proven/probable IC, with the testing of whole blood being superior to testing serum, and specificity of PCR testing increased through sequential positivity.

Given this data, one could question why *Candida* PCR testing has not gained widespread acceptance. Unfortunately, significant limitations remain; while the performance of PCR for the detection of candidaemia is excellent, blood culture is also a sensitive test when the organism is present in the circulation, and the presence of the organism justifies the molecular testing of whole blood, targeting the intact yeast cell. However, IC is more than just candidaemia, and the optimal specimen choice when testing for IC in the absence of candidaemia remains unclear [9]. Given antigens, such as mannan and (1-3)-β-D-glucan (BDG), are readily detected in serum, it is feasible that cell-free *Candida* DNA will also be present, and the molecular methods for testing serum/plasma are technically straightforward. Detection of free DNA may be compromised by the methods used to test whole blood. The sensitivity of the T2Candida when testing whole blood was significantly less for deep-seated candidosis (45%) compared to candidaemia (83%); it is not clear whether this reflects the impact of sample choice and subsequent processing, the limited availability of target in the absence of candidaemia or both, but nevertheless the T2Candida outperformed conventional microbiological approaches [12].

The optimal sample choice for the molecular detection of IC remains unclear; will testing whole blood limit the detection of free DNA that may be present in cases of deep-seated candidosis lacking candidaemia, and conversely will PCR testing of serum/plasma underperform for the detection of candidaemia? This uncertainty of optimal sample choice, or understanding of performance limitation according to disease manifestation, highlights the standardization that remains required for *Candida* PCR to gain widespread acceptance, but this is being addressed through the efforts of the Fungal PCR initiative (www.fpcri.eu) and by the availability of commercial *Candida* PCR kits. To date, the T2Candida is the only commercial platform with extensive clinical validation, and lack of validation limits the incorporation of alternative assays.

As discussed, the performance of PCR for the diagnosis of IC lacking candidaemia is variable. A recent study evaluating the performance of a real-time multiplex PCR for the detection of intra-abdominal candidosis (IAC) when testing serum prior to starting antifungal therapy generated a poor sensitivity of 25% compared to 94% for BDG, although specificity was far superior (91% vs 29%) [13]. Interestingly, 30 cases required blood transfusion before surgery and sampling for *Candida* PCR; for 25 of these patients, PCR testing of pre-transfusional plasma samples increased sensitivity to 64%, indicating a haemodilution effect on target DNA post-transfusion. It also raises the question of optimal sample type for the molecular detection of IAC. A previous study showed that testing plasma was superior to whole blood for the detection of IC, with no significant difference in performance between serum and plasma, although detection of candidaemia (59%) was lower than the other manifestations (88–89%) when testing serum/plasma [14]. This implies there is no difference between *Candida* PCR testing of serum or plasma, although significant differences have been noted for PCR detection of invasive aspergillosis, where it was hypothesized that clot formation in serum samples trapped potential target that remained available in plasma [15, 16]. A previous evaluation of the above multiplex real-time PCR generated much improved sensitivity (96%) when testing serum/plasma from intensive care patients predominantly presenting with candidaemia, highlighting the difficulties in diagnosing deep-seated candidosis [17]. Conversely, the MICAFEM study which utilized the same PCR assay demonstrated a poor sensitivity (21–31%) when

testing serum compared to blood or abdominal fluid culture, with samples taken prior to empirical antifungal therapy in ICU patients at high risk of invasive candidosis [18]. It is unclear why sensitivity was so different to the previous study but could reflect the limitations of testing serum (targeting free DNA) when the organism is present. The study also utilized residual blood, with the majority taken for culture, and required both PCR replicates to be positive if a sample was determined positive. Given the low burden of candidaemia (<1 CFU/ml), PCR positivity will typically be in the non-reproducible range (>35 cycles), and therefore, including non-reproducible positives may enhance sensitivity. Indeed, for disease of low incidence, such as IC, excluding disease through a high negative predictive value represents the optimal use of testing; it is therefore unwise and inaccurate to consider samples where one replicate is positive as negative. In the study of Leon and colleagues, also using the same multiplex real-time PCR assay to test ICU patients with severe abdominal conditions, sensitivity was 84%, with *Candida* PCR considered positive when DNA was detected in a single sample [19]. While specificity was poor (33%), it was improved when PCR positivity was confirmed by another biomarker positivity (e.g. 60% when BDG ≥200pg/ml). Nevertheless, positive predictive values associated with combined positivity remained significantly <50%, highlighting the difficulty of confirming disease in this cohort.

As indicated by the meta-analysis, the PCR detection of candidaemia is likely to be accurate, but the presence of PCR positivity in blood may not be due to candidaemia, but associated with other forms of IC. It is unlikely that molecular tests will provide a definitive answer for the diagnosis of invasive yeast infections. The results, whether positive or negative, should enhance clinical understanding of the likelihood of infection and whether to treat or withhold therapy based on probability. Strategies for incorporating and interpreting non-culture diagnostics for candidaemia and intra-abdominal candidosis in various at-risk populations have been proposed using post-test predictive values and potential thresholds for action [20].

The use of *Candida* PCR blood testing could also provide an early indicator of infection, with a murine model demonstrating PCR positivity in blood within 24 h of infective inoculum, albeit for a limited time due to likely renal uptake/infection [21]. The clinical utility of molecular-based testing demonstrated a significant reduction in time to diagnosis and subsequent treatment (31 h) compared to blood culture (67.5 h) for the diagnosis of candidaemia, with PCR-positive patients associated with potentially shorter ICU admissions and lower mortality [22]. The cost-effectiveness of *Candida* PCR to stop or de-escalate empirical antifungal therapy in ICU patients with suspected fungal peritonitis was demonstrated through a decision tree model, where empirical echinocandin therapy regulated by PCR testing was the most effective strategy, likely to be enhanced when echinocandin costs reduce [23].

A summary of selected non-commercial *Candida* PCR studies are shown in Table 1.

Candida PCR Testing in Paediatrics

IC, in particular candidaemia, is a major cause of IFD in hospitalized paediatric patients; although the incidence is declining in the developed world, it remains associated with significant attributable mortality [31]. Molecular tests represent promising diagnostics, but clinical validation is limited, and the smaller sample volumes attainable from paediatrics, especially neonatal patients, may affect sensitivity [32]. In a neonatal study evaluating the performance of the widely evaluated multiplex real-time PCR (described previously), PCR-positive episodes were associated with a trend towards increased mortality and significant increases in multi-organ failure, possibly negated by the use of antifungal therapy [33]. A summary of selected recent studies describing the performance of molecular detection of *Candida* in paediatrics and neonates, including the T2Candida, is shown in Table 2. Overall performance looks good, but further large-scale studies are required to confirm performance and may be addressed by the BIOPIC trial (NCT02220790) investigating fungal biomarkers for diagnosis and response to therapy for paediatric candidaemia.

Commercial *Candida* PCR Assays Other Than the T2Candida

The availability of commercial *Candida* PCR tests provides methodological standardization, quality control and arguably more extensive, multicentre validation. Many molecular tests have been developed to test positive blood cultures, in doing so providing a rapid and accurate identification similar to direct MALDI-TOF identification [37]. Obviously, the benefits of this approach is limited by the documented confines of blood culture, and for centres with access to a MALDI-TOF, their application will be restricted. The application of commercial *Candida* PCR testing direct to the specimen is beneficial, and while a range of commercial, *Candida* PCR assays are available, clinical validation is limited beyond the T2Candida assay [37].

