Laboratory Diagnostics for Fungal Infections

A Review of Current and Future Diagnostic Assays

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KEYWORDS

• Invasive fungal diagnostics • Molecular methods • Serology • Antibody • Antigen

KEY POINTS

- Classic serologic techniques, including immunodiffusion and complement fixation, in addition to fungal antigen detection methods, continue to be used routinely for diagnosis of fungal infections.
- Novel diagnostic tools, including rapid lateral-flow assays for *Cryptococcus* antigen detection, have been developed and have good performance characteristics.
- Identification of fungi from culture isolates can be rapidly and reliably achieved using a variety of molecular methods, including nucleic acid hybridization probes, matrix-assisted laser desorption ionization time of flight mass spectrometry, polymerase chain reaction (PCR), and DNA sequencing.
- The direct identification of fungi from specimens without the need to culture first is still largely limited to selected *Candida* species or to single-target PCR assays but multiplex PCR panels and direct sequencing methods are beginning to appear in the literature.

INTRODUCTION

The diagnosis of fungal infections has evolved dramatically over the past few decades. Although classic fungal culture and traditional serologic techniques continue to be relevant and necessary, the detection and identification of fungi after growth in culture and directly from specimens by molecular techniques is a rapidly evolving diagnostic field. This review focuses on the routinely used methods for detection of antibodies to and antigens from common invasive fungal agents, and presents an update on recently described molecular methods for fungal detection. This includes discussion of broad-range PCR and sequencing, matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry and real-time PCR applications. **Table 1** summarizes the general advantages and limitations associated with each of these applications. Importantly, we do not present information on phenotypic fungal identification through culture and staining techniques. Finally, although we recognize that *Microsporidia* species are fungi, diagnostic testing for this group of organisms are not be covered because testing is often still relegated to parasitology laboratories.

REVIEW OF SEROLOGIC METHODS FOR ANTIBODY AND ANTIGEN DETECTION

Classically, detection of antifungal antibodies relied on traditional techniques, including complement fixation (CF) and immunodiffusion (ID) assays, both originally optimized in the 1940s.

Disclosure Statement: The authors have nothing to disclose.
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Clin Chest Med ■ (2017) ■–■
http://dx.doi.org/10.1016/j.ccm.2017.04.013

Table 1
General advantages and limitations of fungal diagnostic tests

Tests	Advantages	Limitations
Antigen detection	• Rapid TAT (vs culture) • Minimally invasive specimens • Reduces the need to handle potentially infectious fungi in the laboratory • Serial monitoring may be used for early diagnosis and to gauge treatment response	• Potential for cross-reactivity between closely related fungi • Potential cross-reactivity due to therapeutic interventions • Lack of culture isolate for susceptibility testing (ie, *Aspergillus* sp, *Fusarium* sp) • Antigen performance varies depending on specimen and disease state • Pan-fungal nature of β-D-Glucan (BDG) • Mannan antigen: Low sensitivity and specificity when performed alone
Antibody detection	• Rapid TAT (vs culture) • Minimally invasive specimens • Reduces the need to handle potentially infectious fungi in the laboratory	• Lower sensitivity associated with acute infection and in severely immunosuppressed patients • Low specificity associated with antibodies to other closely related fungi • Persistent seropositivity post disease resolution and in patients residing in endemic regions
Nucleic acid probes	• High specificity and sensitivity • Rapid turnaround time (vs culture)	• Need to grow in culture first (delayed TAT) • Potential for cross-reaction with closely related fungi • Limited species-specific probe availability
MALDI-TOF MS	• Rapid TAT (vs morphologic identification) • High specificity • High throughput • Cost-effective	• Need to grow in culture first (delayed TAT) • Lack of accuracy associated with mixed colonies • Inability to perform direct-from-specimen testing • Inability to perform antimicrobial susceptibility testing • Suboptimal breadth of spectral libraries • Inability to differentiate some closely related species
Identification of isolates by DNA Sequencing	• High specificity • Ability to identify novel species • High throughput	• Need to grow in culture first (delayed TAT) • Suboptimal breadth and accuracy of sequence databases. • Longer TAT than probes or MALDI-TOF MS • High cost • Technically challenging
Identification directly from specimens by DNA sequencing	• High specificity • Rapid TAT (no need to wait for culture growth) • Ability to identify organisms that fail to grow in culture	• Low sensitivity • Highly susceptible to environmental contamination of specimens and reagents (false positives) • High cost • Technically challenging
Identification directly from specimens by Real-time PCR	• High sensitivity and specificity • Rapid TAT • Closed system that reduces potential for contamination by environmental fungi or amplicon	• Lack of assay standardization • Lack of commercially available FDA-approved assays • Available for a limited number of fungi; often single targets per assay • The significance of a positive result from a nonsterile source (eg, BAL fluid) may be clinically confounding • Lack of an isolate limits ability to perform antimicrobial susceptibility testing

Abbreviations: BAL, bronchoalveolar lavage; FDA, Food and Drug Administration; MALDI-TOF MS, matrix-assisted laser desorption ionization time of flight mass spectrometry; PCR, polymerase chain reaction; TAT, turnaround time.