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A Systematic Review of Diagnostic Accuracy: Serological and Molecular Tests Compared to Culture for Invasive Fungal Infections

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Abstract: *Invasive fungal infections (IFIs) remain a major clinical challenge, particularly among immunocompromised patients, due to their high morbidity, mortality, and diagnostic complexity. Despite being the gold standard, traditional fungal culture has low sensitivity and takes a long time to produce results. Recently, serological and molecular diagnostic approaches have emerged as potential alternatives to earlier and more precise IFI detection. The goal of this systematic review was to evaluate the effectiveness of various non-culture approaches and their potential impact on diagnostic precision. To detect invasive fungal infections (IFIs), this systematic review compared the diagnostic accuracy of commonly used genetic and serological approaches to fungal culture. A thorough literature search yielded eligible studies testing assays including β-D-glucan, galactomannan, cryptococcal antigen, and PCR-based approaches. A random-effects model was employed to calculate pooled sensitivity and specificity. While several serological techniques demonstrated good specificity and consistency across numerous fungal species, molecular assays were often more sensitive than culture, particularly for early detection. Despite variations in research quality and inconsistency in diagnostic thresholds, the data show that combining serological and molecular approaches significantly improves diagnosis accuracy and may reduce delays associated with culture-based identification. This systematic review demonstrates that for the diagnosis of invasive fungal infections, both molecular and serological diagnostic procedures outperform culture-based approaches. Molecular assays are important for early diagnosis due to their high sensitivity and rapid turnaround time. Serological tests are highly specific and reliable when performed correctly.*

Keywords: *Immunocompromised individuals; Invasive fungal infections; Molecular testing; Serological testing; Culture*

Introduction

Invasive fungal infections (IFI) represent a major medical problem, with an estimated 6.5 million cases per year and 3.8 million deaths. Pathogens, such as *Aspergillus* species *Mucorales* species, *Candida* species. *Cryptococcus* species and other fungi that pose a risk to immunocompromised persons are the source of these diseases. Effective therapy and better patient outcomes depend on prompt and accurate diagnosis (Sedik *et al.*,2024).

Infections brought on by organisms that are either intrinsically resistant to or less sensitive to antifungal medications are examples of invasive fungal diseases (IFDs), a growing global public health concern in immunocompromised and other seriously ill populations. "Optimal patient outcomes depend on early diagnosis, which includes

accurate detection and identification of the causative agent and antifungal resistance" (Kidd *et al*.,2020).

Every year, millions of people have invasive fungal infections, which are challenging to identify. "This has further necessitated the development of inexpensive, user-friendly, highly sensitive, and specific molecular assays that can distinguish between pathogenic and colonized organisms from a variety of clinical specimens" (Jenks *et al*.,2023).

Invasive fungal disease (IFD) continues to be a significant cause of morbidity and mortality in hospitalized patients despite advancements in antifungal treatments. "Routine histological and culture techniques are slow and insensitive, but early diagnosis, which must include species identification, is critical to improving patient outcomes" (Arvanitis *et al*.,2014).

Due to the high rates of morbidity and mortality and the significant financial burden of managing invasive fungal infections (IFIs), accurate identification is essential (Terrero-Salcedo & Powers-Fletcher,2020). It can be difficult to identify IFIs and distinguish them from other infections with comparable clinical presentations, which could result in diagnostic errors that impact not only the health outcomes of individual patients but also the use of antimicrobial drugs and the growing risk of antimicrobial resistance in bacteria (Terrero-Salcedo & Powers-Fletcher,2020). As a result, "better stewardship regarding diagnostic testing for and treatment of IFIs is greatly needed "(Terrero-Salcedo & Powers-Fletcher,2020).

Fungal cultivation is a copper standard at best. If fungal cultures are positive at all, they frequently do so too late in the course of the illness to be clinically helpful (Nguyen *et al*.,2012). "Microscopy is useful for identifying fungi, and it can be quick and sensitive (Hay *et al*.,2019), but it also has limited sensitivity, needs skilled operators, and can be imprecise given the similar microscopic appearance of several fungi" (Mendonca *et al*.,2022). "This has led to the development of molecular and serological tests that have the potential to revolutionize fungal diagnostics in terms of cost-effectiveness, speed, and accuracy" (Mendonca *et al*.,2022).