A recent evaluation compared the performance of the Bruker Fungiplex *Candida* assay with blood culture and the Roche SeptiFast, with the Fungiplex generating excellent sensitivity (100%) and specificity (94%) compared to blood culture when testing whole blood, with sensitivity superior to SeptiFast (60%) [25]. The identification according to the Fungiplex showed good correspondence with culture,

Table 1 A summary of the performance selected non-commercial *Candida* PCR studies

Patient population	Case/ control	Sample type	PCR target gene	PCR range	Sensitivity (%)	Specificity (%)	Ref
Sepsis	10/115	Blood culture	18S rRNA	Pan-*Candida* *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*	>99	90	[24]
Surgical patients with intra-abdominal candidiasis	17/39	Sera or plasma	ITS1 or ITS2 of rRNA	*C. albicans*, *C. parapsilosis*, *C. tropicalis, C. glabrata*, *C. krusei*, *C. guilliermondii*	Post-transfusion fluids = 25% Pre-transfusion fluids = 64%	91	[13]
ICU/sepsis	8/58	EDTA blood	18S rDNA	Fungiplex *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. dubliniensis*	100	94	[25]
	5/58		16S rDNA	SeptiFast *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*	60	96	
ICU	6/175	Serum	ITS1/2 rDNA	*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. guilliermondii*	16	93	[18]
ICU	10/39	Serum	18S rDNA	Fungiplex *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. dubliniensis*	44	87	[26]
ICU	31/233	Serum	ITS1/2 rDNA	*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. guilliermondii*	84	33	[19]
Mammary candidosis	65/89	Breast milk	ITS2	*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. tropicalis*	67	21	[27]
Unavailable	82 patients	Whole blood	5.8S rDNA	*Candida* genus	100	98	[28]
ICU/peritonitis	23/161	Peritoneal fluid	Not Stated	*C. albicans*, *C. glabrata*, *C.* parapsilosis, C. *krusei*, *C. tropicalis*, *C. guilliermondii*, *C. dubliniensis*	94	Not provided	[29]
Haematology	11/61	Whole blood	18S rDNA	*Candida* genus	100	100	[30]
ICU	27/103	Sera	ITS1/ITS2 rDNA	*C. albicans*, *C. parapsilosis*, *C. tropicalis, C. glabrata*, *C. krusei*, *C. guilliermondii*	96	97	[17]
ICU with IC	55/128	Plasma/serum	ITS1, ITS2	*C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*	80	70	[14]

although the authors highlight that not being able to differentiate between the detection of four common causes of IC (*C. albicans*, *C. parapsilosis*, *C. dubliniensis* and *C. tropicalis*) is a limitation.

The review by Camp and colleagues provides extensive background in to the benefits, limitations and validation of currently available commercial *Candida* PCR assays or broad-ranging molecular tests with the capacity to detect *Candida* species [37].

Table 2 The performance of *Candida* PCR tests in paediatric populations

Population	Sample type	Assay	Volume	Case/ control	Sensitivity	Specificity	Reference
Neonates	Whole Blood/Serum/Sterile fluid	In-house	0.2ml	8/147	88%	82%	[33]
Paediatrics (including 24 neonates)	Whole blood	In-house	1–2ml	8/46	100%	89%	[34]
Paediatrics (including neonates)	Whole blood	T2Candida	3ml	4/59	100%	95%	[35]
Paediatrics (no neonates)	Whole blood	T2Candida	2ml (direct application to cartridge)	15/9	100%	100%	[36]

Commercial *Candida* PCR Assays: T2Candida

The T2Candida panel (T2C; T2 Biosystems, Lexington, MA, USA) is an FDA-approved, automated molecular test that detects the five major pathogenic *Candida* spp. (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis*) accounting for >95% of candidaemia, directly in whole blood [38, 39]. The T2Candida system lyses blood cells and *Candida* cells before performing thermostable PCR amplification of the ITS 2 region using pan-*Candida* primers and identification/differentiation of *C. albicans*/*C. tropicalis*, *C. glabrata*/*C.krusei* and *C. parapsilosis* through hybridization of specific probes and magnetic resonance. Results are generated rapidly (4–5h), requiring limited labour and providing a low limit of detection (LOD) 1–3CFU/ml, dependent on species [38].

While the T2 Candida species selection is justified, changes in both the geographic and temporal epidemiology of *Candida* species causing IC, highlight a potential limitation of the test and individual assays for the detection of emerging *Candida* species (e.g. *C. auris*) have been developed (see below). Cases of IC caused by species outside the detection range of the assay should not be excluded when determining assay sensitivity, as clinically they remain a case that was missed by the test, albeit due to design limitations. If these cases are excluded, negative predictive values for the test will be exaggerated, not accounting for the inability of the test to detect other in-frequent causes of IC and the potential for withholding/withdrawing therapy on the basis of a negative T2Candida result be undermined.

The first extensive trial to validate performance of the T2Candida panel to diagnose candidaemia combined the testing of contrived samples and clinical blood compared to blood culture, generating an overall sensitivity and specificity of 91% and 98%, respectively, but clinical cases were limited [40]. The follow-up prospective DIRECT-2 trial 152 patients were enrolled with a diagnostic blood culture performed, of which 36 grew *Candida*, and the T2Candida sensitivity was 89%, with a specificity of 68% (79/116) [41]. A recent systematic review and meta-analysis of the performance of the T2Candida included eight studies, 2717 patients and 525 cases; it generated pooled sensitivity and specificity of 91% and 94%, respectively [42]. Pooled positive (10.2) and negative (0.08) likelihood ratios were sufficient to confirm or exclude IC when associated with positive or negative results, respectively. While these results are very encouraging, the sensitivity (39%) or specificity (59%, 68%) in some studies are significantly below that demonstrated in meta-analysis [41, 43, 44]. Reduced specificity can be explained by evaluating the performance of novel test that has greater sensitivity over the reference method, resulting in the classification of false positive results associated with the novel test, which could equally reflect false negatives associated with the reference method. Given a major justification for the use of the T2Candida is to prevent the use of the unnecessary antifungal therapy, reports of poor sensitivity are concerning [45]. In the MADRID prospective observational study, sensitivity and negative predictive value were 36% and 80%, respectively, potentially due to the influence of prior empirical antifungal therapy or patients presenting with IC in the absence of candidaemia [46]. Conversely, specificity was high and baseline positivity was a poor prognostic marker.

The performance of the T2Candida in the presence or absence of candidaemia has been recently reviewed in detail [47]. In a study evaluating its performance in 48 ICU patients with (n=18) and without (n=30) intra-abdominal candidosis, the sensitivity and specificity were 33% and 93%, respectively [48]. The T2Candida detected both cases that were blood culture–positive and four additional blood culture–negative cases, confirming the limited rates of detection when the organism is absent from the circulation. Two cases receiving antifungal therapy were also negative. The sensitivity of BDG testing in this cohort was 83%, indicating biomarkers were present in the circulation and detection of free DNA in serum/plasma may have improved molecular detection. Combining T2Candida with BDG generated 100% specificity when both tests were positive and 90% negative predictive value if both were negative [48].

The major restriction to the widespread implementation of the T2Candida platform outside of insurance-based healthcare settings is cost (>$200), which is significantly greater than conventional microbiology and even alternative molecular tests [45]. Obviously, incorporating an internal control to avoid reporting false negative results is essential, but the rate of documented invalid results (5–10%) and the cost per sample reflect a significant unrecoverable, additional expense [40, 41]. This cost of T2Candida testing could be offset by reductions in unnecessary antifungal therapy. An analytical decision tree model was developed to compare antifungal use as directed by T2Candida or blood culture with empirical antifungal therapy [45]. In this model, the T2Candida had the potential to reduce unnecessary antifungal therapy by 98%, but nevertheless, when compared to empirical antifungal therapy, T2Candida diagnostic testing increased cost (approximately $300) and was slightly less effective for the management of suspected candidaemia. This differed to a previous cost-modelling exercise, which demonstrated the T2Candida was both more effective and less costly than empirical therapy, highlighting the limitations of modelling and the need for real-life data [49].

Various recent reviews focus specifically on the T2Candida and should be sourced for a full assessment of the topic [50–53].

Molecular Detection of *Candida auris*

The emergence of *Candida auris* as a global multidrug-resistant healthcare-associated pathogen associated with various hospital outbreaks requires the development of rapid and accurate identification methods to optimize infection control measures and restrict the spread of infection, especially during the COVID-19 pandemic [54]. As with the detection of IC, culture has limited sensitivity when testing surveillance or environmental samples, compounded by the delay to result, which is a significant hindrance to timely infection control measures.

Given the problems with misidentification using conventional biochemical methods and the limitations of MALDI-TOF databases lacking *C. auris* at the time of its emergence, PCR was used as an aid in identifying cultured *C. auris*. [55–57]. While potentially improving the specificity of identification, the reliance on culture and associated delay hinders infection control, and direct sample testing was needed to fully embrace the benefits of molecular-based identification [58].

