AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

COMMENTARY

Recognition of Diagnostic Gaps for Laboratory Diagnosis of Fungal Diseases: Expert Opinion from the Fungal Diagnostics Laboratories Consortium (FDLC)

Sean X. Zhang,[a] N. Esther Babady,[b] Kimberly E. Hanson,[c] Amanda T. Harrington,[d] Paige M. K. Larkin,[e] Sixto M. Leal, Jr,[f] Paul M. Luethy,[g] Isabella W. Martin,[h] Preeti Pancholi,[i] Gary W. Procop,[j] Stefan Riedel,[k] Seyedmojtaba Seyedmousavi,[l] Kaede V. Sullivan,[m] Thomas J. Walsh,[n] Shawn R. Lockhart,[o] on behalf of the Fungal Diagnostics Laboratories Consortium (FDLC)

[a]Division of Medical Microbiology, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[b]Clinical Microbiology Service and Infectious Disease Service, Departments of Laboratory Medicine and Medicine, Memorial Sloan Kettering Cancer Center, New York City, New York, USA
[c]Department of Pathology, Section of Clinical Microbiology, University of Utah and ARUP Laboratories, Salt Lake City, Utah, USA
[d]Department of Pathology and Laboratory Medicine, Loyola University Medical Center, Maywood, Illinois, USA
[e]Department of Pathology and Laboratory Medicine, NorthShore University HealthSystem, Evanston, Illinois, USA
[f]Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama, USA
[g]Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland, USA
[h]Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, USA
[i]Department of Pathology, The Ohio State University Wexner Medical Center, Columbus, Ohio, USA
[j]Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, Ohio, USA
[k]Clinical Microbiology Laboratories, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA
[l]Microbiology Service, Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland, USA
[m]Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine at Temple University, Temple University Health System, Philadelphia, Pennsylvania, USA
[n]Division of Infectious Diseases, Weill Cornell Medicine of Cornell University, New York-Presbyterian Hospital, New York City, New York, USA
[o]Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

ABSTRACT Fungal infections are a rising threat to our immunocompromised patient population, as well as other nonimmunocompromised patients with various medical conditions. However, little progress has been made in the past decade to improve fungal diagnostics. To jointly address this diagnostic challenge, the Fungal Diagnostics Laboratory Consortium (FDLC) was recently created. The FDLC consists of 26 laboratories from the United States and Canada that routinely provide fungal diagnostic services for patient care. A survey of fungal diagnostic capacity among the 26 members of the FDLC was recently completed, identifying the following diagnostic gaps: lack of molecular detection of mucormycosis; lack of an optimal diagnostic algorithm incorporating fungal biomarkers and molecular tools for early and accurate diagnosis of *Pneumocystis* pneumonia, aspergillosis, candidemia, and endemic mycoses; lack of a standardized molecular approach to identify fungal pathogens directly in formalin-fixed paraffin-embedded tissues; lack of robust databases to enhance mold identification with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; suboptimal diagnostic approaches for mold blood cultures, tissue culture processing for Mucorales, and fungal respiratory cultures for cystic fibrosis patients; inadequate capacity for fungal point-of-care testing to detect and identify new, emerging or underrecognized, rare, or uncommon fungal pathogens; and performance of antifungal susceptibility testing. In this commentary, the FDLC delineates the most pressing unmet diagnostic needs and provides expert opinion on how to fulfill them. Most importantly, the FDLC provides a robust laboratory network to tackle these diagnostic gaps and ultimately to

Citation Zhang SX, Babady NE, Hanson KE, Harrington AT, Larkin PMK, Leal SM, Jr, Luethy PM, Martin IW, Pancholi P, Procop GW, Riedel S, Seyedmousavi S, Sullivan KV, Walsh TJ, Lockhart SR, on behalf of the Fungal Diagnostics Laboratories Consortium (FDLC). 2021. Recognition of diagnostic gaps for laboratory diagnosis of fungal diseases: expert opinion from the Fungal Diagnostics Laboratories Consortium (FDLC). J Clin Microbiol 59:e01784-20. https://doi.org/10.1128/JCM.01784-20.

Editor Colleen Suzanne Kraft, Emory University

Address correspondence to Sean X. Zhang, szhang28@jhmi.edu, or N. Esther Babady, babadyn@mskcc.org.

The views expressed in this article do not necessarily reflect the views of the journal or of ASM.

Accepted manuscript posted online 27 January 2021

Published 18 June 2021

improve and enhance the clinical laboratory's capability to rapidly and accurately diagnose fungal infections.

The incidence of fungal infections is on the rise and continues to be a serious threat, especially in transplant and hematology-oncology patients. The diagnosis of invasive fungal infections is often delayed, and current methodologies are not always adequate. Less progress for fungal diagnosis has been made in the last several years than has been made in bacterial and viral infection diagnosis. There have been some recent efforts to increase fungal diagnostic capacity, including the commercialization of direct detection of *Aspergillus* and Mucorales in clinical specimens using nucleic acid amplification tests (NAATs; e.g., AsperGenius and MucorGenius from PathoNostics) and lateral flow assays for antigen-based diagnosis of histoplasmosis (e.g., MiraVista from Optimum Imaging Diagnostics), coccidioidomycosis (e.g., IMMY Diagnostics), and aspergillosis (e.g., IMMY Diagnostics and OLM Diagnostics). Still, more work remains to be done to improve fungal diagnostic testing and clinical outcome.

The overall number of fungal infections is relatively small compared to bacterial and viral infections, but the mortality and morbidity associated with these infections is high, making rapid diagnosis of these infections a high priority. In addition, new fungal threats are emerging, including multidrug-resistant *Candida auris* and azole-resistant *Aspergillus fumigatus*. To address these challenges, the Fungal Diagnostic Laboratory Consortium (FDLC) was formed. The FDLC brings together clinical laboratories in North America that routinely provide diagnostic mycology services to large immunocompromised patient populations. The network will facilitate and accelerate research and development to improve and enhance fungal diagnostics and promote collaboration with industry partners to support new assay development, commercialization, clinical validation, U.S. Food and Drug Administration (FDA) clearance, and laboratory implementation.

The FDLC is currently composed of 26 laboratories (see Acknowledgments). The current members were selected by invitation from the three cochairs (E. Babady, S. Lockhart, and S. Zhang). These centers were chosen mainly based on their large testing volume of providing routine fungal diagnostic service and expertise in fungal diagnostics. The FDLC intends to include more members in the near future. Joining FDLC was free and voluntary. The first face-to-face meeting of the FDLC took place at the American Society for Microbiology 2019 Microbe meeting in San Francisco. One of the first action items from the initial meeting was to assess the diagnostic capabilities of the FDLC member laboratories and identify high priority tasks for the FDLC to address. A survey was sent to all 26 laboratories in the United States and Canada. The following top five priorities were identified from the survey results: (i) development of best practices guidelines for fungal diagnostics, (ii) development/validation of standardized NAAT for molecular detection of fungi directly in clinical specimens, (iii) development and multicenter validation of new diagnostic tests, (iv) workforce development, and (v) laboratory capacity building. Specifically, six disease-specific and six method/approach-specific diagnostic gaps were identified (Fig. 1). In this commentary, the FDLC specifically addresses these high priority gaps in fungal diagnostics and provides expert opinion and approaches to fill them.

DIAGNOSTIC GAPS AND EXPERT OPINION

In the sections below, we discuss disease-specific and method/approach-specific gaps concerning fungal diagnoses.

Disease-specific gaps. (i) *Pneumocystis* pneumonia: develop an optimal diagnostic algorithm. *Pneumocystis jirovecii* is a yeast-like fungus that causes *Pneumocystis* pneumonia (PCP), an opportunistic lung infection associated with high mortality in immunocompromised patients (1, 2). National and international societies have recently published guidelines which include the diagnosis of PCP in non-HIV immunocompromised patients (3, 4). For decades, the gold standard for diagnosing PCP has been direct microscopic

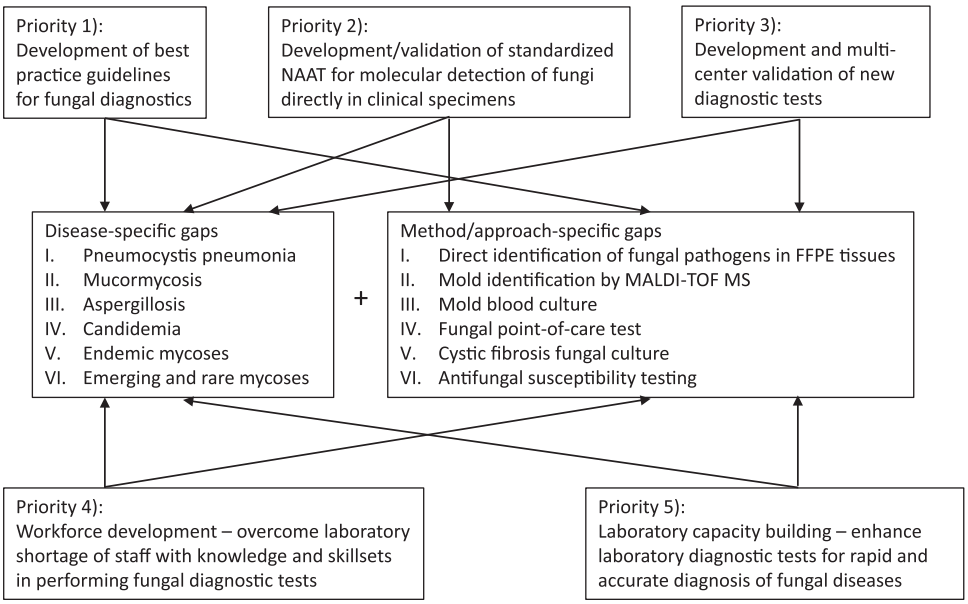

FIG 1 Fungal diagnostic priorities associated with disease-specific and method/approach-specific diagnostic gaps. The figure illustrates identification of the five fungal diagnostic priorities associated six disease- and six method-specific diagnostic gaps that are further delineated in the commentary. FFPE, formalin fixed and paraffin embedded; MALDI-TOF MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry.

identification (ID) of *P. jirovecii* cysts and trophozoites in tissue samples, induced sputum, and bronchoalveolar lavage (BAL) fluid using nonspecific stains such as Grocott-Gomori methenamine-silver, calcofluor white, or a specific stain with fluorescence monoclonal antibodies against *P. jirovecii*. Direct visualization techniques of *P. jirovecii* cyst and/or trophic forms have inferior diagnostic sensitivity in non-HIV patients, which can lead to false-negative test results and missed diagnoses (5).

In recent years, NAATs of respiratory samples have enabled direct detection of *P. jirovecii* DNA (6–8). A *P. jirovecii* NAAT is appealing due to higher sensitivity than direct microscopic methods, especially in non-HIV-infected patients. Different *P. jirovecii* NAAT chemistries and gene targets have been described, but most of the *P. jirovecii* NAAT assays were developed in-house as laboratory-developed tests (LDTs), and it is hard to compare the diagnostic performance of each assay without a standardized approach (9, 10). Furthermore, NAATs may be prone to misinterpretation in cases where *P. jirovecii* is a colonizer (i.e., present without causing disease) rather than the cause of disease (i.e., present in the context of clinical signs or symptoms of acute pneumonia) (11–14). The use of a quantitative *P. jirovecii* NAAT (qNAAT) has been proposed to differentiate between colonization and disease state (3, 4). The cycle threshold (C_T) values obtained by using a qNAAT allow estimation of the fungal burden with the assumption that a higher fungal burden increases the probability of a disease state. However, caution needs to be taken when applying C_T values to correlate the organism load with the disease severity because different factors, including test method variation, may influence C_T values (15). Detection and quantification of *P. jirovecii* DNA using qNAAT in conjunction with establishment of population-specific C_T thresholds might enable differentiation between colonization and infection (16–18). However, this approach is limited by a lack of standardization across *P. jirovecii* qNAAT tests, which impedes the establishment of universal interpretive thresholds.