Aims of this study are:

1. Evaluate the diagnostic accuracy of molecular assays, including PCR and serological testing, compared to fungal culture for detecting invasive fungal infections.
2. Evaluate the effectiveness of molecular and serological diagnostics for diverse fungal infections and clinical situations.
3. Determine the diagnostic value of combining molecular and serological methods for early IFI detection.

Literatures Review

Invasive fungal infections

IFIs are systemic infections caused by the colonization of deep tissues by yeasts or moulds. Superficial fungal infections, unlike IFIs, do not cause major morbidity and mortality (Pagano & Mayor, 2018). Fungal infections such as *candida*, *aspergillus*,

cryptococcus, and *pneumocystis* are the most commonly identified invasive illnesses. Furthermore, *Blastomyces*, *Histoplasma*, *Paracoccidioides*, and *Coccidioides* are endemic fungi that have been related to significant systemic infections in immunocompromised individuals (Malcolm & Chin-Hong, 2013).

"IFDs are common; nevertheless, in many countries, they remain underdiagnosed due to a lack of understanding among healthcare practitioners (Driemeyer *et al*.,2022) and the use of inferior diagnostic methods at healthcare institutions (Salmanton *et al*.,2023). "Furthermore, the treatment of IFD is particularly difficult once diagnosed (Vena *et al*.,2020) due to the lack of effective antifungal medicines (Cornely *et al*.,2019 ; Hoenigl *et al*.,2021), toxicity (Mellinghoff *et al*.,2018), and the requirement for constant monitoring (Vena *et al*.,2020), producing a significant unmet demand for patients and increasing the healthcare burden.

Acute invasive fungal infections (IFI) affect roughly 1.9 million patients annually, while chronic severe fungal infections affect an estimated 3 million persons worldwide (Bongomin *et al*.,2017). "Every year, more than 1.6 million people are expected to die as a result of fungal illnesses. Many of these diseases can be deadly." Despite advances in medical understanding, invasive fungal infections (IFI) remain difficult to detect. "The increasing incidence of IFI, due to immunosuppressive therapies, advances in critical care and new infections highlights the need of early and accurate identification" (Pemán & Ruiz-Gaitán,2025).

Population at Risk for IFD

The number of people at risk for IFD has increased over the last few decades as a result of a variety of disorders and advances in medical techniques that cause immunosuppression. "Candidiasis and aspergillosis are the most common IFDs in immunosuppressed patients (neutropenia, haematologic malignancies, solid and hematopoietic stem cell transplant, chemotherapy, or congenital immunodeficiency disorders), (Enoch et al.,2016)." IFDs caused by *Pneumocystis jiroveci* and *Cryptococcosis* sp. are common in HIV patients (Wang et al.,2017).

Candida albicans and *Aspergillus fumigatus* were the most prevalent species among immunocompromised patients. However, the proportion of non-*albicans Candida* sp., non-*fumigatus Aspergillus* sp., and other moulds outside *Aspergillus* spp. has grown. Other fungi, such as *Candida auris*, can cause severe outbreaks in intensive care units and resistant nosocomial infections. They can be resistant to antifungal medication and have a higher death rate (Černáková et al.,2021).

Invasive candidiasis (IC) is the top fungal infection among hospitalized patients worldwide. "IC causes about 50,000 fatalities each year and affects over 250,000 people worldwide, according to conservative estimates (Kullberg & Arendrup,2015). IC is frequently described in critically unwell patients in the intensive care unit (ICU) (McCarty & Pappas,2016). Risk factors for IC include granulocytopenia, stem cell transplantation, organ transplantation, broad spectrum antimicrobial agents, central venous catheterization, total parenteral nutrition, length of stay in the intensive care unit (ICU), surgery, advanced

life support, and aggressive chemotherapy (McCarty & Pappas,2016). Candidemia is the most common form of invasive candidiasis (IC). Deep or visceral candidiasis can be caused by hematogenous dissemination or direct injection of Candida species into a normally sterile environment, such as the peritoneal cavity. More than 15 *Candida* species can cause candidemia in humans (Wang *et al*.,2014).