The development of a highly sensitive (LOD 1 CFU/PCR reaction) real-time PCR assay for direct testing of patient surveillance swabs and environmental sponges provided detection and significantly increased the environmental detection of *C. auris* over culture (PCR: 22.5% vs culture 12.4% (difference 10.1%, 95% CI: 6.4–14.1)) [59]. While direct PCR testing of patient swabs did not improve rates of detection (PCR: 19.2% vs culture 20.4% (difference 1.2%, 95% CI: –3.8–13.6)), PCR results for both specimens were available within 4 h or processing compared to 4–14 days for culture. The rapid availability of results is beneficial for limiting unnecessary transmission over time. Importantly, this assay also incorporated an internal control PCR to avoid the reporting of false negative results arising due to inhibition of the PCR process that could also undermine infection control procedures [59]. This *C. auris* assay, along with others, has now been fully automated using the BD Max Open System, simplifying technical procedures while generating performance comparable to manual assays, and confirmed the high analytical specificity, with no cross-reactivity noted including closely related species (e.g. *C. haemulonii* and *C. duobushaemulonii*) [60, 61].

Given *C. auris* causes a significant degree of invasive disease, particularly fungaemia direct detection from blood samples would be clinically beneficial, and attempts have been made to test serum and whole blood, but further evaluation of clinical performance is required [62, 63].

Various commercial *C. auris* PCR assays are now available, including the Bruker Fungiplex *Candida auris*, MycoDART-PCR Candida diagnostic panel, MONODOSE CanAur dtec-qPCR and the GENESIG kit that utilizes the primer/probes described by Leach and colleagues, whose performance is described above [59]. Comparison of the OLM AurisID® real-time PCR for direct testing of surveillance swabs without the requirement for DNA extraction with the routine identification using CHROMagar® and MALDI-TOF provided comparable results within a quicker timeframe [64]. Comparison of the OLM and Bruker *C. auris* assays demonstrated slight differences in LOD and analytical specificity when testing higher concentrations of DNA from species closely related to *C. auris* [65]. The T2 *Candida auris* panel provided a fully automated commercial option with performance comparable to culture when testing simulated and clinical axilla/groin swabs [66]. While the development of assays individual to specific emerging species is essential, if the species continues to represent a clinical concern over time, it is important that they are incorporated into broader ranging molecular tests targeting other *Candida* species, improving cost-effectiveness and clinical utility [67].

The mechanism of *C. auris* resistance to fluconazole, voriconazole and micafungin has been studied through PCR sequencing of the *ERG11* and FKS1 genes [68]. Micafungin-resistant isolates harboured a S639F non-synonymous mutation in hotspot 1 of the FKS1 gene that was absent in susceptible isolates. Of the 36 azole-resistant isolates randomly selected for testing, 12 fluconazole-resistant isolates contained a Y132F mutation, while 24 fluconazole-/voriconazole-resistant isolates contained a K143R mutation in the *ERG11* gene. The presence of Y132F and K143R mutations in the *ERG11* gene appear definitive for azole resistance, and rapid molecular tests have been developed to detect the Y132F/K143R and S639F mutations in *ERG11* and *FKS1* genes, respectively [69]. Data linking other mutations with susceptibility profiles are currently conflicting between studies, requiring further large-scale clarification. Whole genome sequencing of *C. auris* may aid our understanding of the molecular mechanism of resistance and has provided insight in to the phylogeny of *C. auris*, initially identifying the presence of 4 distinct clades (South Asia, East Asia, South America and South Africa), with a potential fifth clade recently identified in Iran [70, 71].

Molecular Detection of Resistance in *Candida* Species Other Than *C. auris*

Molecular diagnostics can identify resistance, provided the resistance mechanism has been validated through association with raised or high minimum inhibitory concentrations to a specific antifungal drug, altered drug target, dose-dependent resistance in an animal model or document clinical failures [72•]. In echinocandins, mutations in the 3 FKS genes decrease the sensitivity of glucan synthase to the drug, with resistance dependent on the specific mutation. Fortunately, a small number of FKS mutations are associated with the majority of echinocandin resistance in *Candida*, and several PCR assays not requiring timely DNA sequencing have been

developed [73–75]. To date, testing has been limited to culture so the clinical utility of such strategies is limited.

Azole resistance in *Candida* species is associated with a wide range of mechanisms, potentially excluding real-time PCR approaches, and while these have been developed, DNA sequencing remains the best option for identifying the mutations associated with resistance, limiting clinical application, particularly direct sample testing [72, 76, 77]. Currently, there are no commercial PCR tests to detect mutations associated with antifungal resistance in yeasts. The development of next-generation (whole genome) sequencing may assist in our understanding/detection of resistance, but direct application to clinical samples will likely require PCR amplification of previously selected genes or regions [78].

Next-Generation Sequencing in Mycology

Next-generation sequencing (NGS) represents a revolutionary development in genomic science. In the clinic, much work has focussed on the human genome in order to gain understanding of function and genetic defects that could predispose the individual to certain conditions [10••]. From a microbiological perspective, NGS has helped overcome the limitations of culture when studying the microbiome and mycobiome. Improving our understanding of the composition, anatomical variation, host/microbe and microbe/microbe interaction and imbalance in the microbiome associated with disease development [10••, 79]. On an organism level, antifungal resistance mechanisms and evolutionary/phylogeny of yeast species are being elucidated by NGS [78, 80–83]. Using NGS to analyse RNA transcription can allow us to gain greater understanding of the pathogenicity of fungal species [84]. NGS has potential to enhance typing of organism in potential fungal outbreaks.

However, NGS is a complex procedure and is not without limitations in respect to clinical utility. Obviously, complexity is generally associated with delay, which impacts how information generated by NGS can positively impact management of patients with acute disease. Direct application to the specimen will improve on this, but will likely be reliant on PCR amplification of common genes (e.g. ITS regions), but questions over discriminatory power of this approach have been raised, but may be improved by using novel primers [10••, 85, 86]. This approach has been successfully applied to the investigation of corneal scrapes from non-viral infective keratitis [87].

From a practical standpoint, NGS remains costly, requires specific equipment and technical expertise and generates large amounts of data requiring bioinformatics (software and pipelines) for accurate analysis, allowing use and consistency in diagnostic laboratories [10••, 88••]. Technically, optimization of mycological NGS procedures is similar to other molecular-based processes, requiring optimal sampling, awareness and control of fungal contamination, optimal nucleic acid extraction and efficient PCR amplification [88••]. Databases and procedures are somewhat limited in relation mycology and requires significant attention if NGS is to gain widespread clinical use but also utilize the extensive data generated to the maximum [10••].

Technical Developments

One of the major issues with using molecular methods to detect fungal pathogens, including yeasts, in blood is the limited fungal burden even in the presence of fungaemia. The testing or larger sample volumes (3–10ml) may help to overcome this problem, but complicate sample processing and nucleic acid extraction methods. Recently, pathogens including *Candida tropicalis* have been concentrated in blood using apolipoprotein H, permitting the use of lower volumes (0.2ml) while maintaining analytical LOD (1CFU/ml) [89]. An alternative approach involved the lysis of large blood volumes while preserving and concentrating microbes (including *C. albicans*) using filters in a small volume that could be used for culture or direct molecular testing [90]. In the initial stage of processing, DNase is added to remove human DNA that can interfere with the process, but also removes potential *Candida* DNAemia target in the patient with IC. Recovery of low fungal burdens (3CFU/ml *C. albicans*) was approximately 50%, so it is unclear if this would benefit the detection of candidaemia where lower burdens are typical [90]. Viscoelastic separation and concentration of *C. albicans* in blood significantly improved real-time PCR detection, providing earlier Ct values and an improved LOD [91].

Obviously, efficient nucleic acid extraction remains critical to successful PCR. If targeting the organism (cell-based DNA), then the fungal cell wall represents a significant hurdle that can limit extraction efficiency. The use of enzymes (Zymolase/lyticase) can be used to remove the fungal cell wall (forming spheroplasts), but recombinant enzymes should be used to overcome possible contamination due to the potential fungal origin of these enzymes. The use of lyticase to detect and identify *C. albicans* in contrived blood did not necessarily improve detection of fungi at the lower concentration, but did improve identification success and limit PCR inhibition [92]. Unfortunately, the fungal burdens included were not necessarily clinically relevant (>10^2 CFU/ml), and at lower levels, the effects could have been more evident. Mechanical disruption (bead-beating) of the fungal cell is an alternative, reducing cost and processing time and likely improving efficiency, although may fracture genomic DNA hindering NGS.