1,3-β-D-Glucan (BDG) is an antigenic component of the cell wall of many fungi, including *P. jirovecii*, and is considered a diagnostic adjunct for PCP (19, 20). Although it lacks specificity due to its elevation in various fungal infections, serum BDG testing is often used as a noninvasive means to support the diagnosis of PCP, especially in situations where critical illness precludes invasive diagnostic procedures (20–22). However,

the optimal test cutoff value for the diagnosis of PCP still remains to be determined (23). In addition, more data are needed to evaluate the negative predictive value of BDG testing.

According to the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSGERC) guidelines, the criteria for "proven" PCP remains the detection of cysts in tissues, BAL fluid, or sputum using direct microscopy, while "probable" PCP now includes the use of BDG and NAAT (3). Given the limitations of both BDG and NAAT as described above, an approach that combines both methods could increase the accuracy of PCP diagnosis. In this option, both BDG and *P. jirovecii* NAAT are performed, and the combination of results were interpreted depending on the clinical context and the patient population (24, 25). The development of the best gene target and standardized commercial *P. jirovecii* qNAAT assays and/or quantitative international calibration standards would enable meaningful evaluation of C_T thresholds for defining colonization versus disease state. In addition, future multicenter studies are necessary to bridge the current gap in knowledge surrounding the optimal role of molecular detection of *P. jirovecii* and serum BDG testing either alone or in combination with diagnostic algorithms for PCP in distinct patient populations.

(ii) Mucormycosis (formerly known as zygomycosis). (*a*) *Reevaluation of optimal tissue processing for isolation of Mucorales.* Processing tissue specimens submitted for fungal culture can be done by mincing (cutting a tissue into small pieces) or homogenizing (grinding the whole tissue into suspension) before inoculating onto culture plates. Major clinical microbiology reference texts give particular emphasis to the importance of mincing instead of homogenizing tissue when infection with a member of the mucoraceous molds is suspected (26–28). This is purportedly to avoid damage to fragile hyphal structures of the Mucorales molds, although there is no published data to support this notion. No studies are cited to support this practice, nor could we find such studies when we searched the scientific literature. Indeed, the anecdotal experiences of several laboratories call into question this practice based on the observation that nearly 50% of the tissues that were positive for Mucorales hyphae in calcofluor white stain or in histology yielded no growth in culture using the mincing method for tissue processing (unpublished data). Occasional observations have been made in cases where the tissue submitted for bacterial culture (which was homogenized in a tissue grinder) grew a Mucorales mold, while the tissue concurrently submitted for fungal culture (which was minced) did not yield any fungal growth.

Formal studies are lacking that directly compare the effect of tissue mincing versus grinding on recovery of Mucorales. Homogenization of tissue usually results in releasing microorganisms into the suspension suitable for downstream culture. This tissue process does not show any damage to bacterial culture recovery and actually is recommended for culture isolation of *Histoplasma*; its detrimental effect on the Mucorales molds is therefore called into question by anecdotal experiences. These observations warrant further multicenter studies to directly compare mincing versus grinding in order to discern the best tissue processing strategies to enhance the diagnostic yield of fungal culture for Mucorales.

(*b*) *Develop Mucorales NAAT for early diagnosis of mucormycosis.* Early diagnosis of mucormycosis is key to increase survival outcomes. There are no commercially available Mucorales antigen tests in serum but large amount of circulating cell-free Mucorales DNA in blood has been detected as early as up to 9 days prior to the diagnosis made by culture in patients with mucormycosis (29–32). Therefore, NAAT-based tests may be the most sensitive method to detect Mucorales DNA in blood and serve as a screening and diagnostic assay for early diagnosis. NAATs have been developed for both BAL and blood samples. Depending on the test, results could be available in $<$2 h (33, 34). Early diagnosis of pulmonary mucormycosis in patients with hematological malignancy has been achieved through detection of Mucorales DNA in both serum and whole-blood samples using a pan-Mucorales NAAT assay targeting 18S ribosomal DNA (rDNA) (30, 31). Another Mucorales-specific gene target is the *CotH* gene

encoding spore coat homolog proteins. In both animal model and human infection cases, an NAAT assay targeting the *CotH* gene demonstrated early diagnosis of mucormycosis with better sensitivity seen in urine samples than in BAL and blood samples (35). Despite all efforts made so far to develop sensitive molecular assays to aid in early diagnosis of mucormycosis, Mucorales NAAT is not included in the recent revised EORTC/MSG criteria for probable invasive mold infection (3). In a recent global guideline for diagnosis and management of mucormycosis, detection of Mucorales DNA in serum, as well as in other body fluids, is supported only with moderate strength (36). Lack of standardization and relative rarity in cases prevent implementing a molecular assay in clinical laboratories to improve the speed and sensitivity for diagnosing mucormycosis. Variable factors such as sample type, method of extraction, NAAT target, and NAAT format and conditions contribute to the performance of the NAAT assay. Development of a commercial assay could potentially standardize these variables and facilitate laboratory validation and implementation. Currently, there is only one commercially available Mucorales NAAT assay (MucorGenius; PathNostics) that demonstrated early diagnosis of mucormycosis (32, 34), but the assay has not been approved by the FDA. Multicenter investigations seeking to standardize Mucorales NAAT and assess its clinical utility may improve diagnosis of this rapidly progressive and often fatal infection (37).

(iii) Aspergillosis: NAAT in conjunction with galactomannan for early and accurate diagnosis. Microbiologic diagnosis for invasive aspergillosis (IA) relies on recovery of *Aspergillus* spp. in culture, microscopic examination of sterile samples, and detection of the galactomannan (GM) antigen in serum and BAL fluid. Although useful, these methods lack specificity, are time-consuming, and can result in inconclusive findings. The GM antigen assay is most useful in neutropenic leukemia patients but lacks sensitivity in high-risk hematopoietic stem cell transplant patients on antifungal prophylaxis or in other nonneutropenic immunocompromised patients (38, 39).

The diagnostic utility of NAAT for the diagnosis of IA remains unknown. Although NAATs targeting *Aspergillus* spp. from BAL and blood specimens are commercially available (40), the majority of NAATs for *Aspergillus* species are still LDTs (41). Most utilize real-time PCR (RT-PCR) to detect *Aspergillus* at genus level, but some additionally identify individual species yielding results for: *A. fumigatus*, *A. terreus* (amphotericin B resistance), and other *Aspergillus* spp. (42, 43). *Aspergillus* NAAT is widely used in Europe and recommended in ESCMID guidelines (44, 45). The most recent EORTC/MSG criteria includes *Aspergillus* NAAT in defining proven (NAAT on tissues) or probable IA (NAAT on blood and BAL samples) (3, 38).

The potential advantages of an *Aspergillus* NAAT include a rapid turnaround time, increased clinical specificity compared to GM, the ability to differentiate between *Aspergillus* species, and detection of antifungal resistance markers (46). Disadvantages include the need for specialized equipment, molecular expertise, and false-positive results caused by ubiquitous *Aspergillus* spp. transiently colonizing the respiratory tract or contaminating reagents (e.g., silica in nucleic acid extraction columns; sodium citrate vacutainers). Like culture, detection of *Aspergillus* DNA from nonsterile sites does not necessarily equate to invasive infection. Quantitative NAAT applied to BAL fluid has been explored as a means to increase the predictive value of the NAAT. While higher fungal burdens may be more suggestive of IA, there remains significant overlap in DNA loads between colonization and invasive disease (47).

Given substantial interlaboratory NAAT performance variability, the European *Aspergillus* PCR Initiative (EAPCRI) has led efforts to optimize and standardize *Aspergillus* NAAT testing (48–52). Commercial NAAT assay may also provide standardization and accessibility; AsperGenius (PathoNostics) is the only one commercially available that has been studied and reported with good clinical performance (42). Screening for *Aspergillus* infection in blood samples from high-risk populations not receiving prophylaxis can be done using NAAT (84 to 88% sensitivity; 75 to 76% specificity) or GM (79 to 80% sensitivity; 81 to 86% specificity) (53). An NAAT exhibits higher sensitivity and identifies more patients at risk of IA requiring additional testing (physical exam, chest computed

tomography, and BAL analyses) (53). Screening patient blood using NAAT and GM increases the sensitivity to 99% (when at least one test is positive) and specificity to 98% (when both tests are positive), leading to decreased use of empirical antifungals, earlier detection, and reduced mortality (53–56).

Multicenter clinical trials using optimized testing protocols are needed to evaluate the utility of the *Aspergillus* NAAT in conjunction with GM. Twice-weekly screening with serum NAAT and GM in high-risk populations with hematologic malignancy is expected to mitigate prophylactic antifungal use while enabling prompt treatment initiation with high sensitivity. Likewise, the combined use of GM and NAAT on BAL samples from symptomatic individuals may increase diagnostic accuracy. As part of well-designed prospective studies, additional work is required to determine the clinically relevant quantitative thresholds that best differentiate patients with IA and low levels of fungal DNA from specimens that may be contaminated or have high background signal.

Azole resistance in *A. fumigatus* is increasing, attributable to mutations (L98H, TR34, T289A, and Y121F) in the *CYP51A*, the gene encoding lanosterol 14α demethylase (57, 58). Multicenter evaluations on the clinical utility of NAAT to rapidly detect *A. fumigatus CYP51A* azole resistance mutations and species, such as *A. terreus*, with clinically actionable innate resistance patterns are needed and would be expected to show more rapid time to appropriate therapy relative to traditional methods given low culture sensitivity and long ID and mold antifungal susceptibility testing (AST) turnaround times. Opportunities to incorporate the detection of cryptic *Aspergillus* species, such as *A. lentulus* or *A. calidoustus*, with reduced azole susceptibility may also be of significant value.

(iv) Candidemia: optimize rapid test algorithm. Candidemia is the third to the fourth most common bloodstream infection in health care settings, and the predominant severe fungal infection developing in critically ill patients in intensive care units (ICUs) (59). Other patients with malignancy, transplantation, immunosuppression, abdominal surgery, prolonged broad antibacterial use, and injection drug use are at high risk of developing candidemia (60–62). Overall crude mortality rate during the hospitalization with candidemia is approximately 25% (63). Delayed diagnosis and initiation of inappropriate antifungal treatment are associated with higher mortality rates (64).

Recent progress has been made to speed up the detection and ID of *Candida* spp. that cause candidemia. Details on the FDA-cleared methods for the rapid ID of *Candida* spp. from blood are summarized in Table 1. The most widely adopted methods involving nucleic acid detection assays applied to aliquots from positive blood culture bottles, showing highly accurate and significantly speedy time to organism identification (65–68). Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) has also been used directly on positive blood culture aliquots (69, 70). This approach requires blood lysis and protein extraction up front, which adds additional hands-on time and complexity. In theory, MALDI-TOF MS incorporating robust spectral databases would be expected to be able to identify the broadest spectrum of *Candida* species compared to the targeted molecular methods.