Diagnosis methods

"The diagnosis of invasive or chronic fungal infections is difficult, particularly in immunocompromised hosts. 'Microscopy and culture are still the gold standard but they are not sensitive enough. It is proposed that non-culture-based approaches should be used in addition to conventional methods for improved diagnostic yield" (Lass-Flörl *et al*.,2021). "Current laboratory procedures for the diagnosis of IFI include direct microscopy, fungal culture and identification of the pathogen, histopathology, antigen detection and PCR tests for detection of fungal DNA" (Salmanton-García *et al*.,2023).

"It should be noted that the diagnostic classification of IFI (proven or probable) according to the criteria of published diagnostic guidelines for different host conditions will depend on the technique used for the diagnosis". For the confirmed IFI diagnosis, culture or histopathology for the fungal pathogen in sterile specimens is required, and for the probable IFI diagnosis, serological or molecular approaches for the detection of antigens or fungal DNA, respectively, are applied (Donnelly *et al*.,2020; Dannaoui,2022).

Conventional microbiological techniques are not sensitive enough to diagnose fungal infections. Blood cultures may fail to detect candidemia in up to 50% of cases (Clancy & Nguyen,2013). For fungal infections, culture sensitivity is significantly lower. This has resulted in the development of noncultural serologic and molecular diagnostics for diagnosis of invasive fungal illness. "Serologic tests include Aspergillus galactomannan antigen and 1-3 beta-D-glucan for fungal infections other than cryptococcosis and zygomycosis and are part of the diagnostic criteria for IFI" (De Pauw *et al*.,2008). "Galactomannan detection in BAL fluid has higher sensitivity and specificity for the diagnosis of invasive pulmonary aspergillosis than in serum (Zou *et al*.,2012). "The acceptance of PCR-based tests has not been as good, mainly due to the lack of standardization in the type of clinical sample (serum, plasma, tissue), primer selection ("pan-fungal", species specific) and PCR format (qualitative versus quantitative, real time)" (Alanio & Bretagne,2014). These tests have diagnostic potential, supported by systematic reviews. (Table 1).

Table 1: A comparison of laboratory methods for the conclusive identification of invasive fungal infections.

Technique	Strengths	Weaknesses	Diagnostic utility	References
"Direct microscopy"	•Rapid findings (<5 min) • Easy to implement • Economic	• Limited sensitivity (50%) • Not specific enough • Unknown genus or species • Required expertise	• Candidiasis • Hyphomycosis • Zygomycosis • Pheomycosis • Cryptococcosis	(Donnelly *et al*.,2020 ; Sattler *et al*.,2021)

"Mycological culture "	• Isolation of the fungus pathogen • Identification down to species level • Antifungal susceptibility testing • Long-term strains storage • Quantified data	• Prolonged (>48 h) • Targeted treatment with long latency • Prone to contamination • False negative outcomes possible (50%) • False-positive results may be due to sterile/deep specimens	• Candidiasis • Hyphomycosis • Zygomycosis • Pheomycosis • Cryptococcosis	(Bao *et al.*,2021)
"Blood culture"	• Best diagnostic method for candidemia and other systemic yeast infections • Detection of resistance to antifungal agents	• Prolonged duration (>48 hours) • Potential for false-negative outcomes (50%) • The lysis-centrifugation approach is advised for endemic mycosis. • Not applicable for systemic filamentous fungal mycoses (with exceptions)	• Candidemia • Systemic mycosis by: ✓ *Fusarium* ✓ *Scedosporium* ✓ *T. marneffei* ✓ *Lomentospora*	(Hamam *et al.*,2021)
"Fungal identification from culture": •Morphologic identification laboratories	Most frequent method for identification of filamentous fungus	•Not beneficial for yeast • Takes time • May require special culture media • Requires trained people or reference	Any mycoses caused by filamentous or dimorphic fungi	(Honarvar *et al.*,2018)
• Chromogenic media	• Simple to use • Presumptive identification of the most prevalent species of Candida • Economical • Particularly helpful in surveillance cultures and	• Time-consuming (48 hours) • It is impossible to identify rare or rarely species	Candidiasis	(Pini *et al.*,2019;Robert *et al.*,2020)