Unlike the molecular detection of *Aspergillus*, attempts to optimize *Candida* PCR have been limited, possibly by early studies demonstrating comparative performance between methods at that time [93, 94]. A recent study compared 11

automated extraction methods for the detection of the five main *Candida* species in contrived whole blood samples, demonstrating wide-ranging efficiency (31–81%) and variation for the detection of different *Candida* species [95]. Combining off-board bead-beating with the BioMerieux easyMAG provided optimal performance. Other studies comparing nucleic acid extraction methods have confirmed the variable detection of low fungal burdens and demonstrated the benefits of testing larger sample volumes and other specimen types [96–98]. Nevertheless, questions regarding the optimal technique remain, and the Fungal PCR Initiative (www.fpcri.eu) is performing strategic evaluations in order to address these issues. The availability of external quality assurance programmes (e.g. QCMD *Candida* PCR proficiency panel) is essential in ensuring consistent performance between methods/centres, underpinning regulatory approval (ISO15189) of routine diagnostic testing.

A summary of selected technical advances in the last decade, not discussed in the text, is shown in Table 3.

Molecular Detection of Yeasts Other Than *Candida*

The development of individual assays specific to species/genera other than *Candida* is limited by the relatively low incidence of non-*Candida* invasive yeast infection or the availability of alternative, simple, yet accurate diagnostics (e.g. cryptococcal lateral flow assay). A PCR test specific for the detection of *Trichosporon* species was described over 20 years ago and more recently a test specific for the black yeast *Exophiala dermatiditis* in cystic fibrosis patients was reported [123, 124]. While other non-*Candida* yeast species/genera specific PCR assays likely exist, they remain limited, and it makes more sense to target rarer fungal infections using PCR tests with a broad range of detection, incorporating both *Candida* and non-*Candida* yeasts, possibly other filamentous fungi and bacteria. However, it is important that such tests possess the ability to provide a species/genera level of identification, as simply confirming the presence of fungi without identification has limited positive predictive value, compounded by potential contamination and prevents administering species-directed antifungal therapy [125]. If pan-fungal PCR assays require sequencing to confirm identification, then the delay in getting a result also minimizes the benefit of testing but may utility the testing of histological or tissue biopsies [126].

A recent yeast panel multiplex PCR, not requiring formal nucleic acid extraction, targeting 21 species of *Candida, Trichosporon*, *Rhodotorula*, *Cryptococcus* and *Geotrichum*, was developed [127•]. Initially, it was designed to improve identification of yeasts in the absence of MALDI-TOF, with which it generated 100% concordance when identifying 800 clinical isolates. While this approach is beneficial, providing robustness of identification in resource-limited settings, direct specimen testing would be advantageous, and a multiplex real-time PCR targeting *Candida, Aspergillus* and *Rhizopus* generated a sensitivity and specificity of 89% and 100%, respectively, in a pilot study [128].

For the direct, molecular detection of yeasts, especially non-*Candida* species, pan-fungal PCR approaches likely combined with broad-ranging microbiological detection will most likely provide the optimal diagnostic route. Most of the broad target approaches, including commercial options, simply target common *Candida* with filamentous moulds and/or a range of bacteria in a single test or simply lack validation for direct specimen testing of unusual yeasts [24, 129, 130]. One approach testing plasma and combining a range of multiplex real-time PCR assays generated good sensitivity for the detection of *Candida* (85%); specificity was excellent 99.5%, but while the assay targeted a range of fungi (*Aspergillus* sp., *Mucorales* sp., hyalohyphomycoses and endemic fungi), yeast detection was limited to *C. albicans*, *C. glabrata* and *C. krusei* [130].

Unfortunately, systems using PCR/electrospray ionization-mass spectrometry (e.g. Abbott IRIDICA), with the capacity to replicate the range of detection provided by culture, but with greater sensitivity when directly testing a range of samples, appear to be currently unavailable [131–135]. Other broad-ranging approaches (e.g. Luminex xTAG technology) only have limited evaluation, and developments appear to have stalled [136]. Subsequently, the molecular detection of yeast species other than *Candida* requires significant development, which it is unlikely to get.

Concluding Remarks

The impact of molecular diagnostics for invasive yeast infections has been limited by a lack of standardization and commercial assays. With both being addressed, it is hoped that molecular testing will become routine practice, making the potential benefits widely available. The emergence of multidrug-resistant species (i.e. *C. auris*) has demonstrated how advantageous molecular testing can be for the diagnosis and emergence of novel species. NGS will be essential in gaining an understanding

Table 3 A summary of recent technical developments in the molecular diagnosis of invasive yeast infections

Purpose	Application	Technology	Reference
Improve identification of yeasts	Culture—Differentiation of cryptic species of *C. albicans*, *C. glabrata* and *C. parapsilosis*	Low-cost multiplex PCR with specific primers—differentiation based on amplicon size	[99]
	Detection and ID of *Candida* direct from blood	Real-time PCR with high-resolution melt-curve analysis	[100]
	Differentiation of isolated *Candida* species	Real-time PCR with high-resolution melt-curve analysis	[101]
	Identification of *Candida* species	Real-time PCR with high-resolution melt-curve analysis	[102]
	Differentiation of cryptic *C. parapsilosis* species	Exon-primed intron-crossing PCR assay combined with restriction enzyme analysis—MnSOD gene	[103]
	Identification of *C. albicans*	Loop-mediated isothermal amplification assay	[104]
	Identification of *C. parapsilosis* complex	Loop-mediated isothermal amplification assay	[105]
Improve utility of blood culture yeast diagnosis	Identification of clinically relevant yeasts and bacteria in positive blood cultures	Prove-it Sepsis Microarray assay	[106]
	Rapid identification of 15 fungal pathogens direct from blood culture	GenMark Dx ePlex microfluidic and electrochemical detection system	[107]
	Pan-candidal and bacterial detection direct from blood culture	GenMark Dx ePlex microfluidic and electrochemical detection system	[108]
	Rapid identification of seven *Candida* species direct from blood culture	Antimicrobial polymers and CHIP detection targeting the 28S rRNA gene	[109]
	Rapid identification of bacteria and *Candida* species direct from blood culture	Punch-it NA-Sample kit and reverse blot hybridization assay	[110]
Improve direct diagnosis of yeast infections	Detection of *C. albicans* and other genitourinary pathogens direct from urine or vaginal swabs	DNA chip (STDetect)	[111]
	Detection of mixed fungal infections in tissue	Broad-range PCR (28S rRNA and ITS2), sequencing Isentio RipSeq tool and Fluorescence *in situ* hybridization	[112]
	Detection of bacterial and fungal pathogens in ocular samples from patients with suspected endophthalmitis	Direct PCR amplification of 16S and 18s rDNA and sequencing	[113]
	Detection of bacterial and fungal pathogens in vitreous fluid from patients with suspected endophthalmitis	PCR and DNA microarray analysis of the ITS1 region	[114]
	Detection of four *Candida* species direct from blood	Asymmetric PCR and fluorescence polarization assay	[115]
	Detection of *Candida* and *Aspergillus* direct from blood	Multiplex PCR targeting 18S and 28s RNA genes and semi-automated surface-enhanced Raman scattering assay	[116]
	Detection of the five main *Candida* species direct from blood	Reverse-transcriptase real-time PCR of 18S/28S rRNA genes	[117]
	Direct detection of *C. albicans* direct from blood	Polymerase spiral reaction targeting the ITS2 region	[118]
	Direct detection of *C. albicans* direct from blood	Microfluidic real-time PCR	[119]
	Direct detection of *C. albicans* in oral exfoliative cytology samples	Loop-mediated isothermal amplification	[120]
	Direct detection of bacterial and yeast infections in blood	PCR-reverse blot hybridization, using the REBA Sepsis-ID assay	[121]
Other potential clinical applications	Use of qPCR to determine viable cells in response to antifungal therapy	Ethidium bromide pre-staining of cultures with and without antifungal treatment, using qPCR to determine cell viability	[122]

of the molecular mechanisms of resistance in established yeast pathogens and identifying potential new antifungal targets. Unfortunately, beyond *Candida* species, the use of specific molecular testing is limited by the low incidence of that particular disease, and potential methods (PCR/electrospray ionization-mass spectrometry) with the capacity to replicate the detection range of culture appear no longer widely available. With an

ever-increasing population at risk of yeast infections, the range of potential pathogens will undoubtedly evolve, and it is essential that technology be embraced to ensure an accurate and timely diagnosis.

Declarations

Ethics Approval Not required

Conflict of Interest P. Lewis White**:** Performed diagnostic evaluations and received meeting sponsorship from Associates of Cape Cod, Bruker, Dynamiker and Launch Diagnostics; speakers' fees, expert advice fees and meeting sponsorship from Gilead; and speaker and expert advice fees from F2G and speaker fees from IMMY, MSD and Pfizer. Is a founding member of the European *Aspergillus* PCR Initiative.Matthijs Backx: Speakers' fees, expert advice fees and meeting sponsorship from Gilead. Meeting sponsorship from AbbVie.