Current commercial blood culture systems are estimated to detect invasive candidiasis (IC) with a sensitivity of approximately 50% (71). While the aforementioned commercially available rapid diagnostic tests are capable of expediting identification of the *Candida* spp., they still rely on blood culture turning positive, and thus they may not increase the speed and sensitivity for the detection of IC. A single platform (T2Candida) has been FDA cleared for the direct detection of the five most common *Candida* spp. in whole blood, greatly speeding the time to candidiasis diagnosis (72, 73). However, given the limited number of organisms detected by T2 combined with a clinical sensitivity of approximately 90%, routine blood culture is still required as a back-up. Direct detection of *Candida* species from blood using molecular methods may increase sensitivity compared to culture but its clinical benefits and costs are unclear (74). Developing a sensitive molecular test of blood also needs to take into consideration the prevalence of candidemia in a patient population. For example, in

TABLE 1 FDA-cleared methods for the rapid identification of *Candida* species in blood[a]

Parameter	PNA-FISH	FISH	Nested multiplex PCR with melt curve analysis	Multiplex NAAT with DNA hybridization and electrochemical detection	Protein extraction, followed by MALDI-TOF MS	Multiplex PCR with DNA hybridization and T2 MR detection
Test name (manufacturer)	Yeast TrafficLight (AdvanDx)	Accelerate Pheno System (Accelerate)	FilmArray BCID (Biofire)	ePlex BCID-FP (GenMark)	Sepsityper[f] with MALDI Biotyper (Bruker) or VITEK MS (bioMérieux)	T2Candida Panel (T2 Biosystems)
Approach	Blood culture	Blood culture	Blood culture	Blood culture	Blood culture	Direct EDTA blood
Organisms[b]						
C. albicans	●	●	●[c]	●	●	●
C. auris			●	●	●	
C. dubliniensis				●	●	
C. famata				●	●	
C. glabrata	●	●	●	●	●	●
C. guilliermondii				●	●	
C. keyr				●	●	
C. krusei	●		●	●	●	●
C. lusitaniae				●	●	
C. parapsilosis	●		●	●	●	●
C. tropicalis	●		●	●	●	●
Run time	90 min	90 min	60 min	90 min	75 min	3–5 h
Manual steps[d]	Moderate	Minimal	Minimal	Minimal	Moderate	Minimal
Accuracy[e]	96%	98–99%	99–100%	99–100%	56–100%	SN 89–91%, SP 99%

[a]FDA, Food and Drug Administration; PNA, peptide nucleic acid; FISH, fluorescent *in situ* hybridization; BCID, blood culture identification; SN, sensitivity; SP, specificity.
[b]The Yeast TrafficLight and T2Candida panel detected but could not differentiate *C. albicans* from *C. parapsilosis* or *C. glabrata* from *C. krusei*.
[c]The FilmArray BCID2 includes *C. auris*.
[d]Minimal steps include sample pipetting only. Moderate steps may include pipetting, mixing, centrifugation, and/or slide preparation.
[e]Accuracy in blood culture aliquot studies represents the overall agreement compared to a reference standard. For MALDI-TOF MS studies, species identification scores below the study's stated threshold were considered incorrect.
[f]Sepsityper protocols vary across studies, and some reports have used laboratory-developed extraction methods. Sepsityper is CE marked but is not FDA cleared.

settings with low prevalence of candidemia in a general inpatient population (e.g., ≤1%), PPV of T2Candida has been estimated to range from 15 to 31%, which does not strongly support its use (74). Multicenter controlled clinical trials enrolling the optimal patient population for direct detection of *Candida* would help to maximize the clinical outcome and impact (e.g., reductions in length of hospital stay and mortality rate) of direct testing and contain costs. In addition, coupling rapid *Candida* identification with rapid phenotypic antifungal susceptibility testing may help to optimize antifungal use.

(v) Endemic mycoses: improve availability and performance of diagnostics. The endemic mycoses in North America are histoplasmosis, blastomycosis, and coccidioidomycosis. Since each of the dimorphic fungi has a morphologic mimic when grown in culture, microscopic preparations with features suggestive of an endemic fungus should be confirmed with a genetic probe (i.e., AccuProbe; Hologic, Inc., San Diego, CA [this commercial product may be phased out soon]) or another molecular method (e.g., DNA sequencing). It has been demonstrated that MALDI-TOF MS was able to identify *Histoplasma*, *Coccidioides* from the mycelial phase of the colonies (75, 76), but biosafety precaution needs to be taken due to potential laboratory exposure to the organisms.

Genus- and/or species-specific NAAT assays are available that target endemic fungi (77–90). Although useful to confirm the presence of a pathogen in respiratory specimens and tissues, these assays have not been widely adopted. In contrast, a broad-range NAAT with 28S rRNA sequencing, which enables the identification of most fungi in clinical specimens, exhibits a higher diagnostic yield (91–94). Importantly, these methods are not a replacement for culture, as demonstrated by Stempak et al. (95), but rather an adjunct that is most useful when fungi are detected in an anatomic pathology specimen but either they did not grow in culture or a corresponding specimen was not submitted for culture.

Antigen detection tests are available for each of the etiologic agents of the endemic mycoses. *Histoplasma* antigen assays are commercially available for both urine and serum specimens (96–100). There are conflicting data in the literature regarding the superiority of one of these assays over another, but these authors slightly favor a urine specimen over a serum specimen (101–104). Libert et al. (105), however, demonstrate that either one of these is sufficient in the workup of a patient with suspected histoplasmosis, but ordering both is diagnostically duplicative and increases cost without increasing value. *Histoplasma* antigen detection testing is most useful for the diagnosis of disseminated histoplasmosis, moderately useful for localized pulmonary histoplasmosis, and not useful for remote disease (97). Antigen detection testing for *Blastomyces dermatitidis* and *Coccidioides* species is also commercially available, but it is used considerably less than the *Histoplasma* antigen assays (106–111). These have been particularly useful for the diagnosis of central nervous system (CNS) disease caused by these fungi (108, 109, 111). Cross-reactivity with substrates from other fungi may occur with these assays and offer opportunities for optimization of future diagnostics (112–114).

Fungal serologic studies are a means of demonstrating that an individual who has the ability to mount an immunologic response has been infected by one of the members of the endemic mycoses. The classic methods used for fungal serology testing are technically complex complement fixation and immunodiffusion (115, 116). The development of low-complexity serologic assays may increase incorporation into clinical laboratory test menus. However, serologic assays for endemic mycoses are of limited to no use during early infection due to the time required for antibody production. In contrast, a rising titer between acute- and convalescent-phase sera is supportive of recent and possibly ongoing infection (115). However, false-positive IgM serology in coccidioidomycosis has been observed in an enzyme immunoassay (117). In addition, serologic tests may be useful when assessing a patient with a solitary pulmonary nodule, particularly when combined with imaging studies and needle biopsy or fine-needle aspiration cytology.

Correlation of direct examination and culture with histopathologic/cytologic findings is a best practice. Enhanced education on organism morphology in tissue and the associated host tissue response would mitigate inaccurate diagnosis and improve patient care. For example, the ability to distinguish *H. capsulatum* from *Candida glabrata* based on a neutrophilic (not granulomatous) response, *Blastomyces* from *Cryptococcus* based on a pyogranulomatous host response, and *Coccidioides* endospores from yeasts based on ruptured spherules, granulomas, and eosinophils provides extremely helpful clinically actionable information with significant implications on the choice of antifungal agents and patient morbidity/mortality (118, 119).

The detection and identification of the etiologic agents of the endemic mycoses is important for the accuracy of diagnosis and the direction of antifungal therapy. The combined, but appropriate use of culture, histologic/cytologic studies, antigen testing, serology, and molecular studies afford the timely detection and accurate identification of these important fungal pathogens.

(vi) Fungal infections caused by emerging and underrecognized rare fungal pathogens. Some fungal organisms are newly emerging or not frequently encountered, but they can cause severe infections and fatal outcomes. Importantly, they pose significant diagnostic challenges to clinical laboratories. The best example of newly emerging fungal pathogen is *Candida auris*. It is particularly problematic in health care settings due to its multidrug-resistant nature and propensity to cause invasive disease associated with high morbidity and mortality in vulnerable patient populations (120, 121). As hospital systems move toward routine screening for *C. auris* colonization (122), the inability to correctly and rapidly identify *C. auris* hinders infection prevention efforts, furthering transmission. While correct identification of *C. auris* can be done by MALDI-TOF MS (123, 124), misidentifications occur with biochemical reaction-based commercial systems, i.e., Vitek 2, MicroScan, and API tests (123–126). Laboratory-developed NAATs have been successful in the identification of *C. auris* (127–132), with some methods performed directly on the specimen, reducing turnaround time, and others performed on automated platforms, increasing throughput. We recommend a multicenter approach for developing and evaluating an easy-to-use, cost-effective, rapid, standardized *C. auris* NAAT directly from the patient specimen.

Rare or uncommon yeasts causing bloodstream infection include but are not limited to uncommon *Candida* species, *Cryptococcus* spp. (other than *C. neoformans* and *C. gattii*), *Trichosporon*, *Rhodotorula*, *Malassezia*, *Geotrichum*, and *Saprochaete*. Most of the bloodstream infections caused by these rare yeasts are either catheter line associated or due to breakthrough on antifungal treatment (133–137). Current automated commercial blood culture systems (BD Bactec Myco/F Lytic bottle, bioMérieux BacT/Alert, and Thermo Fisher VersaTREK) are able to recover these organisms from blood culture except for *Malassezia* (that requires lipid supplement that is not present in these commercial blood culture systems), but the performance of commercial blood culture systems for detection and isolation of rare yeasts in blood culture is still not well characterized. Current FDA-cleared rapid molecular assays (Biofire FilmArray BCID, GenMark ePlex BCID-FP, and Accelerate Pheno System) for direct identification of yeasts in positive blood culture do not cover these rare yeasts, except for *Rhodotorula* covered by GenMark ePlex BCID-fungal panel (68). MALDI-TOF MS can also be applied to identify rare yeasts directly on positive blood cultures, e.g., the Bruker Sepsityper (138), but this method may not work well if there is a mixed infection in blood or if that yeast is not in database. If the identification is inconclusive by MALDI-TOF MS, fungal DNA sequencing needs to be performed by targeting the D1D2, ITS, and IGS regions. Determining antifungal susceptibility of rare yeasts is another challenge. Most clinical laboratories rely on commercial antifungal susceptibility testing systems (YeastOne and Vitek), but the performance of these platforms may not be reliable compared to the reference broth microdilution. In addition, there are no Clinical and Laboratory Standards Institute (CLSI) breakpoints or epidemiologic cutoff values (ECVs) to interpret MICs for rare yeast, so little guidance of antifungal drug of choices is provided to clinicians treating patients infected with rare yeasts.

Relatively speaking, any mold infection other than invasive aspergillosis and mucormycosis would be considered a rare mold infection, of which fusariosis and *Lomentospora prolificans* infection represent the two most important mold infections in immunocompromised patients (139). While isolation of *Fusarium* from blood or biopsy tissues often supports the diagnosis of the infection, recovery of *Fusarium* in respiratory samples does not necessarily indicate infection; other laboratory findings and the clinical context of the patient are necessary to make the diagnosis. The serum *Aspergillus* GM level is often elevated in patients with invasive fusariosis (140, 141). Most *Fusarium* culture isolates can be reliably identified to species or species complex level by MALDI-TOF MS (142, 143). If these methods are inconclusive, then sequencing identification targeting translation elongation factor-1α region can be applied (144). Isolation of *Lomentospora prolificans* from non-cystic fibrosis (non-CF) patients with underlying immunocompromised conditions is usually indicative of infection and often results in poor clinical outcome (145). *L. prolificans* tends to grow slowly on fungal culture media but can be correctly identified by MALDI-TOF MS or microscopic features if the organism sporulates well (142). Since neither a *Fusarium*- or *L. prolificans*-specific biomarker nor an FDA-approved NAAT-based molecular assay is available for rapid and early diagnosis of the diseases directly from clinical samples, clinical laboratories primarily rely on culture-based methods to support the diagnosis of these rare mold infections. Such an inadequate diagnostic approach was also seen in diagnosis of other rare mold infections caused by members of dematiaceous fungi (e.g., *Alternaria*, *Bipolaris*, *Exophiala*, *Phialophora*, *Rhinocladiella*, etc.), as well as members of hyaline hyphomycetes (e.g., *Acremonia*, *Paecilomyces*, *Purpureocillium*, *Rasamsonia*, *Scopulariopsis*, etc.).