	mixed infections			
• **Biochemical identification**	• Good for common species of yeasts • Economical	• Arduous and protracted (>48 h) • Elevated incidence of misidentification for rare or novel species. • Supplanted by mass spectrometry in clinical laboratories	• Common yeasts infections	(Mauri *et al.*,2024)
• **MALDI-TOF**	• Fast findings (<10 min) Simple to perform •Lower cost per analysis •Correct species identification of yeasts and some moulds at the species level • Separation of species that are closely related • Direct ID from blood culture potential	•Requires colony growth on solid culture medium •Requires pre-extracted features •Databases provide accurate live updates for the rarest and new species •Most moulds still difficult to detect: ◦Small number of mould spectra ◦Contamination of the spectra by culture media ◦Extended protein extraction ◦ Staff training required	• Yeasts infections • Aspergillosis • Fusariosis • Scedosporiosis	(Arvanitis *et al.*,2014; Rajasingham *et al.*,2019)
• **Molecular identification by automated platforms**	• Quick results (<90 min) • Easy to operate • Excellent sensitivity and specificity • Correct identification of common Candida and Cryptococcus species	• High cost per test • Special equipment required • Rare species on fungus panels. • Isolates from blood culture only	• Fungemia	(Bryant *et al.*,2020; Lin *et al.*,2022)
•**DNA sequencing for fungal isolates**	• Gold standard for molecular identification of fungus	• Time-consuming and laborious (>24 h)	• Any mycoses caused by yeasts, filamentous, or dimorphic fungi	(Wickes & Wiederhold,2018)

• Maximum specificity • Inexpensive • Public databases (large)	• Extensive technological knowledge. • PCR amplification step required. • May require interpreting data • Not much commercialized.

Mycological culture

"Despite the integration of numerous novel laboratory diagnostic techniques in recent decades, mycological culture continues to be the gold standard for diagnosing invasive fungal infections (IFI), as it uniquely facilitates the isolation, comprehensive identification, and preservation of the causative pathogen, along with the subsequent assessment of its susceptibility to antifungal agents" (Bao *et al.*,2021).

"While most fungi thrive in standard clinical bacteriology culture media (blood agar, chocolate agar), it is advisable to inoculate samples in specialized mycological media, such as Sabouraud dextrose agar with chloramphenicol (SDCA), brain heart infusion agar (BHIA), or inhibitory mould agar (IMA), to enhance fungal proliferation." IMA is advised for the optimum separation of filamentous fungus (Bao *et al.*,2021). "After seeding, plates must be incubated at 30 ◦C, which is the optimal growth temperature for the majority of pathogenic fungi." In the absence of humidity in the incubator, it is prudent to position a container of water adjacent to the culture plates. Fungal cultures of valuable or hard-to-acquire specimens should be cultured for 3 to 4 weeks prior to disposal. "Incubation at 30 ◦C and 37 ◦C should be conducted separately for suspected infections caused by Zygomycetes or dimorphic fungi" (Cornely *et al.*,2019).

Blood culture is the most effective approach for detecting fungemia, despite its low overall sensitivity (50-95%). The automated continuous monitoring methods utilized for bacteria are also advised for yeast recovery from blood; however, if a systemic infection by a dimorphic fungus is suspected, the lysis-centrifugation procedure seems to offer superior benefits. Nevertheless, neither of the two blood culture techniques has proven viable for the recovery of the majority of filamentous fungi (Lu *et al.*,2019).

Blood cultures for yeasts necessitate 1–3 days for growth, followed by an additional 1–2 days for organism identification and susceptibility testing, often leading to delays in the commencement of targeted treatment. The turnaround time for candidemia can fluctuate based on the species, generally ranging from 14 to 72 hours, as certain species, such Candida glabrata, exhibit slower growth rates than others. Nevertheless, in cases with profound candidiasis absent candidemia, the sensitivity of blood cultures is significantly diminished, and the detection period is considerably prolonged)" Tabak *et al.*,2018).