Jessica S. Price and Alan Cordey declare that they have no conflict of interest.

References

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

1. Ullmann AJ, Cornely OA, Donnelly JP, Akova M, Arendrup MC, Arikan-Akdagli S, et al. ESCMID guideline for the diagnosis and management of Candida diseases 2012: developing European guidelines in clinical microbiology and infectious diseases. Clin Microbiol Infect. 2012;18(SUPPL.7):1–8.
2. Cuenca-Estrella M, Verweij PE, Arendrup MC, Arikan-Akdagli S, Bille J, Donnelly JP, et al. ESCMID guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. Clin Microbiol Infect. 2012;18(SUPPL.7):9–18.
3. Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. J Clin Microbiol. 2011;49(2):665–70.
4. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Crit Rev Microbiol. 2010;36(1):1–53.
5. Tumbarello M, Posteraro B, Trecarichi EM, Fiori B, Rossi M, Porta R, et al. Biofilm production by Candida species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. J Clin Microbiol. 2007;45(6):1843–50.
6. Golan Y, Wolf MP, Pauker SG, Wong JB, Hadley S. Empirical anti- *Candida* therapy among selected patients in the intensive care unit: a cost-effectiveness analysis. Ann Intern Med. 2005;20:143(12).
7. Marklein G, Josten M, Klanke U, Müller E, Horré R, Maier T, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J Clin Microbiol. 2009;47(9):2912–7.
8. McMullan R, Metwally L, Coyle PV, Hedderwick S, McCloskey B, O'Neill HJ, et al. A prospective clinical trial of a real-time polymerase chain reaction assay for the diagnosis of candidemia in nonneutropenic, critically ill adults. Clin Infect Dis. 2008;46(6):890–6.
9. Clancy CJ, Nguyen MH. Finding the missing 50% of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013;56(9):1284–92.
10. •• Consortium OPATHY, Gabaldón T. Recent trends in molecular diagnostics of yeast infections: from PCR to NGS. FEMS Microbiol Rev. 2019;43(5):517–47 **Excellent, extensive review on the potential applications of next generation sequencing for yeasts.**
11. Ricotta EE, Lai YL, Babiker A, Strich JR, Kadri SS, Lionakis MS, et al. Invasive candidiasis species distribution and trends, United States, 2009-2017. Infect Dis Ther. 2021;223(7):1295–302.
12. Arendrup MC, Andersen JS, Kristian Holten M, et al. Diagnostic performance of T2Candida among ICU patients with risk factors for invasive candidiasis. Open Forum Infect Dis. 2019;6(5):ofz136.
13. Fortún J, Buitrago MJ, Gioia F, de la Pedrosa Gómez-Ga E, Alvarez ME, Martín-Dávila P, et al. Roles of the multiplex real-time PCR assay and beta-D-glucan in a high-risk population for intra-abdominal candidiasis (IAC). Med Mycol. 2020;58(6):789–96.
14. Nguyen MH, Wissel MC, Shields RK, Salomoni MA, Hao B, Press EG, et al. Clancy CJ Performance of Candida real-time polymerase chain reaction, beta-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. Clin Infect Dis. 2012;54(9):1240–8.
15. White PL, Barnes RA, Springer J, Klingspor L, Cuenca-Estrella M, Morton CO, et al. EAPCRI. Clinical performance of aspergillus PCR for testing serum and plasma: a study by the European Aspergillus PCR Initiative. J Clin Microbiol. 2015;53(9):2832–7.
16. Loeffler J, Mengoli C, Springer J, Bretagne S, Cuenca-Estrella M, Klingspor L, et al. European Aspergillus PCR Initiative. Analytical comparison of in vitro-spiked human serum and plasma for PCR-based detection of Aspergillus fumigatus DNA: a study by the European Aspergillus PCR Initiative. J Clin Microbiol. 2015;53(9):2838–45.
17. Fortún J, Meije Y, Buitrago MJ, Gago S, Bernal-Martinez L, Pemán J, et al. Clinical validation of a multiplex real-time PCR assay for detection of invasive candidiasis in intensive care unit patients. J Antimicrob Chemother. 2014;69(11):3134–41.
18. Nieto M, Robles JC, Causse M, Gutiérrez L, Cruz Perez M, Ferrer R, et al. Merino P; MICAFEM Study Group, GEIPC (SEIMC) and GTEIS (SEMICYUC).Polymerase chain reaction versus blood culture to detect Candida species in high-risk patients with suspected invasive candidiasis: the MICAFEM study. Infect Dis Ther. 2019;8(3):429–44.
19. León C, Ruiz-Santana S, Saavedra P, Castro C, Loza A, Zakariya I, et al. Contribution of Candida biomarkers and DNA detection for the diagnosis of invasive candidiasis in ICU patients with severe abdominal conditions. Crit Care. 2016;20(1):149.
20. Clancy CJ, Nguyen MH. Rapid diagnosis of invasive candidiasis: ready for prime-time? Curr Opin Infect Dis. 2019;32(6):546–52.
21. He ZX, Zhao HH, Wang FK. PCR-detectable Candida DNA exists a short period in the blood of systemic candidiasis murine model. Open Life Sci. 2020;15(1):677–82.
22. Bloos F, Bayer O, Sachse S, Straube E, Reinhart K, Kortgen A. Attributable costs of patients with candidemia and potential implications of polymerase chain reaction-based pathogen detection on antifungal therapy in patients with sepsis. J Crit Care. 2013;28(1):2–8.
23. Pagès A, Iriart X, Molinier L, Georges B, Berry A, Massip P, et al. Cost effectiveness of Candida polymerase chain reaction detection and empirical antifungal treatment among patients with suspected fungal peritonitis in the intensive care unit. Value Health. 2017;20(10):1319–28.