Clinical laboratories should be vigilant in detecting any emerging fungal pathogen and recognizing rare or uncommon opportunistic fungal pathogens. A multicenter approach to understand the incidence, clinical features (including risk factors), and antifungal susceptibility profiles of these rare fungal infections is needed. This could be accomplished through a joint effort to develop and validate non-culture-based molecular diagnostic approaches to achieve early and rapid diagnosis of these rare but severe and life-threatening fungal infections.

Method/approach-specific gaps. (i) Direct ID of fungal pathogens in FFPE tissues. Due to a variety of reasons, tissue for fungal culture is not always submitted with the surgical pathology, leading to all tissues being fixed in formalin and resulting in a diagnosis based solely on histopathological evidence of fungus in formalin-fixed and paraffin-embedded (FFPE) tissue. However, histopathological ID based on morphologic features of fungi (especially molds) is prone to error (146). Lack of culture and erroneous ID based on morphologies on histology render molecular detection of fungi from FFPE tissue particularly helpful in identifying the infectious agent in tissue to the species level (26).

Molecular ID of fungi in FFPE tissue has been successfully achieved by panfungal DNA sequencing ID, targeting ITS, D1/D2, 18S regions (118, 147). Sequencing of hyaline septate molds from FFPE tissue may identify the following clinically actionable items based on species-level IDs: amphotericin B resistance (*A. terreus*, *Scedosporium apiospermum* complex, and *Purpureocillium lilacinum* [formerly *Paecilomyces lilacinus*]), increased azole resistance (cryptic *Aspergillus* species like *A. lentulus* and *Paecilomyces variotii*), intrinsic voriconazole resistance (*Rasamsonia* spp.), and resistance to all current antifungal agents (*Lomentospora prolificans*) (148–150).

Additional benefits to species-level ID by tissue sequencing include the detection of pigmented molds with a predilection to cause CNS lesions (*Exophiala dermatitidis*, *Cladophialaophora bantiana*, etc.), ID of the potential source of dermatophyte infections (geophilic, zoophilic, or anthropophilic), and alterations in medical therapy (*C. gattii* versus *C. neoformans*, *Blastomyces* versus unencapsulated *Cryptococcus*, and *Histoplasma capsulatum* versus other small yeasts), and tissue processing contaminant versus true infection (151–154).

Although sequencing can identify the isolate to the species level, it also has the potential to detect mutations associated with antifungal resistance, such as *Aspergillus*

CYP51A mutations (155, 156). Novel assays targeting resistance markers and studies generating more species-level AST data correlated with antifungal resistance markers are needed to enhance clinically actionable interpretations of molecular data. Given the expected complexity of such reports, there is also an opportunity for the production of composite infectious disease pathology/microbiology reports to ensure that important test results are not overlooked.

While FFPE tissue sequencing is advantageous for the reasons outlined above, there are important considerations before implementing this assay into routine clinical use. First, protocols for processing, DNA extraction, targeted amplification, sequencing, and bioinformatics analysis are not standardized and vary in their ability to correctly identify fungal organisms (157). Second, fungal databases are limited in comparison to bacterial databases, which can lead to a mis-ID or lack of ID (158). Third, FFPE tissue blocks are processed and handled in a nonsterile manner and thus are prone to contamination. To increase specificity, only FFPE tissue blocks containing histopathologic evidence of fungi should be sequenced.

A targeted next-generation sequencing (NGS) approach has also recently been explored in ID of fungi in FFPE tissue (159). NGS is beneficial for polymicrobial fungal infections, but it is costly and requires highly skilled and trained technologists performing time-consuming manual procedures. Optimizing, automating, streamlining, and standardizing the process would allow more labs to pursue NGS sequencing of FFPE tissue.

(ii) Enhance mold ID by MALDI-TOF MS. Two MALDI-TOF MS platforms, Vitek MS (bioMérieux, Durham, NC) and Bruker MS (Bruker Daltonics, Billerica, MA), are FDA approved and becoming available in more clinical laboratories. ID of molds using MALDI-TOF MS can be simpler, significantly faster, and more accurate than conventional morphology-based ID. However, it lags behind bacteria and yeasts for utility in clinical microbiology laboratories (142, 160). There are several hurdles to laboratory implementation of MALDI-TOF MS for routine ID of molds. The first is the standardization and expansion of mold databases. Different molds grow better on different culture media, and they grow differently on solid media than on liquid culture. In addition, when molds are grown on plates, unlike most bacteria and yeasts, they have different growth phases and rates that can influence protein expression profiles. For example, a 2-day-old *Aspergillus* isolate on Czapek's agar may not give the same profile as the same isolate at 5 days or as a 2-day-old isolate on Sabouraud dextrose agar. Even within the same *Aspergillus* colony, extraction from the portion predominately containing spores versus the young hyphal mass will generate different proteomic spectra that affects the MADLI-TOF MS ID scores. One way to mitigate this problem is to expand databases to include multiple growth conditions and mold preparations (spores versus hyphae) so that a single validated database can be used for molds grown under diverse conditions. This can be achieved through the sharing of a validated set of mold isolates and the creation of a centralized database to which validated spectra can be added. The other way is to convert molds into hyphal structures by growing them in a liquid culture medium. Once in liquid culture, molds stop sporulation and convert to hyphae within 72 h. The advantage of this method is to eliminate the variation caused by media and to achieve a standard preparation from the hyphal mass, but the limitation is that it adds additional culture time and additional laboratory burden of both time and equipment.

A second significant hurdle to the adoption and expansion of mold ID by MALDI-TOF MS is the variability in each manufacturer's database. To date, only the Vitek MS mold database has been approved by the FDA for clinical use, while the Bruker Biotyper mold database remains under research-use-only conditions in the United States (161). The number of identifiable organisms in each database differs, with the Vitek MS containing 79 species in the version 3.0 Knowledge Base and the Bruker MS containing 180 species in its V3 library (162). Both of these instruments have shown varying success with the manufacturer-provided databases (161), with Vitek MS users

reporting a higher ID rate than those with the Bruker MS. One way to increase successful ID is to supplement the manufacturer's databases with clinical isolates. These home-grown databases have not only shown improved ID rates, but they also allow for the expansion of libraries by the addition of species that may not be as commonly encountered in some laboratories (163–166). The free distribution of continuously updated and curated databases would provide a significant benefit to clinical laboratories. The creation of MALDI-TOF MS depositories for fungal isolates by groups of clinical laboratories could be the first step in the development of standard databases. These groups could be defined by which instrument they use and could be further refined by geographical area or fungi of interest (e.g., *Aspergillus* species, dematiaceous molds, etc.). Using a standardized extraction procedure, these groups could produce database updates, provide validation sets containing representative fungal isolates used for database production, and create standardized instructional material, similar to proficiency testing materials.

(iii) Improve detection and isolation of molds in blood culture. Fungemia, the presence of fungi in the blood, can occur as a result of disseminated fungal infection in patients with malignancies and other forms of immunosuppression (61). Molds are isolated at a much lower rate than *Candida* but are still important pathogens in this vulnerable patient population since they can have a high rate of mortality (167). The most commonly isolated mold from blood specimens is *Fusarium* spp. (168), with isolation of *Fusarium* from 60 to 70% of blood specimens from patients with disseminated fusariosis. Fungemia due to other molds is rare. *Aspergillus* spp., the most common cause of invasive mold infections, are rarely isolated from blood specimens, with one study finding only 6% of patients with documented invasive aspergillosis had positive blood cultures (169). In addition, as *Aspergillus* spp. can be common environmental contaminants, determining the clinical significance of a fungal blood culture growing *Aspergillus* may prove difficult. Other molds, such as *Scedosporium apiospermum*, *Lomentospora prolificans*, and *Paecilomyces* spp., may be emerging causes of fungemia in cancer patients (167) but are rarely recovered from blood culture.

Several commercial blood culture systems have been employed by clinical laboratories to isolate yeast and mold in blood from patients suspected of having invasive fungal infections. These systems include isolator tubes (Wampole Laboratories, Cranbury, NJ), the BD Bactec Myco/F lytic bottle (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD), BacT/Alert (bioMérieux, Inc., Durham, NC), and VersaTREK (Thermo Fisher Scientific). The overall performance data for these automated blood culture systems on recovering molds in blood culture are scant, with *Fusarium* spp. being the most common. Among these systems, the isolator tube is particularly designed to support isolation of molds. However, its performance on isolating molds is not well known. Of >9,000 pediatric isolator tubes collected from children over a 10-year period, <0.3% recovered a mold (170). The isolator tubes were also prone to false-positive growth due to environmental contamination (171). Although in theory the presence of molds in blood would be inevitable in patients with invasive mold infection due to hematogenous dissemination, it is not clear whether these molds are intrinsically inert to growth in blood or whether these particular commercial blood culture systems are suboptimal to support mold growth in blood. These questions need to be further investigated to find a way to improve the detection and isolation of molds in blood culture. Alternatively, the use of culture-independent methods, including targeted PCR (e.g., aspergillosis PCR), and cell-free NGS are currently emerging as novel approaches to detect molds directly in blood samples and will need further validation (53, 172, 173). Since the incidence of fungemia caused by molds is low in each individual academic center, a multicenter joint effort would be needed in order to optimize and standardize a laboratory protocol for mold blood culture.

(iv) Fungal point-of-care testing. The immunochromatography technologies have led to the development of lateral flow assays (LFAs) that meet the requirements for point-of-care tests (POCT) for the detection of fungal infections. The POCT are rapid (in minutes), easy to perform, and affordable. One of the best samples is the FDA-approved

cryptococcal antigen LFA (IMMY Diagnostics, Norman, OK) with a sensitivity and specificity of >98% in serum and cerebrospinal fluid and a sensitivity of 85% in urine for the diagnosis of cryptococcosis in HIV and non-HIV patients (174, 175). Two commercial LFAs were recently available for the diagnosis of invasive pulmonary aspergillosis (IPA): AspLFD (OLM Diagnostics, United Kingdom), and IMMY so°na *Aspergillus* GM LFA (IMMY, USA). The AspLFD uses the JF5 monoclonal antibody to detect an extracellular glycoprotein (mannoprotein) antigen secreted by actively growing *Aspergillus* species, whereas the IMMY GM LFA uses two monoclonal antibodies against *Aspergillus* GM. Both assays showed a good performance for the diagnosis of IPA in BAL fluids from adult hematology patients and nonneutropenic patients (176, 177). Both LFAs are CE marked (the manufacturer's declaration that the product meets EU standards for health, safety, and environmental protection and indicates that the product may be sold freely in any part of the European Economic Area, regardless of its country of origin) in Europe but not FDA cleared in the United States. A *Histoplasma* urine antigen LFA has also recently been developed by MiraVista Diagnostics for the rapid detection of disseminated histoplasmosis, showing a sensitivity and a specificity of 96 and 94%, respectively, in patients with AIDS (178). More recently, a *Coccidioides* LFA assay (IMMY Diagnostics) combined with procalcitonin testing demonstrated correct diagnosis of 77% cases of coccidioidomycosis in a patient cohort from an area of endemicity (179). Overall, the current landscape of the fungal POCT indicates that development of new generation of affordable and rapid LFAs may have the potential to increase clinical laboratory capacity for early and rapid diagnosis of invasive fungal infections.

(v) Optimize and standardize fungal culture procedure for cystic fibrosis patients. While lung function decline in CF patients has been associated with the recovery of *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, and possibly *Staphylococcus aureus* in respiratory cultures, the role of fungi in CF pathogenesis is not well understood (180). The American CF Foundation Patient Registry reported in 2019 that prevalence of the most commonly isolated mold, *Aspergillus* spp. was about 17% in adults (181). However, because the CF Foundation does not explicitly recommend use of selective fungal media in their clinical care guidelines (which are the basis for accreditation of a program as a CF Care Center), it is difficult to know the true prevalence in this population (182). Clinically, recovery of mold in a CF patient's sputum has been increasingly reported, leading to possible association with decreased lung function, but it is unclear whether mold is a marker of advanced lung disease or a causative agent of lung function decline (180, 183–185).