Blood cultures exhibit a low diagnostic yield in invasive mycoses induced by filamentous fungus, as these infections typically lack a detectable hematogenous phase of dissemination. Filamentous fungus in blood cultures should generally be regarded as

contaminants, except in cases of mycoses caused by *Aspergillus terreus*, *Fusarium* spp., *Pseudoallescheria/Scedosporium* spp., *Lomentospora prolificans*, or *T. marneffei*. Blood cultures yield positive results in 40% to 50% of invasive fusariosis patients and in 70% of systemic infections caused by *Pseudoallescheria/Scedosporium* or *L. prolificans* (Table 1).

Table 2: Fungal culture sensitivity for different fungal species and TAT in invasive illness.

Fungus	Sample type and disease form	Culture sensitivity	TAT	References
Aspergillus	Sputum in chronic pulmonary disorders	30%	48–92 hours	(Denning,2021)
Candida	Hemorrhage in potential sepsis	30–50%	36–128 hours	(Mikulska *et al.*,2010; Avni *et al.*,2011)
Pneumocystis	Non-culturable	0%	NA	(Liu *et al.*,2018)
Cryptococcus	Cerebrospinal fluid in meningitis	80–95%	Up to 7 days	(Abassi *et al.*,2015; Boulware *et al.*,2014)
Histoplasma	Sputum in chronic pulmonary conditions	<50%	3–12 weeks	(Baker *et al.*,2020)
Mucorales	Samples from various organs	50%	3–5 days	(Roden *et al.*,2005)

Molecular methodologies

Upon the recovery of a fungal isolate, often from an automated blood culture system, various PCR-based techniques can be employed to detect pathogenic fungi. FilmArray® (bioMérieux) is a completely automated system that consolidates sample preparation, multiplex PCR amplification, and pathogen detection/identification in approximately one hour. The newly developed Biofire® Blood Culture Identification 2 (BCID2) Panel for this platform can identify 33 pathogens responsible for sepsis, comprising 26 bacterial species and 7 yeast species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. auris*, and *Cryptococcus gattii/Cryptococcus neoformans*), as well as 10 antimicrobial resistance genes. The panel's detection of microorganisms exhibited superior sensitivity and specificity (>98% and >99%, respectively) compared to cultures (Lu *et al.*,2019).

The inaugural handheld platform for Real Time PCR globally is the newly commercialized Molecular MouseTM system (Alifax, Italy). The pre-prepared lab-on-chip cartridges contain all lyophilized reagents in each micro-well and facilitate up to 6 concurrent multiplex operations. The five cartridges of the Sepsis Panel can identify 44 significant clinical bacteria and 20 antibiotic resistance genes from a positive blood culture in approximately one hour. The cartridges comprise 15 genera or species of Gram-negative bacteria, 20 genera or species of Gram-positive bacteria, and 9 species of *Candida*, including *C. albicans*, *C. auris*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. tropicalis*. Currently, there is few data regarding the efficacy of this new platform in a clinical context, and none pertains to bloodstream infections caused by *Candida*. The new device demonstrated constant alignment with conventional techniques for monomicrobial blood cultures; however, it did not entirely concur with the results of polymicrobial samples (Mauri *et al.*,2024).

The ePlex® system (GenMark Diagnostics, USA) is a comprehensive random-access multiplex PCR platform designed for syndromic diagnosis. It encompasses the fundamental procedures necessary for the detection of pathogens extracted from positive sterile sample cultures. Three panels have been created to identify 56 bacteria responsible for sepsis and 10 antibiotic resistance genes within 90 minutes, contingent upon the gram stain result of a positive blood culture. The Gram-negative Panel focuses on 21 bacterial genera or species and 6 resistance genes, whereas the Gram-positive Panel addresses 20 genera or species and 4 resistance genes. The Fungal Pathogen Panel identifies 15 fungal genera or species: *C. albicans, C. auris, Candida dubliniensis, C. famata, C. glabrata, Candida guilliermondii, Candida kefyr, Candida lusitaniae, C. parapsilosis, C. tropicalis, C. krusei, C. neoformans* sensu lato, *C. gattii* sensu lato, *Fusarium*, and *Rhodotorula*. The ePlex® method for fungal pathogens exhibits a worldwide sensitivity of 99.8–100% in blood cultures, alongside a specificity of 100% " (Bryant *et al*.,2020).