24. Wang HY, Kim S, Kim H, Kim J, Kim Y, Park SD, et al. Real-time PCR TaqMan assay for rapid screening of bloodstream infection. Ann Clin Microbiol Antimicrob. 2014;13:3.
25. Fuchs S, Lass-Flörl C, Posch W. Diagnostic performance of a novel multiplex PCR assay for candidemia among ICU patients. J Fungi (Basel). 2019;5(3):86.
26. McKeating C, White PL, Posso R, Palmer M, Johnson E, McMullan R. Diagnostic accuracy of fungal PCR and beta-d-glucan for detection of candidaemia: a preliminary evaluation. J Clin Pathol. 2018;71(5):420–4.
27. Mutschlechner W, Karall D, Hartmann C, Streiter B, Baumgartner-Sigl S, Orth-Höller D, et al. Mammary candidiasis: molecular-based detection of Candida species in human milk samples. Eur J Clin Microbiol Infect Dis. 2016;35(8):1309–13.
28. Guo Y, Yang JX, Liang GW. A real-time PCR assay based on 5.8S rRNA gene (5.8S rDNA) for rapid detection of Candida from whole blood samples. Mycopathologia. 2016;181(5-6):405–13.
29. Corrales I, Giménez E, Aguilar G, Delgado C, Puig J, Izquierdo A, et al. Detection of fungal DNA in peritoneal fluids by a PCR DNA low-density microarray system and quantitation of serum (1-3)-β-D-glucan in the diagnosis of peritoneal candidiasis. Med Mycol. 2015;53(2):199–204.
30. Ashrafi M, Nabili M, Shokohi T, Janbabaie G, Hedayati MT, Ali-Moghaddam K. A real time PCR assay on blood for diagnosis of invasive candidiasis in immunocompromised patient. Curr Med Mycol. 2015;1(1):35–41.
31. Walsh TJ, Katragkou A, Chen T, Salvatore CM, Roilides E. Invasive Candidiasis in infants and children: recent advances in epidemiology, diagnosis, and treatment. J Fungi (Basel). 2019;5(1):11.
32. Calley JL, Warris A. Recognition and diagnosis of invasive fungal infections in neonates. J Inf Secur. 2017;74(Suppl 1):S108–13.
33. Ramos JT, Villar S, Bouza E, Bergon-Sendin E, Perez Rivilla A, Collados CT, et al. Performance of a quantitative PCR-based assay and beta-d-glucan detection for diagnosis of invasive candidiasis in very-low-birth-weight preterm neonatal patients (CANDINEO Study). J Clin Microbiol. 2017;55(9):2752–64.
34. Taira CL, Okay TS, Delgado AF, Ceccon ME, de Almeida MT, Del Negro GM. A multiplex nested PCR for the detection and identification of Candida species in blood samples of critically ill paediatric patients. BMC Infect Dis. 2014;14:40.
35. Cendejas-Bueno E, Falces-Romero I, Laplaza-González M, Escosa-García L, Schuffelmann-Gutierrez C, Romero-Gómez MP, et al. Candidemia diagnosis with T2 nuclear magnetic resonance in a PICU: a new approach. Pediatr Crit Care Med. 2021;22(2):e109–14.
36. Hamula CL, Hughes K, Fisher BT, Zaoutis TE, Singh IR, Velegraki A. T2Candida provides rapid and accurate species identification in pediatric cases of candidemia. Am J Clin Pathol. 2016;145(6):858–61.
37. Camp I, Spettel K, Willinger B. Molecular methods for the diagnosis of invasive candidiasis. J Fungi. 2020;6:101.
38. Neely LA, Audeh M, Phung NA, Min M, Suchocki A, Plourde D, et al. T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci Transl Med. 2013;5:182ra54.
39. Pfaller MA, Jones RN, Castanheira M. Regional data analysis of Candida non-albicans strains collected in United States medical sites over a 6-year period, 2006-2011. Mycoses. 2014;57(10):602–11.
40. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis. 2015;60(6):892–9.
41. Clancy CJ, Pappas PG, Vazquez J, Judson MA, Kontoyiannis DP, Thompson GR 3rd, et al. Detecting infections rapidly and easily for candidemia trial, part 2 (DIRECT2): a prospective, multicenter study of the T2Candida panel. Clin Infect Dis. 2018.
42. Tang DL, Chen X, Zhu CG, Li ZW, Xia Y, Guo XG. Pooled analysis of T2 Candida for rapid diagnosis of candidiasis. BMC Infect Dis. 2019;19(1):79.
43. Mylonakis E, Zacharioudakis IM, Clancy CJ, Nguyen MH, Pappas PG. Efficacy of T2 magnetic resonance assay in monitoring candidemia after initiation of antifungal therapy: the serial therapeutic and antifungal monitoring protocol (STAMP) trial. J Clin Microbiol. 2018;56(4)
44. Dwivedi S, Ordaya E, Kezlarian B, Kenny R, Tibbetts R. Novel T2 magnetic resonance assay compared to standard blood cultures for detection of candidemia. Open Forum Infect Dis. 2016.
45. Walker B, Powers-Fletcher MV, Schmidt RL, Hanson KE. Cost-effectiveness analysis of multiplex PCR with magnetic resonance detection versus empiric or blood culture-directed therapy for management of suspected candidemia. J Clin Microbiol. 2016;54(3):718–26.
46. Muñoz P, Vena A, Machado M, Gioia F, Martínez-Jiménez MC, Gómez E, et al. T2MadRid study group.T2Candida MR as a predictor of outcome in patients with suspected invasive candidiasis starting empirical antifungal treatment: a prospective pilot study. J Antimicrob Chemother. 2018;73(suppl_4):iv6–iv12.
47. Zacharioudakis IM, Zervou FN, Mylonakis E. Use of T2MR in invasive candidiasis with and without candidemia. Future Microbiol. 2018;13:1165–73.
48. Lamoth F, Clancy CJ, Tissot F, Squires K, Eggimann P, Flückiger U, et al. Performance of the T2Candida panel for the diagnosis of intra-abdominal candidiasis. Open Forum Infectious Diseases 2020;7(3).
49. Bilir SP, Ferrufino CP, Pfaller MA, Munakata J. The economic impact of rapid Candida species identification by T2Candida among high-risk patients. Future Microbiol. 2015;29:1–12.
50. Pfaller MA, Wolk DM, Lowery TJ. T2MR and T2Candida: novel technology for the rapid diagnosis of candidemia and invasive candidiasis. Future Microbiol. Future Medicine Ltd. 2016;11:103–17.
51. Zacharioudakis IM, Zervou FN. Mylonakis E.T2 magnetic resonance assay: overview of available data and clinical implications. J Fungi (Basel). 2018;4(2):45.
52. Monday LM, Parraga Acosta T, Alangaden. T2Candida for the diagnosis and management of invasive Candida infections. GJ Fungi (Basel). 2021;7(3):178.
53. Clancy CJ, Nguyen MH. T2 magnetic resonance for the diagnosis of bloodstream infections: charting a path forward. J Antimicrob Chemother. 2018;73(suppl_4):iv2–5.
54. de Almeida JN Jr, Francisco EC, Hagen F, Brandão IB, Pereira FM, Presta Dias PH, et al. Emergence of Candida auris in Brazil in a COVID-19 intensive care unit. J Fungi (Basel). 2021;7(3):220.
55. Theill L, Dudiuk C, Morales-Lopez S, Berrio I, Rodríguez JY, Marin A, et al. Single-tube classical PCR for Candida auris and Candida haemulonii identification. Rev Iberoam Micol. 2018;35(2):110–1.
56. Ruiz-Gaitán AC, Fernández-Pereira J, Valentin E, Tormo-Mas MA, Eraso E, Pemán J, et al. Molecular identification of Candida auris by PCR amplification of species-specific GPI protein-encoding genes. Int J Med Microbiol. 2018;308(7):812–8.
57. Kordalewska M, Zhao Y, Lockhart SR, Chowdhary A, Berrio I, Perlin DS. Rapid and accurate molecular identification of the emerging multidrug-resistant pathogen Candida auris. J Clin Microbiol. 2017;55(8):2445–52.
58. Sexton DJ, Kordalewska M, Bentz ML, Welsh RM, Perlin DS, Litvintseva AP. Direct detection of emergent fungal pathogen Candida auris in clinical skin swabs by SYBR green-based quantitative PCR assay. J Clin Microbiol. 2018;56(12):e01337–18.