Most clinical laboratories do not routinely include fungal selective media in CF cultures. Fungi in CF cultures are often recovered from bacterial culture media, which are suboptimal due to lack of antibiotics to suppress bacterial growth and nutrients to support fungal growth. Also, bacterial cultures are typically incubated for shorter periods of time compared to fungal cultures. As a result, slow-growing fungi may be missed. In a recent prospective study, sputum samples from a cohort of CF patients in a single medical center were cultured simultaneously with and without fungal selective media. Inclusion of fungal selective media significantly increased recovery of fungal organisms from 26 to 65% (186). Similar findings were reported by other medical centers (187, 188). Although interest in the role of fungi in pulmonary decline in CF patients is developing, the lack of a widely accepted and standardized CF fungal culture protocol is challenging investigations into the role of fungi.

Development of a standardized and comprehensive fungal culture protocol would require consideration of the following issues. Fungal selective media can be made in-house, but the labor and skill required limit this option to reference laboratories. In contrast, several commercially available fungal media (inhibitory mold agar, Sabouraud agar, and brain heart infusion agar) are readily available and can be easily fit into a clinical laboratory's workflow. The aforementioned studies confirmed the performance of these commercial fungal selective media to enhance fungal recovery in CF sputum samples (183, 186). In addition, frequency of culture, optimal incubation temperatures and duration, and use of a mucolytic agent during specimen processing may also

enhance fungal recovery (187). Multicenter efforts to evaluate optimal culturing practices are needed to develop an evidence-based culture protocol (189). Once a standardized protocol is in place, studies to understand and determine the role of fungi in CF patients (airway colonization versus real infection in conjunction with clinical context) could be further explored.

(vi) Antifungal susceptibility testing. Antifungal resistance has been reported for all existing antifungal agents in diverse groups of medically important fungi, including *Candida* and *Aspergillus* species (190). Although certain species harbor known intrinsic resistance patterns, many fungi exhibit variable resistance necessitating testing of the individual isolate to obtain clinically actionable data. Similar to bacterial AST, CLSI reference methods for fungi include broth microdilution and disk diffusion. However, the available breakpoints are limited to a few of the most common pathogenic yeasts, and only one breakpoint is available for molds (*A. fumigatus* and voriconazole) (191, 192). Continued expansion of disk diffusion and MIC breakpoints to less-common bug/drug combinations, novel drug classes, and emerging pathogens, such as *Candida auris*, is essential to improve patient outcome and adapt to new challenges in antifungal resistance (193).

CLSI breakpoint incorporation requires accurate species-level identification paired with MIC, pharmacokinetic/pharmacodynamics, and clinical outcome data. However, given the relatively low frequency compared to bacterial infections, these data are difficult to obtain for less common yeasts and molds. In the interim, yeast and mold ECVs are providing some guidance on AST interpretation for wild-type (WT) and non-WT isolates (194). To move beyond ECVs toward accurate clinically actionable breakpoints will require well-coordinated and longitudinal multicenter studies involving paired clinical outcome data combined with accurate species-level identification and CLSI reference method MIC data provided by specialized mycology reference laboratories.

Although there are several standardized CLSI phenotypic methods available, many clinical labs in the United States do not perform yeast susceptibility testing. Mold susceptibility testing is restricted to a few reference laboratories (195). In contrast to the CLSI broth microdilution reference method, the disk diffusion reference method and commercial gradient diffusion strips are relatively simple (195). There are several commercial automated platforms available for susceptibility testing of yeasts, including Vitek2 and YeastOne (195). Future development of yeast AST on such commercial automated platforms may increase access to many clinical laboratories for in-house streamlined yeast AST, mitigating excess turnaround time and improving time to effective antifungal therapy.

In contrast, there are no commercial platforms for susceptibility testing of molds. While the College of American Pathologists offers proficiency testing for yeast susceptibility testing, it does not offer proficiency testing for mold susceptibility testing. For these reasons, mold susceptibility testing is offered only by a few large reference laboratories in the United States, with relatively long turnaround times. More commercial assays for fungal susceptibility testing, especially platforms that support mold susceptibility testing, are needed. Multicenter studies aimed at identifying the accuracy and clinical utility of in-house commercial gradient diffusion strips and other commercial platforms versus send-out testing are merited. Since three new classes of mold-active antifungals are currently in development, there may soon be multiple good choices for the treatment of mold infections. In this context, mold antifungal susceptibility testing will be essential for maximizing effective therapy (196).

SUMMARY

Improvement of fungal diagnostics cannot rely on a single technology. Instead, it needs to be equipped with an array of diagnostic tools, including MALTI-TOF MS, fungal biomarkers, antigen and antibody tests, and NAAT. Utilizing the strength of combined technologies would allow us to fulfill the diagnostic gaps and optimize the test algorithms for diagnosis of pneumocystis pneumonia, mucormycosis, aspergillosis, candidemia, endemic mycoses, and emerging and rare fungal infections. Likewise,

TABLE 2 Summary of fungal disease-specific and method/approach-specific diagnostic gaps and proposals to fill the gaps[a]

Approach	Method								Diagnostic gap	Proposal
	Microscopy/ histology	Culture	MALDI-TOF MS	Sequencing ID	BDG	GM	Antigen/ antibody	NAAT		
Disease specific										
Pneumocystis pneumonia	+/+	−	−	+	+	−	−/+	+	NAAT cannot differentiate colonization and real infection; BDG has low specificity	Standardize NAAT; NAAT (particularly qNAAT) coupled with BDG for accurate diagnosis
Mucormycosis	+/+	+	+	+	−	−	−/−	+	Culture/histology lacks sensitivity and speed for diagnosis	Define optimal tissue process for culture; standardize NAAT for screening and early diagnosis
Aspergillosis	+/+	+	+	+	+	+	+/−	+	Lack of NAAT standardization; insufficient data to understand the utility of NAAT in conjunction with GM	Standardize NAAT; multicenter clinical trials to evaluate NAAT in conjunction with GM to optimize diagnosis
Candidemia	+/+	+	+	+	+	−	+/−	+	Non-culture-based direct detection of *Candida* species in blood	Clinical trials are needed to address the feasibility of implementing non-culture-based rapid detection platforms and determine their clinical impact and outcomes
Endemic mycoses	+/+	+	+	+	+	−	+/+	+	Lack of availability and ability to perform antigen and antibody assays in clinical laboratories	Development of low-complexity antigen/ serology assays may increase clinical laboratories' capabilities in timely diagnosis of disease
Emerging and rare fungal infections	+/+	+	+	+	+	−	−/−	+	Lack of knowledge and capabilities to detect and recognize these emerging, rare or uncommon fungal pathogens	Multicenter efforts to understand the prevalence, characteristics, and clinical features of these rare fungal infections
Method/approach specific										
Fungal ID in FFPE									Difficulty with identification to species of fungal pathogens present in FFPE tissues	Standardize molecular approach to directly identify fungal pathogens present in FFPE tissues
Mold ID by MALDI-TOF MS									Limited diagnostic spectra for molds in MALDI databases	Standardize and expand MALDI mold database
Mold blood culture									Suboptimal recovery of mold from blood culture	Multicenter efforts to optimize and standardize mold blood culture
Fungal POCT									Lack of POCT for rapid diagnosis of fungal diseases	Implement low-complexity and affordable LFA assays to increase clinical laboratory capacity for fungal diagnosis
Fungal culture for CF									Lack of standardize laboratory approach for fungal culture in CF	Standardize CF fungal culture protocol
AST									Increasing clinical need for antifungal drug susceptibility testing, particularly for molds	Multicenter efforts to develop a feasible and affordable approach for mold susceptibility testing in clinical laboratories

[a]AST, antifungal susceptibility testing; BDG, 1,3-β-D-glucan; CF, cystic fibrosis; FFPE, formalin fixed and paraffin embedded; GM, galactomannan; ID, identification; NAAT, nucleic acid amplification test; qNAAT, quantitative NAAT; POCT, point-of-care test; +, available; −, not available.

strategies and approaches to enhance fungal ID and detection include direct ID of fungi in FFPE tissues, recovery of molds in blood culture, mold ID by MALDI-TOF MS, isolation of Mucorales in tissue culture, streamlining fungal culture for CF, access to fungal POCT, and performing AST. Furthermore, standardization of NAAT and the development of a qNAAT assay will help clinical laboratories to determine clinical relevance of test results. Since a majority of fungi identified in clinical specimens could come from environment, interpretation of fungal diagnostic testing results needs to take the clinical context of the patient into consideration, as well as other laboratory findings, to support fungal diagnosis. A composite diagnostic approach, such as incorporating NAAT and fungal biomarkers, would enhance sensitivity and specificity for the diagnosis of fungal infections.

Improvement of fungal diagnostics cannot rely on a single medical center or institution. Instead, it needs to a concerted multicenter effort. This is the first time that clinical laboratories from the United States and Canada have come together to tackle these diagnostic gaps (Table 2). The FDLC will take a multicenter approach to generate robust data to consolidate optimal diagnostic algorithms and draw consensus guideline that clinical laboratories can follow. The FDLC will collaborate with industry partners for commercial assay development, clinical validation, and FDA approval. The availability of commercial diagnostics would provide standardization and accessibility to facilitate laboratory implementation. The FDLC will also work closely with clinical colleagues to conduct diagnostic-method-driven clinical trials to determine the optimal diagnostic algorithms that would ultimately improve patient clinical outcomes.

ACKNOWLEDGMENTS

A.T.H. received a research grant, consulting fee, and speaking honoraria from BioFire. S.M.L. received research funding from IMMY diagnostics. S.X.Z. received research funding from IMMY diagnostics and Vela diagnostics. S.S. was supported by the Intramural Research Program of the National Institutes of Health, Clinical Center, Department of Laboratory Medicine.

The Fungal Diagnostics Laboratories Consortium (FDLC) consists of the following laboratories and medical centers: ARUP Laboratories, Utah, USA; Beth Israel Deaconess Medical Center, Massachusetts, USA; CDC Mycotic Diseases Branch, Georgia, USA; Cleveland Clinic, Ohio, USA; Dartmouth-Hitchcock Medical Center, New Hampshire, USA, Duke University Medical Center, North Carolina, USA; Fungus Testing Laboratory, Texas, USA: Johns Hopkins Medical Institutions, Maryland, USA; Laboratoire de Sante Publique du Quebec, Quebec, Canada; London Health Sciences Center, Ontario, Canada; Loyola University Medical Center, Illinois, USA; Mayo Clinic, Arizona, USA; Mayo Clinic, Minnesota, USA; Memorial Sloan Kettering Cancer Center, New York, USA; New York-Presbyterian Hospital and Weill Cornell Medicine, New York, USA; NIH Microbiology Service, Maryland, USA; NorthShore University HealthSystem, Illinois, USA; The Ohio State University Wexner Medical Center, Ohio, USA; Public Health Ontario Laboratory, Ontario, Canada; St. Jude Children's Research Hospital, Tennessee, USA; Stanford Medical Center, California, USA; Temple University Health System, Pennsylvania, USA; University of Alabama at Birmingham, Alabama, USA; University of Maryland Medical Center, Maryland, USA; University of Texas Southwestern, Texas, USA; and Wake Forest Medical Center, North Carolina, USA.