DNA sequencing is currently the definitive method employed for mould detection in several clinical mycology laboratories. "However, it possesses constraints." Identifying fungi to the species level remains a significant difficulty and typically necessitates data from various genetic targets. The high cost of testing, limited commercial availability, and issues with comparison databases restrict the extensive application of sequencing for accurate identification (Wickes & Wiederhold,2018Wickes & Wiederhold,2018).

Serological techniques

As previously stated, timely diagnosis of IFI is crucial for initiating antifungal treatment; yet, existing methods for definitive diagnosis frequently suffer from delays or inadequate sensitivity. "Serological methods have gained significant relevance in diagnosing fungal infections, particularly those induced by *Candida* and *Aspergillus* species, as they can identify specific antigens or antibodies in the host's blood or sterile fluids, including (1,3)-d-glucan, mannan antigen, anti-mannan antibody, anti-germ tube antibodies, galactomannan antigen, and glucuroxylomannan antigen." These approaches can be employed for the non-invasive diagnosis of probable IFI; nevertheless, they possess certain limitations (Table 3). The assay for (1→3)-β-D-glucan (BDG) is significant among the serological techniques. This assay has been validated for many fungal infections and delivers data potentially days prior to the availability of positive cultures, facilitating early infection identification (Bregón-Villahoz *et al*.,2025).

The application of contemporary serologic assays, including those for the detection of -d-glucan and galactomannan, has enhanced the diagnostic precision of probable invasive fungal infection cases. The amalgamation of these assays with clinical and radiological data may facilitate earlier intervention, albeit at the cost of heightened false positives and the necessity for meticulous clinical correlation. Molecular diagnostics, particularly polymerase chain reaction (PCR), provide swift species-specific identification from clinical specimens. MALDI-TOF mass spectrometry has enhanced diagnostic efficacy, especially in yeast identification; nonetheless, challenges persist for filamentous fungi (Pemán & Ruiz-Gaitán,2025).

Table 3: A comparison of commercial serological and PCR-based methods for diagnosing invasive mycoses.

Technique	Strengths	Weaknesses	Diagnostic utility	References
Beta-d-glucan	• Pan-fungal • Quantitative • Sensitivity 85–96% • Specificity 80–92%	• False positives resulting from various clinical conditions and pathogens • Ineffective for *Cryptococcus* and Zygomycetes infections	•Mycological criteria for suspected invasive fungal infection (IFI) • Instrument for the screening and surveillance of *Candida* and Pneumocystis infections	De Carolis *et al.*,2020 ; Yi *et al.*,2021)
Galactomannan	• Effective against Aspergillus and other mould infections • Helpful in BAL fluid and serum • Quantitative • Sensitivity 80-90% • 85–95% specific	• False positives resulting from host diseases, beta-lactam medicines, chemotherapy, and other factors	• Mycological criteria for suspected invasive aspergillosis • Additionally positive in IFI for *Scedosporium*, *Fusarium*, *Geotrichum*, and *Histoplasma*. • alternative moulds. • Appropriate for early identification and assessment of treatment response	(Albert *et al.*, 2024; Donnelly *et al.*,2020)
Mannan & Antimannan	• Combined use (Ag+Ab) may be positive prior to blood culture • 50-80 % sensitivity • Specificity 70 to 80%	• Candida infections only • Used in conjunction with PCR or other serological biomarkers	• Mycological criteria for possible invasive candidiasis • Useful for diagnosis and monitoring	(Huang *et al.*,2021)
Anti-germ tube antibodies	○ Differentiate between colonisation and infection. ○ Elevated negative predictive value	•Limited sensitivity and specificity	• The incorporation of BDG detection enhances the diagnosis of invasive candidiasis.	Pappas *et al.*,2016; Patterson& Donnelly,2019)
Glucuroxylomannan	○ Elevated sensitivity, specificity, and predictive values	Reduced precision in localised pulmonary or cutaneous infections	•Highly beneficial in disseminated and meningeal cryptococcosis	Liang *et al.*,2024 ; Price *et al.*,2023)