59. Leach L, Zhu Y, Chaturvedi S. Development and validation of a real-time PCR assay for rapid detection of Candida auris from surveillance samples. J Clin Microbiol. 2018;56(2):e01223–17.
60. Leach L, Russell A, Zhu Y, Chaturvedi S, Chaturvedi V. A rapid and automated sample-to-result Candida auris real-time PCR assay for high-throughput testing of surveillance samples with the BD Max Open System. J Clin Microbiol. 2019;57(10):e00630–19.
61. Lima A, Widen R, Vestal G, Uy D, Silbert S. A TaqMan probe-based real-time PCR assay for the rapid identification of the emerging multidrug-resistant pathogen Candida auris on the BD Max System. J Clin Microbiol. 2019;57(7):e01604–18.
62. Arastehfar A, Fang W, Daneshnia F, Al-Hatmi AM, Liao W, Pan W, et al. Novel multiplex real-time quantitative PCR detecting system approach for direct detection of Candida auris and its relatives in spiked serum samples. Future Microbiol. 2019;14:33–45.
63. Walchak RC, Buckwalter S, Zinsmaster NM, et al. *Candida auris* Direct detection from surveillance swabs, blood, and urine using a laboratory-developed PCR method. J Fungi. 2020;6(4):224.
64. Mulet Bayona JV, Garcia CS, Palop NT, Cardona CG. Validation and implementation of a commercial real-time PCR assay for direct detection of *Candida auris* from surveillance samples. Mycoses. 2021;64:612–5. https://doi.org/10.1111/myc.13250. Online ahead of print.
65. Sattler J, Noster J, Brunke A, Plum G, Wiegel P, Kurzai O, et al. Comparison of two commercially available qPCR kits for the detection of Candida auris. J Fungi (Basel). 2021;7(2):154.
66. Sexton DJ, Bentz ML, Welsh RM, Litvintseva AP. Evaluation of a new T2 magnetic resonance assay for rapid detection of emergent fungal pathogen Candida auris on clinical skin swab samples. Mycoses. 2018;61(10):786–90.
67. Jainlabdin MH, Batra A, Paredes ES, et al. Single-tube, dual channel pentaplexing for the identification of Candida strains associated with human infection. Sci Rep. 2019;9(1):14692.
68. Ahmad S, Khan Z, Al-Sweih N, Alfouzan W, Joseph L. Candida auris in various hospitals across Kuwait and their susceptibility and molecular basis of resistance to antifungal drugs. Mycoses. 2020;63(1):104–12.
69. Hou X, Lee A, Jiménez-Ortigosa C, Kordalewska M, Perlin DS, Zhao Y. Rapid detection of ERG11-associated azole resistance and FKS-associated echinocandin resistance in Candida auris. Antimicrob Agents Chemother. 2018;63(1):e01811-1.
70. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, et al. Simultaneous emergence of multidrug-resistant Candida auris on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis. 2017;64:134–40.
71. Chow NA, de Grrot T, Badali H, et al. Potential fifth clade of Candida auris, Iran, 2018. Emerg Infect Dis. 2019;25:1780–1.
72.• Perlin DS, Wiederhold NP. Culture-independent molecular methods for detection of antifungal resistance mechanisms and fungal identification. J Infect Dis. 2017;216(suppl_3):S458–S46 **Comprehensive review of antifungal resistance mechanisms.**
73. Balashov SV, Park S, Perlin DS. Assessing resistance to the echinocandin antifungal drug caspofungin in Candida albicans by profiling mutations in FKS1. Antimicrob Agents Chemother. 2006;50:2058–63 59.
74. Pham CD, Bolden CB, Kuykendall RJ, Lockhart SR. Development of a Luminex based multiplex assay for detection of mutations conferring resistance to echinocandins in Candida glabrata. J Clin Microbiol. 2014;52:790–5 60.
75. Zhao Y, Nagasaki Y, Kordalewska M, Press EG, Shields RK, Nguyen MH, et al. Rapid detection of FKS-associated echinocandin resistance in Candida glabrata. Antimicrob Agents Chemother. 2016;60:6573–7.
76. Teo JQ, Lee SJ, Tan AL, Lim RS, Cai Y, Lim TP, et al. Molecular mechanisms of azole resistance in Candida bloodstream isolates. BMC Infect Dis. 2019;19(1):63.
77. Paul S, Dadwal R, Singh S, Shaw D, Chakrabarti A, Rudramurthy SM, et al. Rapid detection of ERG11 polymorphism associated azole resistance in Candida tropicalis. PLoS One. 2021;16(1):e0245160.
78. Biswas C, Chen SC, Halliday C, Martinez E, Rockett RJ, Wang Q, et al. Whole genome sequencing of Candida glabrata for detection of markers of antifungal drug resistance. J Vis Exp. 2017;130:56714.
79. White LP, Price JS. Recent advances and novel approaches in laboratory-based diagnostic mycology. J Fungi (Basel). 2021;7(1):41.
80. Garnaud C, Botterel F, Sertour N, Bougnoux ME, Dannaoui E, Larrat S, et al. Next-generation sequencing offers new insights into the resistance of Candida spp. to echinocandins and azoles. J Antimicrob Chemother. 2015;70(9):2556–65.
81. Hagen F, Khayhan K, Theelen B, et al. Recognition of seven species in the *Cryptococcus gattii/Cryptococcus neoformans* species complex. Fungal Genet Biol. 2015;78:16–48.
82. Pryszcz LP, Németh T, Gácser A, et al. Genome comparison of *Candida orthopsilosis* clinical strains reveals the existence of hybrids between two distinct subspecies. Genome Biol Evol. 2014;6:1069–78.
83. Schroder MS, Martinez de San Vicente K, Prandini THR, et al. Multiple origins of the pathogenic yeast *Candida orthopsilosis* by separate hybridizations between two parental species. PLoS Genet. 2016;12:e1006404.
84. Bruno VM, Shetty AC, Yano J, et al. Transcriptomic analysis of vulvovaginal candidiasis identifies a role for the NLRP3 inflammasome. MBio. 2015;6:e00182–15.
85. Huseyin CE, O'Toole PW, Cotter PD, Scanlan PD. Forgotten fungi-the gut mycobiome in human health and disease. FEMS Microbiol Rev. 2017;41:479–511.
86. Usyk M, Zolnik CP, Patel H, Levi MH, Burk RD. Novel ITS1 Fungal Primers for Characterization of the Mycobiome. mSphere. 2017;2(6):e00488-1.
87. Holmgaard DB, Barnadas C, Mirbarati SH, O'Brien Andersen L, Nielsen HV, Stensvold CR. Detection and identification of Acanthamoeba and other nonviral causes of infectious keratitis in corneal scrapings by real-time PCR and next-generation sequencing-based 16S-18S gene analysis. J Clin Microbiol. 2021;59(2):e02224–0.
88.•• Nilsson RH, Anslan S, Bahram M, et al. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev. 2019;17:95–109 **A review of the technical aspects and limitations of next generation sequencing.**
89. Vutukuru MR, Sharma DK, Ragavendar MS, Schmolke S, Huang Y, Gumbrecht W, et al. A rapid, highly sensitive and culture-free detection of pathogens from blood by positive enrichment. J Microbiol Methods. 2016;131:105–9.
90. Boardman AK, Campbell J, Wirz H, Sharon A, Sauer-Budge AF. Rapid microbial sample preparation from blood using a novel concentration device. PLoS One. 2015;10(2):e0116837.
91. Nam J, Jang WS, Hong DH, Lim CS. Viscoelastic separation and concentration of fungi from blood for highly sensitive molecular diagnostics. Sci Rep. 2019;9(1):3067.
92. Goldschmidt P, Degorge S, Merabet L, Chaumeil C. Enzymatic treatment of specimens before DNA extraction directly influences molecular detection of infectious agents. PLoS One. 2014;9(6):e94886.
93. Reichard U, Buchheidt D, Lass-Flörl C, Loeffler J, Lugert R, Ruhnke M, et al. Interlaboratory comparison of PCR-based identification of Candida and Aspergillus DNA in spiked blood samples. Mycoses. 2012;55(5):426–34.