REFERENCES

1. Williams KM, Ahn KW, Chen M, Aljurf MD, Agwu AL, Chen AR, Walsh TJ, Szabolcs P, Boeckh MJ, Auletta JJ, Lindemans CA, Zanis-Neto J, Malvezzi M, Lister J, de Toledo Codina JS, Sackey K, Chakrabarty JL, Ljungman P, Wingard JR, Seftel MD, Seo S, Hale GA, Wirk B, Smith MS, Savani BN, Lazarus HM, Marks DI, Ustun C, Abdel-Azim H, Dvorak CC, Szer J, Storek J, Yong A, Riches MR. 2016. The incidence, mortality and timing of *Pneumocystis jirovecii* pneumonia after hematopoietic cell transplantation: a CIBMTR analysis. Bone Marrow Transplant 51:573–580. https://doi.org/10.1038/bmt.2015.316.
2. Sepkowitz KA. 2002. Opportunistic infections in patients with and patients without acquired immunodeficiency syndrome. Clin Infect Dis 34:1098–1107. https://doi.org/10.1086/339548.
3. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, Clancy CJ, Wingard JR, Lockhart SR, Groll AH, Sorrell TC, Bassetti M, Akan H, Alexander BD, Andes D, Azoulay E, Bialek R, Bradsher RW, Bretagne S, Calandra T, Caliendo AM, Castagnola E, et al. 2020. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and

Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis 71:1367–1376. https://doi.org/10.1093/cid/ciz1008.
4. Fishman JA, Gans H, AST Infectious Diseases Community of Practice. 2019. *Pneumocystis jirovecii* in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant 33:e13587. https://doi.org/10.1111/ctr.13587.
5. Limper AH, Offord KP, Smith TF, Martin WJ, II. 1989. *Pneumocystis carinii* pneumonia. Differences in lung parasite number and inflammation in patients with and without AIDS. Am Rev Respir Dis 140:1204–1209. https://doi.org/10.1164/ajrccm/140.5.1204.
6. Fan LC, Lu HW, Cheng KB, Li HP, Xu JF. 2013. Evaluation of PCR in bronchoalveolar lavage fluid for diagnosis of *Pneumocystis jirovecii* pneumonia: a bivariate meta-analysis and systematic review. PLoS One 8:e73099. https://doi.org/10.1371/journal.pone.0073099.
7. Lu Y, Ling G, Qiang C, Ming Q, Wu C, Wang K, Ying Z. 2011. PCR diagnosis of *Pneumocystis* pneumonia: a bivariate meta-analysis. J Clin Microbiol 49:4361–4363. https://doi.org/10.1128/JCM.06066-11.
8. Tia T, Putaporntip C, Kosuwin R, Kongpolprom N, Kawkitinarong K, Jongwutiwes S. 2012. A highly sensitive novel PCR assay for detection of *Pneumocystis jirovecii* DNA in bronchoalveolar lavage specimens from immunocompromised patients. Clin Microbiol Infect 18:598–603. https://doi.org/10.1111/j.1469-0691.2011.03656.x.
9. Gits-Muselli M, White PL, Mengoli C, Chen S, Crowley B, Dingemans G, Fréalle E, R LG, Guiver M, Hagen F, Halliday C, Johnson G, Lagrou K, Lengerova M, Melchers WJG, Novak-Frazer L, Rautemaa-Richardson R, Scherer E, Steinmann J, Cruciani M, Barnes R, Donnelly JP, Loeffler J, Bretagne S, Alanio A. 2020. The Fungal PCR Initiative's evaluation of in-house and commercial *Pneumocystis jirovecii* qPCR assays: toward a standard for a diagnostics assay. Med Mycol 58:779–788. https://doi.org/10.1093/mmy/myz115.
10. Liu B, Totten M, Nematollahi S, Datta K, Memon W, Marimuthu S, Wolf LA, Carroll KC, Zhang SX. 2020. Development and evaluation of a fully automated molecular assay targeting the mitochondrial small subunit rRNA gene for the detection of *Pneumocystis jirovecii* in bronchoalveolar lavage fluid specimens. J Mol Diagn 22:1482–1493. https://doi.org/10.1016/j.jmoldx.2020.10.003.
11. Maskell NA, Waine DJ, Lindley A, Pepperell JC, Wakefield AE, Miller RF, Davies RJ. 2003. Asymptomatic carriage of *Pneumocystis jirovecii* in subjects undergoing bronchoscopy: a prospective study. Thorax 58:594–597. https://doi.org/10.1136/thorax.58.7.594.
12. Nevez G, Raccurt C, Vincent P, Jounieaux V, Dei-Cas E. 1999. Pulmonary colonization with *Pneumocystis carinii* in human immunodeficiency virus-negative patients: assessing risk with blood $CD4^+$ T cell counts. Clin Infect Dis 29:1331–1332. https://doi.org/10.1086/313478.
13. Khodadadi H, Mirhendi H, Mohebali M, Kordbacheh P, Zarrinfar H, Makimura K. 2013. *Pneumocystis jirovecii* colonization in non-HIV-infected patients based on nested-PCR detection in bronchoalveolar lavage samples. Iran J Public Health 42:298–305.
14. Fritzsche C, Riebold D, Fuehrer A, Mitzner A, Klammt S, Mueller-Hilke B, Reisinger EC. 2013. *Pneumocystis jirovecii* colonization among renal transplant recipients. Nephrology (Carlton) 18:382–387. https://doi.org/10.1111/nep.12054.
15. Rhoads D, Peaper DR, She RC, Nolte FS, Wojewoda CM, Anderson NW, Pritt BS. 2020. College of American Pathologists (CAP) Microbiology Committee Perspective: caution must be used in interpreting the cycle threshold (Ct) value. Clin Infect Dis https://doi.org/10.1093/cid/ciaa1199.
16. Fauchier T, Hasseine L, Gari-Toussaint M, Casanova V, Marty PM, Pomares C. 2016. Detection of *Pneumocystis jirovecii* by quantitative PCR to differentiate colonization and pneumonia in immunocompromised HIV-positive and HIV-negative patients. J Clin Microbiol 54:1487–1495. https://doi.org/10.1128/JCM.03174-15.
17. Perret T, Kritikos A, Hauser PM, Guiver M, Coste AT, Jaton K, Lamoth F. 2020. Ability of quantitative PCR to discriminate *Pneumocystis jirovecii* pneumonia from colonization. J Med Microbiol 69:705–711. https://doi.org/10.1099/jmm.0.001190.
18. Pinana JL, Albert E, Gomez MD, Perez A, Hernandez-Boluda JC, Montoro J, Salavert M, Gonzalez EM, Tormo M, Gimenez E, Villalba M, Balaguer-Rosello A, Hernani R, Bueno F, Borras R, Sanz J, Solano C, Navarro D. 2020. Clinical significance of *Pneumocystis jirovecii* DNA detection by real-time PCR in hematological patient respiratory specimens. J Infect 80:578–606. https://doi.org/10.1016/j.jinf.2020.01.001.
19. Theel ES, Jespersen DJ, Iqbal S, Bestrom JE, Rollins LO, Misner LJ, Markley BJ, Mandrekar J, Baddour LM, Limper AH, Wengenack NL, Binnicker MJ. 2013. Detection of (1,3)-β-D-glucan in bronchoalveolar lavage and serum samples collected from immunocompromised hosts. Mycopathologia 175:33–41. https://doi.org/10.1007/s11046-012-9579-y.
20. Theel ES, Doern CD. 2013. β-D-Glucan testing is important for diagnosis of invasive fungal infections. J Clin Microbiol 51:3478–3483. https://doi.org/10.1128/JCM.01737-13.
21. Karageorgopoulos DE, Qu JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME. 2013. Accuracy of β-D-glucan for the diagnosis of *Pneumocystis jirovecii* pneumonia: a meta-analysis. Clin Microbiol Infect 19:39–49. https://doi.org/10.1111/j.1469-0691.2011.03760.x.
22. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, Morinobu A, Nishimura K, Kumagai S. 2012. Diagnostic accuracy of serum 1,3-β-D-glucan for *Pneumocystis jirovecii* pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol 50:7–15. https://doi.org/10.1128/JCM.05267-11.
23. Tasaka S. 2015. Pneumocystis pneumonia in human immunodeficiency virus-infected adults and adolescents: current concepts and future directions. Clin Med Insights Circ Respir Pulm Med 9:19–28. https://doi.org/10.4137/CCRPM.S23324.
24. Damiani C, Le Gal S, Da Costa C, Virmaux M, Nevez G, Totet A. 2013. Combined quantification of pulmonary *Pneumocystis jirovecii* DNA and serum (1→3)-β-D-glucan for differential diagnosis of *Pneumocystis* pneumonia and *Pneumocystis* colonization. J Clin Microbiol 51:3380–3388. https://doi.org/10.1128/JCM.01554-13.
25. Morjaria S, Frame J, Franco-Garcia A, Geyer A, Kamboj M, Babady NE. 2019. Clinical Performance of 1,3-β-D-glucan for the diagnosis of pneumocystis pneumonia (PCP) in cancer patients tested with PCP polymerase chain reaction. Clin Infect Dis 69:1303–1309. https://doi.org/10.1093/cid/ciy1072.
26. CLSI. 2012. Principles and practice for detection of fungi in clinical specimens: direct examination and culture. Approved guideline M54-A. Clinical and Laboratory Standards Institute, Wayne, PA.
27. Berkow E, McGowan K. 2019. Mycology: specimen collection, transportation, and processing, p 1016–2024. *In* Carroll K, Pfaller M, Landry M (ed), Manual of clinical microbiology, 12th ed. ASM Press, Washington, DC.
28. Leber A. 2016. Clinical microbiology procedure handbook, 4th ed, vol 2. ASM Press, Washington, DC.
29. Legrand M, Gits-Muselli M, Boutin L, Garcia-Hermoso D, Maurel V, Soussi S, Benyamina M, Ferry A, Chaussard M, Hamane S, Denis B, Touratier S, Guigue N, Fréalle E, Jeanne M, Shaal JV, Soler C, Mimoun M, Chaouat M, Lafaurie M, Mebazaa A, Bretagne S, Alanio A. 2016. Detection of circulating Mucorales DNA in critically ill burn patients: preliminary report of a screening strategy for early diagnosis and treatment. Clin Infect Dis 63:1312–1317. https://doi.org/10.1093/cid/ciw563.
30. Millon L, Herbrecht R, Grenouillet F, Morio F, Alanio A, Letscher-Bru V, Cassaing S, Chouaki T, Kauffmann-Lacroix C, Poirier P, Toubas D, Augereau O, Rocchi S, Garcia-Hermoso D, Bretagne S. 2016. Early diagnosis and monitoring of mucormycosis by detection of circulating DNA in serum: retrospective analysis of 44 cases collected through the French Surveillance Network of Invasive Fungal Infections (RESSIF). Clin Microbiol Infect 22:810 e811–810 e818.
31. Millon L, Larosa F, Lepiller Q, Legrand F, Rocchi S, Daguindau E, Scherer E, Bellanger AP, Leroy J, Grenouillet F. 2013. Quantitative polymerase chain reaction detection of circulating DNA in serum for early diagnosis of mucormycosis in immunocompromised patients. Clin Infect Dis 56:e95-101. https://doi.org/10.1093/cid/cit094.
32. Mercier T, Reynders M, Beuselinck K, Guldentops E, Maertens J, Lagrou K. 2019. Serial detection of circulating Mucorales DNA in invasive mucormycosis: a retrospective multicenter evaluation. J Fungi (Basel) 5:113. https://doi.org/10.3390/jof5040113.
33. Springer J, Lackner M, Ensinger C, Risslegger B, Morton CO, Nachbaur D, Lass-Flörl C, Einsele H, Heinz WJ, Loeffler J. 2016. Clinical evaluation of a Mucorales-specific real-time PCR assay in tissue and serum samples. J Med Microbiol 65:1414–1421. https://doi.org/10.1099/jmm.0.000375.
34. Guegan H, Iriart X, Bougnoux ME, Berry A, Robert-Gangneux F, Gangneux JP. 2020. Evaluation of MucorGenius Mucorales PCR assay for the diagnosis of pulmonary mucormycosis. J Infect 81:311–317. https://doi.org/10.1016/j.jinf.2020.05.051.
35. Baldin C, Soliman SSM, Jeon HH, Alkhazraji S, Gebremariam T, Gu Y, Bruno VM, Cornely OA, Leather HL, Sugrue MW, Wingard JR, Stevens DA, Edwards JE, Jr, Ibrahim AS. 2018. PCR-based approach targeting Mucorales-specific gene family for diagnosis of mucormycosis. J Clin Microbiol 56:e00746-18. https://doi.org/10.1128/JCM.00746-18.

36. Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger B, Hoenigl M, Jensen HE, Lagrou K, Lewis RE, Mellinghoff SC, Mer M, Pana ZD, Seidel D, Sheppard DC, Wahba R, Akova M, Alanio A, Al-Hatmi AMS, Arikan-Akdagli S, Badali H, Ben-Ami R, et al. 2019. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. Lancet Infect Dis 19:e405–e421. https://doi.org/10.1016/S1473-3099(19)30312-3.
37. Rocchi S, Scherer E, Mengoli C, Alanio A, Botterel F, Bougnoux ME, Bretagne S, Cogliati M, Cornu M, Dalle F, Damiani C, Denis J, Fuchs S, Gits-Muselli M, Hagen F, Halliday C, Hare R, Iriart X, Klaassen C, Lackner M, Lengerova M, Letscher-Bru V, Morio F, Nourrisson C, Posch W, Sendid B, Springer J, Willinger B, White PL, Barnes RA, Cruciani M, Donnelly JP, Loeffler J, Millon L. 2020. Interlaboratory evaluation of Mucorales PCR assays for testing serum specimens: a study by the fungal PCR Initiative and the Modimucor Study Group. Med Mycol 59:126–138. https://doi.org/10.1093/mmy/myaa036.
38. Patterson TF, Thompson GR, III, Denning DW, Fishman JA, Hadley S, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Nguyen MH, Segal BH, Steinbach WJ, Stevens DA, Walsh TJ, Wingard JR, Young JA, Bennett JE. 2016. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 63:e1–e60. https://doi.org/10.1093/cid/ciw326.
39. Marr KA, Laverdiere M, Gugel A, Leisenring W. 2005. Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. Clin Infect Dis 40:1762–1769. https://doi.org/10.1086/429921.
40. Rath PM, Steinmann J. 2018. Overview of commercially available PCR assays for the detection of *Aspergillus* spp. DNA in patient samples. Front Microbiol 9:740. https://doi.org/10.3389/fmicb.2018.00740.
41. Cruciani M, Mengoli C, Barnes R, Donnelly JP, Loeffler J, Jones BL, Klingspor L, Maertens J, Morton CO, White LP. 2019. Polymerase chain reaction blood tests for the diagnosis of invasive aspergillosis in immunocompromised people. Cochrane Database Syst Rev 9:CD009551. https://doi.org/10.1002/14651858.CD009551.pub4.
42. White PL, Posso RB, Barnes RA. 2017. Analytical and clinical evaluation of the PathoNostics AsperGenius assay for detection of invasive aspergillosis and resistance to azole antifungal drugs directly from plasma samples. J Clin Microbiol 55:2356–2366. https://doi.org/10.1128/JCM.00411-17.
43. White PL, Perry MD, Moody A, Follett SA, Morgan G, Barnes RA. 2011. Evaluation of analytical and preliminary clinical performance of Myconostica MycAssay Aspergillus when testing serum specimens for diagnosis of invasive aspergillosis. J Clin Microbiol 49:2169–2174. https://doi.org/10.1128/JCM.00101-11.
44. Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, Lass-Flörl C, Lewis RE, Munoz P, Verweij PE, Warris A, Ader F, Akova M, Arendrup MC, Barnes RA, Beigelman-Aubry C, Blot S, Bouza E, Brüggemann RJM, Buchheidt D, Cadranel J, Castagnola E, et al. 2018. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect 24(Suppl 1): e1–e38. https://doi.org/10.1016/j.cmi.2018.01.002.
45. White PL, Wingard JR, Bretagne S, Löffler J, Patterson TF, Slavin MA, Barnes RA, Pappas PG, Donnelly JP. 2015. Aspergillus polymerase chain reaction: systematic review of evidence for clinical use in comparison with antigen testing. Clin Infect Dis 61:1293–1303. https://doi.org/10.1093/cid/civ507.
46. Barnes RA, White PL, Morton CO, Rogers TR, Cruciani M, Loeffler J, Donnelly JP. 2018. Diagnosis of aspergillosis by PCR: clinical considerations and technical tips. Med Mycol 56:60–72. https://doi.org/10.1093/mmy/myx091.
47. Luong ML, Clancy CJ, Vadnerkar A, Kwak EJ, Silveira FP, Wissel MC, Grantham KJ, Shields RK, Crespo M, Pilewski J, Toyoda Y, Kleiboeker SB, Pakstis D, Reddy SK, Walsh TJ, Nguyen MH. 2011. Comparison of an *Aspergillus* real-time polymerase chain reaction assay with galactomannan testing of bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. Clin Infect Dis 52:1218–1226. https://doi.org/10.1093/cid/cir185.
48. White PL, Barnes RA, Springer J, Klingspor L, Cuenca-Estrella M, Morton CO, Lagrou K, Bretagne S, Melchers WJ, Mengoli C, Donnelly JP, Heinz WJ, Loeffler J, EAPCRI. 2015. Clinical performance of *Aspergillus* PCR for testing serum and plasma: a study by the European Aspergillus PCR Initiative. J Clin Microbiol 53:2832–2837. https://doi.org/10.1128/JCM.00905-15.
49. White PL, Mengoli C, Bretagne S, Cuenca-Estrella M, Finnstrom N, Klingspor L, Melchers WJ, McCulloch E, Barnes RA, Donnelly JP, Loeffler J, European *Aspergillus* PCR Initiative (EAPCRI). 2011. Evaluation of *Aspergillus* PCR protocols for testing serum specimens. J Clin Microbiol 49:3842–3848. https://doi.org/10.1128/JCM.05316-11.
50. Springer J, White PL, Hamilton S, Michel D, Barnes RA, Einsele H, Löffler J. 2016. Comparison of performance characteristics of *Aspergillus* PCR in testing a range of blood-based samples in accordance with international methodological recommendations. J Clin Microbiol 54:705–711. https://doi.org/10.1128/JCM.02814-15.
51. Loeffler J, Mengoli C, Springer J, Bretagne S, Cuenca-Estrella M, Klingspor L, Lagrou K, Melchers WJ, Morton CO, Barnes RA, Donnelly JP, White PL, European *Aspergillus* PCR Initiative. 2015. 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 53:2838–2845. https://doi.org/10.1128/JCM.00906-15.
52. Morton CO, White PL, Barnes RA, Klingspor L, Cuenca-Estrella M, Lagrou K, Bretagne S, Melchers W, Mengoli C, Caliendo AM, Cogliati M, Debets-Ossenkopp Y, Gorton R, Hagen F, Halliday C, Hamal P, Harvey-Wood K, Jaton K, Johnson G, Kidd S, Lengerova M, Lass-Florl C, Linton C, Millon L, Morrissey CO, Paholcsek M, Talento AF, Ruhnke M, Willinger B, Donnelly JP, Loeffler J. 2017. Determining the analytical specificity of PCR-based assays for the diagnosis of IA: what is *Aspergillus*? Med Mycol 55:402–413. https://doi.org/10.1093/mmy/myw093.
53. Arvanitis M, Ziakas PD, Zacharioudakis IM, Zervou FN, Caliendo AM, Mylonakis E. 2014. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. J Clin Microbiol 52:3731–3742. https://doi.org/10.1128/JCM.01365-14.
54. Aguado JM, Vázquez L, Fernández-Ruiz M, Villaescusa T, Ruiz-Camps I, Barba P, Silva JT, Batlle M, Solano C, Gallardo D, Heras I, Polo M, Varela R, Vallejo C, Olave T, López-Jiménez J, Rovira M, Parody R, Cuenca-Estrella M, Spanish Network for Research in Infectious Diseases. 2015. Serum galactomannan versus a combination of galactomannan and polymerase chain reaction-based Aspergillus DNA detection for early therapy of invasive aspergillosis in high-risk hematological patients: a randomized controlled trial. Clin Infect Dis 60:405–414. https://doi.org/10.1093/cid/ciu833.
55. Morrissey CO, Chen SC, Sorrell TC, Milliken S, Bardy PG, Bradstock KF, Szer J, Halliday CL, Gilroy NM, Moore J, Schwarer AP, Guy S, Bajel A, Tramontana AR, Spelman T, Slavin MA, Australasian Leukaemia Lymphoma Group and the Australia and New Zealand Mycology Interest Group. 2013. Galactomannan and PCR versus culture and histology for directing use of antifungal treatment for invasive aspergillosis in high-risk haematology patients: a randomised controlled trial. Lancet Infect Dis 13:519–528. https://doi.org/10.1016/S1473-3099(13)70076-8.
56. Springer J, Morton CO, Perry M, Heinz WJ, Paholcsek M, Alzheimer M, Rogers TR, Barnes RA, Einsele H, Loeffler J, White PL. 2013. Multicenter comparison of serum and whole-blood specimens for detection of *Aspergillus* DNA in high-risk hematological patients. J Clin Microbiol 51:1445–1450. https://doi.org/10.1128/JCM.03322-12.
57. Buil JB, Zoll J, Verweij PE, Melchers WJG. 2018. Molecular detection of azole-resistant *Aspergillus fumigatus* in clinical samples. Front Microbiol 9:515. https://doi.org/10.3389/fmicb.2018.00515.
58. Walker TA, Lockhart SR, Beekmann SE, Polgreen PM, Santibanez S, Mody RK, Beer KD, Chiller TM, Jackson BR. 2018. Recognition of azole-resistant aspergillosis by physicians specializing in infectious diseases, United States. Emerg Infect Dis 24:111–113. https://doi.org/10.3201/eid2401.170971.
59. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 39:309–317. https://doi.org/10.1086/421946.
60. Cesaro S, Tridello G, Blijlevens N, Ljungman P, Craddock C, Michallet M, Martin A, Snowden JA, Mohty M, Maertens J, Passweg J, Petersen E, Nihtinen A, Isaksson C, Milpied N, Rohlich PS, Deconinck E, Crawley C, Ledoux MP, Hoek J, Nagler A, Styczynski J. 2018. Incidence, risk factors, and long-term outcome of acute leukemia patients with early candidemia after allogeneic stem cell transplantation: a study by the Acute Leukemia and Infectious Diseases Working Parties of European Society for Blood and Marrow Transplantation. Clin Infect Dis 67:564–572. https://doi.org/10.1093/cid/ciy150.
61. Cornely OA, Gachot B, Akan H, Bassetti M, Uzun O, Kibbler C, Marchetti O, de Burghgraeve P, Ramadan S, Pylkkanen L, Ameye L, Paesmans M, Donnelly JP, Donnelly PJ, EORTC Infectious Diseases Group. 2015. Epidemiology and outcome of fungemia in a cancer Cohort of the Infectious Diseases Group (IDG) of the European Organization for Research and