	○ Rapid performance ○ Inexpensive		•Beneficial when used alongside BG for the diagnosis of invasive *Trichosporon* and *Magnusiomyces* infections • Essential for diagnosis and monitoring	
Lateral flow assays	•Fast findings (<20 min) •High specificity(90-95%%) •Simple performance • Price low	• Less sensitive when used alone (70-85%) • Results weakly positive, difficult to evaluate without a digital readout.	• Point of care testing • Ability to diagnose infections with *Aspergillus*, *Cryptococcus*, and *Histoplasma* • Helpful in resource-constrained contexts	(Abdallah *et al.*,2021 ; White *et al.*,2020)
PCR: ***Aspergillus* spp.** ***Candida* spp.** ***P. jirovecii*** **Zygomycetes**	•High sensitivity • Sensitivity 75-95% • Specificity 80-90%	• Risk of contamination • False negatives outcomes• Inability to separate colonization from infection in non-sterile specimens	• Diagnosis of invasive aspergillosis, invasive candidiasis, mucormycosis, PCP, especially in high-risk populations . • Incorporated with additional biomarkers for increased accuracy	(Huang *et al.*,2021; Dos Santos *et al.*,2024)

Previous Studies

1. Meta-analyses show that quantitative PCR (qPCR) is more sensitive than traditional methods for identifying invasive fungal disease (IFD), outperforming microscopy, culture, and serological testing (Brown *et al.*,2025).
2. "The advent of molecular diagnostic technologies has significantly enhanced the realm of fungal diagnostics and identification, offering numerous tests for the diagnosis and/or screening of at-risk patients for invasive fungal diseases (IFDs), characterised by rapid turnaround times and encompassing a broad range of IFDs"(Kidd *et al.*,2020).
3. Non-culture diagnostics are now essential for assessing suspected invasive fungal infections (IFI) due to the use of serological and fungal antigen detection assays, as well as targeted and pan-fungal molecular approaches. These methods provide faster and more sensitive results. Nonetheless, none of these tests has showed sufficient sufficiency to replace the gold standard as independent assessments, and they should

be used in conjunction with host and radiographic features to effectively manage at-risk people (Terrero-Salcedo & Powers-Fletcher,2020).

4. "Serological and PCR-based methodologies have markedly improved the detection of fungal infections, facilitating earlier diagnosis and more accurate species identification compared to conventional culture methods"(Pemán& Ruiz-Gaitán,2025).
5. "The novel methodologies must be integrated with traditional assays, positioning them optimally within the diagnostic framework to yield the most reliable and precise diagnosis" (Fang *et al*.,2023).
6. "Standardization for multiplex assays, broad-range PCRs, and molecular diagnostics of antifungal resistance is anticipated; however, it encounters greater challenges compared to single-target diagnostics" (Pham *et al*.,2024).

Conclusion

Previous research has demonstrated that serological and molecular diagnostic approaches outperform traditional culture methods for diagnosing invasive fungal infections (IFIs). Molecular testing, particularly PCR-based procedures, are speedy and sensitive detection tools that can help with disease detection early on. Serologic tests like β-D-glucan and galactomannan are extremely specific and effective in therapeutic situations. Culture is a useful method for species identification and antifungal susceptibility testing, but it should not be the sole diagnostic tool. Rapid non-culture diagnostics combined with culture confirmation are expected to enhance patient outcomes.

Recommendations

1. Use a variety of diagnostic tools: Combining serological and molecular tests with culture improves diagnostic accuracy, particularly in high-risk people.
2. Testing methodologies must be standardized in order to reduce inter-study variability and increase clinical comparability.
3. Expand laboratory capacity: Health care providers must establish the infrastructure for molecular diagnostics and train staff to use new tests correctly.
4. Additional research is needed to determine the diagnostic performance for specific patient groups, as well as the cost-effectiveness and utility of combination biomarker panels.
5. While non-culture-based technologies enable early detection, fungal culture remains indispensable for definitive species identification and guiding targeted antifungal therapy.

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