94. White P, Barton R, Guiver M, et al. A consensus on fungal polymerase chain reaction diagnosis?: a United Kingdom-Ireland evaluation of polymerase chain reaction methods for detection of systemic fungal infections. J Mol Diag. 2006;8(3):376–84.
95. Menu E, Landier J, Prudent E, Ranque S, L'Ollivier C. Evaluation of 11 DNA automated extraction protocols for the detection of the 5 mains Candida species from artificially spiked blood. J Fungi (Basel). 2021;7(3):228.
96. Dalla-Costa LM, Morello LG, Conte D, Pereira LA, Palmeiro JK, Ambrosio A, et al. Comparison of DNA extraction methods used to detect bacterial and yeast DNA from spiked whole blood by real-time PCR. J Microbiol Methods. 2017;140:61–6.
97. Mazoteras P, Bispo PJ, Höfling-Lima AL, Casaroli-Marano RP. DNA extraction methods for panbacterial and panfungal PCR detection in intraocular fluids. Curr Eye Res. 2015;40(7):697–706.
98. Loonen AJ, Bos MP, van Meerbergen B, Neerken S, Catsburg A, Dobbelaer I, et al. Comparison of pathogen DNA isolation methods from large volumes of whole blood to improve molecular diagnosis of bloodstream infections. PLoS One. 2013;8(8):e72349.
99. Arastehfar A, Fang W, Pan W, Liao W, Yan L, Boekhout T. Identification of nine cryptic species of Candida albicans, C. glabrata, and C. parapsilosis complexes using one-step multiplex PCR. BMC Infect Dis. 2018;18(1):480.
100. Nawar NN, Behiry IK, Yousef RHA, Emara MA. Application of high-resolution melting PCR to detect the genomic fungal ITS 2 region. Lab Med. 2020;51(1):66–73.
101. Eghtedar Nejad E, Ghasemi Nejad Almani P, Mohammadi MA, Salari S. Molecular identification of Candida isolates by real-time PCR-high-resolution melting analysis and investigation of the genetic diversity of Candida species. J Clin Lab Anal. 2020;34(10):e23444.
102. Nemcova E, Cernochova M, Ruzicka F, Malisova B, Freiberger T, Nemec P. Rapid identification of medically important Candida isolates using high resolution melting analysis. PLoS One. 2015;10(2):e0116940.
103. Feng X, Wu Z, Ling B, Pan S, Liao W, Pan W, et al. Identification and differentiation of Candida parapsilosis complex species by use of exon-primed intron-crossing PCR. J Clin Microbiol. 2014;52(5):1758–61.
104. Fallahi S, Babaei M, Rostami A, Mirahmadi H, Arab-Mazar Z, Sepahvand A. Diagnosis of Candida albicans: conventional diagnostic methods compared to the loop-mediated isothermal amplification (LAMP) assay. Arch Microbiol. 2020;202(2):275–82.
105. Trabasso P, Matsuzawa T, Fagnani R, Muraosa Y, Tominaga K, Resende MR, et al. Isolation and drug susceptibility of Candida parapsilosis sensu lato and other species of C. parapsilosis complex from patients with blood stream infections and proposal of a novel LAMP identification method for the species. Mycopathologia. 2015;179:53–62.
106. Aittakorpi A, Kuusela P, Koukila-Kähkölä P, Vaara M, Petrou M, Gant V, et al. Accurate and rapid identification of Candida spp. frequently associated with fungemia by using PCR and the microarray-based Prove-it Sepsis assay. J Clin Microbiol. 2012;50(11):3635–40.
107. Zhang SX, Carroll KC, Lewis S, Totten M, Mead P, Samuel L, et al. Multicenter evaluation of a PCR-based digital microfluidics and electrochemical detection system for the rapid identification of 15 fungal pathogens directly from positive blood cultures. J Clin Microbiol. 2020;58(5):e02096–19.
108. Carroll KC, Reid JL, Thornberg A, Whitfield NN, Trainor D, Lewis S, et al. Clinical performance of the novel GenMark Dx ePlex Blood Culture ID Gram-Positive panel. J Clin Microbiol. 2020;58(4):e01730–19.
109. Ao W, Klonoski J, Berlinghoff E, Jensen J, Afroz T, Munns D, et al. Rapid detection and differentiation of clinically relevant Candida species simultaneously from blood culture by use of a novel signal amplification approach. J Clin Microbiol. 2017;56(1):e00982–17.
110. Kim J, Wang HY, Kim S, Park SD, Yu K, Kim HY, et al. Evaluation of the Punch-it NA-Sample kit for detecting microbial DNA in blood culture bottles using PCR-reverse blot hybridization assay. J Microbiol Methods. 2016;128:24–30.
111. Kweon OJ, Choi JH, Song UH, Park AJ. Performance evaluation of a DNA chip assay in the identification of major genitourinary pathogens. J Microbiol Methods. 2015;109:117–2.
112. Rickerts V, McCormick Smith I, Mousset S, Kommedal O, Fredricks DN. Deciphering the aetiology of a mixed fungal infection by broad-range PCR with sequencing and fluorescence in situ hybridisation. Mycoses. 2013;56(6):681–6.
113. Selva Pandiyan A, Siva Ganesa Karthikeyan R, Rameshkumar G, Sen S, Lalitha P. Identification of bacterial and fungal pathogens by rDNA gene barcoding in vitreous fluids of endophthalmitis patients. Semin Ophthalmol. 2020;35(7-8):358–64.
114. Sakai T, Kohzaki K, Watanabe A, Tsuneoka H, Shimadzu M. Use of DNA microarray analysis in diagnosis of bacterial and fungal endophthalmitis. Clin Ophthalmol. 2012;6:321–6.
115. He Y, Geng W, Wang P, Xi L, Wang Z, Wu G, et al. Simultaneous detection of four common oral Candida species from blood samples by the fluorescence polarization assay. Cell Biochem Biophys. 2015;71(2):919–24.
116. White PL, Hibbitts SJ, Perry MD, Green J, Stirling E, Woodford L, et al. Evaluation of a commercially developed semiautomated PCR-surface-enhanced Raman scattering assay for diagnosis of invasive fungal disease. J Clin Microbiol. 2014;52(10):3536–43.
117. Ogata K, Matsuda K, Tsuji H, Nomoto K. Sensitive and rapid RT-qPCR quantification of pathogenic Candida species in human blood. J Microbiol Methods. 2015;117:128–35.
118. Jiang X, Dong D, Bian L, Zou D, He X, Ao D, et al. Rapid detection of Candida albicans by polymerase spiral reaction assay in clinical blood samples. Front Microbiol. 2016;7:916.
119. Schell WA, Benton JL, Smith PB, Poore M, Rouse JL, Boles DJ, et al. Evaluation of a digital microfluidic real-time PCR platform to detect DNA of Candida albicans in blood. Eur J Clin Microbiol Infect Dis. 2012;31(9):2237–45.
120. Noguchi H, Iwase T, Omagari D, Asano M, Nakamura R, Ueki K, et al. Rapid detection of Candida albicans in oral exfoliative cytology samples by loop-mediated isothermal amplification. J Oral Sci. 2017;59:541–7.
121. Wang HY, Kim J, Kim S, Park SD, Kim HY, Choi HK, et al. Performance of PCR-REBA assay for screening and identifying pathogens directly in whole blood of patients with suspected sepsis. J Appl Microbiol. 2015;119(5):1433–42.
122. Agustí G, Fittipaldi M, Morató J, Codony F. Viable quantitative PCR for assessing the response of Candida albicans to antifungal treatment. Appl Microbiol Biotechnol. 2013;97(1):341–9.
123. Nagai H, Yamakami Y, Hashimoto A, Tokimatsu I, Nasu M. PCR detection of DNA specific for Trichosporon species in serum of patients with disseminated trichosporonosis. J Clin Microbiol. 1999;37(3):694–9.
124. Nagano Y, Elborn JS, Millar BC, Goldsmith CE, Rendall J, Moore JE. Development of a novel PCR assay for the identification of the black yeast, Exophiala (Wangiella) dermatitidis from adult patients with cystic fibrosis (CF). J Cyst Fibros. 2008;7(6):576–80.
125. Jordanides NE, Allan EK, Mclintock LA, et al. A prospective study of real-time panfungal PCR for the early diagnosis of invasive fungal infection in haemato-oncology patients. Bone Marrow Transplant. 2005;35(4):389–95.
126. Buitrago MJ, Aguado JM, Ballen A, Bernal-Martinez L, Prieto M, Garcia-Reyne A, et al. Efficacy of DNA amplification in tissue biopsy samples to improve the detection of invasive fungal disease. Clin Microbiol Infect. 2013;19(6):E271–7.

127.• Arastehfar A, Fang W, Pan W, Lackner M, Liao W, Badiee P, et al. YEAST PANEL multiplex PCR for identification of clinically important yeast species: stepwise diagnostic strategy, useful for developing countries. Diagn Microbiol Infect Dis. 2019;93(2):112–9 **Development of a novel approach to identify a wide range of yeasts, not requiring MALDI-TOF, for use in resource limited settings.**

128. Carvalho-Pereira J, Fernandes F, Araújo R, Springer J, Loeffler J, Buitrago MJ, et al. Multiplex PCR based strategy for detection of fungal pathogen DNA in patients with suspected invasive fungal infections. J Fungi (Basel). 2020;6(4):308.

129. Gosiewski T, Jurkiewicz-Badacz D, Sroka A, Brzychczy-Włoch M, Bulanda M. A novel, nested, multiplex, real-time PCR for detection of bacteria and fungi in blood. BMC Microbiol. 2014;14:144.

130. Senchyna F, Hogan CA, Murugesan K, Moreno A, Ho DY, Subramanian A, Schwenk HT, Budvytiene I, Costa HA, Gombar S, Banaei N. Clinical accuracy and impact of plasma cell-free DNA fungal PCR panel for non-invasive diagnosis of fungal infection. Clin Infect Dis. 2021;ciab158

131. Strålin K, Rothman RE, Özenci V, Barkataki K, Brealey D, Dhiman N, et al. Performance of PCR/electrospray ionization-mass spectrometry on whole blood for detection of bloodstream microorganisms in patients with suspected sepsis. J Clin Microbiol. 2020;58(9):e01860–19.

132. Ullberg M, Lüthje P, Mölling P, Strålin K, Özenci V. Broad-range detection of microorganisms directly from bronchoalveolar lavage specimens by PCR/electrospray ionization-mass spectrometry. PLoS One. 2017;12(1):e0170033.

133. Metzgar D, Frinder MW, Rothman RE, Peterson S, Carroll KC, Zhang SX, et al. The IRIDICA BAC BSI assay: rapid, sensitive and culture-independent identification of bacteria and Candida in blood. PLoS One. 2016;11(7):e0158186.

134. Chang YT, Wang HC, Wang MC, Wu AB, Sung JM, Sun HS, et al. Rapid identification of bacteria and Candida pathogens in peritoneal dialysis effluent from patients with peritoneal dialysis-related peritonitis by use of multilocus PCR coupled with electrospray ionization mass spectrometry. J Clin Microbiol. 2014;52(4):1217–9.

135. Bacconi A, Richmond GS, Baroldi MA, Laffler TG, Blyn LB, Carolan HE, et al. Improved sensitivity for molecular detection of bacterial and Candida infections in blood. J Clin Microbiol. 2014;52(9):3164–74.

136. Balada-Llasat JM, LaRue H, Kamboj K, Rigali L, Smith D, Thomas K, et al. Detection of yeasts in blood cultures by the Luminex xTAG fungal assay. J Clin Microbiol. 2012;50(2):492–4.