Treatment of Cancer (EORTC 65031). Clin Infect Dis 61:324–331. https://doi.org/10.1093/cid/civ293.
62. Zhang AY, Shrum S, Williams S, Petnic S, Nadle J, Johnston H, Barter D, VonBank B, Bonner L, Hollick R, Marceaux K, Harrison L, Schaffner W, Tesini BL, Farley MM, Pierce RA, Phipps E, Mody RK, Chiller TM, Jackson BR, Vallabhaneni S. 2019. The changing epidemiology of candidemia in the United States: injection drug use as an increasingly common risk factor: active surveillance in selected sites, United States, 2014-2017. Clin Infect Dis 71:1732–1737. https://doi.org/10.1093/cid/ciz1061.
63. Tsay SV, Mu Y, Williams S, Epson E, Nadle J, Bamberg WM, Barter DM, Johnston HL, Farley MM, Harb S, Thomas S, Bonner LA, Harrison LH, Hollick R, Marceaux K, Mody RK, Pattee B, Shrum Davis S, Phipps EC, Tesini BL, Gellert AB, Zhang AY, Schaffner W, Hillis S, Ndi D, Graber CR, Jackson BR, Chiller T, Magill S, Vallabhaneni S. 2020. Burden of candidemia in the United States, 2017. Clin Infect Dis 71:e449–e453. https://doi.org/10.1093/cid/ciaa193.
64. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, Bearden DT. 2006. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis 43:25–31. https://doi.org/10.1086/504810.
65. Pancholi P, Carroll KC, Buchan BW, Chan RC, Dhiman N, Ford B, Granato PA, Harrington AT, Hernandez DR, Humphries RM, Jindra MR, Ledeboer NA, Miller SA, Mochon AB, Morgan MA, Patel R, Schreckenberger PC, Stamper PD, Simner PJ, Tucci NE, Zimmerman C, Wolk DM. 2018. Multicenter evaluation of the accelerate PhenoTest BC kit for rapid identification and phenotypic antimicrobial susceptibility testing using morphokinetic cellular analysis. J Clin Microbiol 56:e01329-17. https://doi.org/10.1128/JCM.01329-17.
66. Salimnia H, Fairfax MR, Lephart PR, Schreckenberger P, DesJarlais SM, Johnson JK, Robinson G, Carroll KC, Greer A, Morgan M, Chan R, Loeffelholz M, Valencia-Shelton F, Jenkins S, Schuetz AN, Daly JA, Barney T, Hemmert A, Kanack KJ. 2016. Evaluation of the FilmArray blood culture identification panel: results of a multicenter controlled trial. J Clin Microbiol 54:687–698. https://doi.org/10.1128/JCM.01679-15.
67. Simor AE, Porter V, Mubareka S, Chouinard M, Katz K, Vermeiren C, Fattouh R, Matukas LM, Tadros M, Mazzulli T, Poutanen S. 2018. Rapid identification of *Candida* species from positive blood cultures by use of the FilmArray blood culture identification panel. J Clin Microbiol 56:e01387-18. https://doi.org/10.1128/JCM.01387-18.
68. Zhang SX, Carroll KC, Lewis S, Totten M, Mead P, Samuel L, Steed LL, Nolte FS, Thornberg A, Reid JL, Whitfield NN, Babady NE. 2020. 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 58:e02096-19. https://doi.org/10.1128/JCM.02096-19.
69. Jeddi F, Yapo-Kouadio GC, Normand AC, Cassagne C, Marty P, Piarroux R. 2017. Performance assessment of two lysis methods for direct identification of yeasts from clinical blood cultures using MALDI-TOF mass spectrometry. Med Myco 55:185–192. https://doi.org/10.1093/mmy/myw038.
70. Yan Y, He Y, Maier T, Quinn C, Shi G, Li H, Stratton CW, Kostrzewa M, Tang YW. 2011. Improved identification of yeast species directly from positive blood culture media by combining Sepsityper specimen processing and Microflex analysis with the matrix-assisted laser desorption ionization Biotyper system. J Clin Microbiol 49:2528–2532. https://doi.org/10.1128/JCM.00339-11.
71. Clancy CJ, Nguyen MH. 2013. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis 56:1284–1292. https://doi.org/10.1093/cid/cit006.
72. Clancy CJ, Pappas PG, Vazquez J, Judson MA, Kontoyiannis DP, Thompson GR, Garey KW, Reboli A, Greenberg RN, Apewokin S, Lyon GM, Ostrosky-Zeichner L, Wu AHB, Tobin E, Nguyen MH, Caliendo AM. 2018. Detecting infections rapidly and easily for Candidemia Trial, Part 2 (DIRECT2): a prospective, multicenter study of the T2Candida Panel. Clin Infect Dis 66:1678–1686. https://doi.org/10.1093/cid/cix1095.
73. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, Groeger JS, Judson MA, Vinagre YM, Heard SO, Zervou FN, Zacharioudakis IM, Kontoyiannis DP, Pappas PG. 2015. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis 60:892–899. https://doi.org/10.1093/cid/ciu959.
74. Clancy CJ, Nguyen MH. 2018. T2 magnetic resonance for the diagnosis of bloodstream infections: charting a path forward. J Antimicrob Chemother 73:iv2–iv5. https://doi.org/10.1093/jac/dky050.
75. Valero C, Buitrago MJ, Gago S, Quiles-Melero I, García-Rodríguez J. 2018. A matrix-assisted laser desorption/ionization time of flight mass spectrometry reference database for the identification of *Histoplasma capsulatum*. Med Mycol 56:307–314. https://doi.org/10.1093/mmy/myx047.
76. Porte L, Valdivieso F, Wilmes D, Gaete P, Díaz MC, Thompson L, Munita JM, Alliende R, Varela C, Rickerts V, Weitzel T. 2019. Laboratory exposure to *Coccidioides*: lessons learnt in a non-endemic country. J Hosp Infect 102:461–464. https://doi.org/10.1016/j.jhin.2019.03.006.
77. Babady NE, Buckwalter SP, Hall L, Le Febre KM, Binnicker MJ, Wengenack NL. 2011. Detection of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from culture isolates and clinical specimens by use of real-time PCR. J Clin Microbiol 49:3204–3208. https://doi.org/10.1128/JCM.00673-11.
78. Bialek R, Ernst F, Dietz K, Najvar LK, Knobloch J, Graybill JR, Schaumburg-Lever G. 2002. Comparison of staining methods and a nested PCR assay to detect *Histoplasma capsulatum* in tissue sections. Am J Clin Pathol 117:597–603. https://doi.org/10.1309/MH5B-GAQ2-KY19-FT7P.
79. Bialek R, Feucht A, Aepinus C, Just-Nubling G, Robertson VJ, Knobloch J, Hohle R. 2002. Evaluation of two nested PCR assays for detection of *Histoplasma capsulatum* DNA in human tissue. J Clin Microbiol 40:1644–1647. https://doi.org/10.1128/jcm.40.5.1644-1647.2002.
80. Bialek R, Kern J, Herrmann T, Tijerina R, Cecenas L, Reischl U, Gonzalez GM. 2004. PCR assays for identification of *Coccidioides posadasii* based on the nucleotide sequence of the antigen 2/proline-rich antigen. J Clin Microbiol 42:778–783. https://doi.org/10.1128/jcm.42.2.778-783.2004.
81. Binnicker MJ, Buckwalter SP, Eisberner JJ, Stewart RA, McCullough AE, Wohlfiel SL, Wengenack NL. 2007. Detection of *Coccidioides* species in clinical specimens by real-time PCR. J Clin Microbiol 45:173–178. https://doi.org/10.1128/JCM.01776-06.
82. Burgess JW, Schwan WR, Volk TJ. 2006. PCR-based detection of DNA from the human pathogen Blastomyces dermatitidis from natural soil samples. Med Mycol 44:741–748. https://doi.org/10.1080/13693780600954749.
83. Elias NA, Cuestas ML, Sandoval M, Poblete G, Lopez-Daneri G, Jewtuchowicz V, Iovannitti C, Mujica MT. 2012. Rapid identification of *Histoplasma capsulatum* directly from cultures by multiplex PCR. Mycopathologia 174:451–456. https://doi.org/10.1007/s11046-012-9567-2.
84. Guedes H. L d M, Guimarães AJ, Muniz M. d M, Pizzini CV, Hamilton AJ, Peralta JM, Deepe GS, Zancopé-Oliveira RM. 2003. PCR assay for identification of *Histoplasma capsulatum* based on the nucleotide sequence of the M antigen. J Clin Microbiol 41:535–539. https://doi.org/10.1128/jcm.41.2.535-539.2003.
85. Martagon-Villamil J, Shrestha N, Sholtis M, Isada CM, Hall GS, Bryne T, Lodge BA, Reller LB, Procop GW. 2003. Identification of *Histoplasma capsulatum* from culture extracts by real-time PCR. J Clin Microbiol 41:1295–1298. https://doi.org/10.1128/jcm.41.3.1295-1298.2003.
86. Mitchell M, Dizon D, Libke R, Peterson M, Slater D, Dhillon A. 2015. Development of a real-time PCR assay for identification of *Coccidioides immitis* by use of the BD Max system. J Clin Microbiol 53:926–929. https://doi.org/10.1128/JCM.02731-14.
87. Muraosa Y, Toyotome T, Yahiro M, Watanabe A, Shikanai-Yasuda MA, Kamei K. 2016. Detection of *Histoplasma capsulatum* from clinical specimens by cycling probe-based real-time PCR and nested real-time PCR. Med Mycol 54:433–438. https://doi.org/10.1093/mmy/myv106.
88. Pounder JI, Hansen D, Woods GL. 2006. Identification of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides* species by repetitive-sequence-based PCR. J Clin Microbiol 44:2977–2982. https://doi.org/10.1128/JCM.00687-06.
89. Sidamonidze K, Peck MK, Perez M, Baumgardner D, Smith G, Chaturvedi V, Chaturvedi S. 2012. Real-time PCR assay for identification of *Blastomyces dermatitidis* in culture and in tissue. J Clin Microbiol 50:1783–1786. https://doi.org/10.1128/JCM.00310-12.
90. Wiwanitkit V. 2010. TaqMan real-time PCR assay for *Coccidioides*. Med Mycol 48:679; author reply 680. https://doi.org/10.3109/13693780903496625.
91. Hall L, Doerr KA, Wohlfiel SL, Roberts GD. 2003. Evaluation of the MicroSeq system for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. J Clin Microbiol 41:1447–1453. https://doi.org/10.1128/JCM.41.4.1447-1453.2003.
92. Hall L, Wohlfiel S, Roberts GD. 2003. Experience with the MicroSeq D2 large-subunit ribosomal DNA sequencing kit for identification of commonly encountered, clinically important yeast species. J Clin Microbiol 41:5099–5102. https://doi.org/10.1128/jcm.41.11.5099-5102.2003.
93. Hall L, Wohlfiel S, Roberts GD. 2004. Experience with the MicroSeq D2 large-subunit ribosomal DNA sequencing kit for identification of

filamentous fungi encountered in the clinical laboratory. J Clin Microbiol 42:622–626. https://doi.org/10.1128/jcm.42.2.622-626.2004.
94. Sandhu GS, Kline BC, Stockman L, Roberts GD. 1995. Molecular probes for diagnosis of fungal infections. J Clin Microbiol 33:2913–2919. https://doi.org/10.1128/JCM.33.11.2913-2919.1995.
95. Stempak LM, Vogel SA, Richter SS, Wyllie R, Procop GW. 2019. Routine broad-range fungal polymerase chain reaction with DNA sequencing in patients with suspected mycoses does not add value and is not cost-effective. Arch Pathol Lab Med 143:634–638. https://doi.org/10.5858/arpa.2017-0299-OA.
96. Wheat LJ. 2006. Improvements in diagnosis of histoplasmosis. Expert Opin Biol Ther 6:1207–1221. https://doi.org/10.1517/14712598.6.11.1207.
97. Wheat LJ. 2006. Histoplasmosis: a review for clinicians from non-endemic areas. Mycoses 49:274–282. https://doi.org/10.1111/j.1439-0507.2006.01253.x.
98. Wheat LJ, Kohler RB, Tewari RP. 1986. Diagnosis of disseminated histoplasmosis by detection of *Histoplasma capsulatum* antigen in serum and urine specimens. N Engl J Med 314:83–88. https://doi.org/10.1056/NEJM198601093140205.
99. Falci DR, Monteiro AA, Braz Caurio CF, Magalhães TCO, Xavier MO, Basso RP, Melo M, Schwarzbold AV, Ferreira PRA, Vidal JE, Marochi JP, Godoy CSM, Soares RBA, Paste A, Bay MB, Pereira-Chiccola VL, Damasceno LS, Leitão T, Pasqualotto AC. 2019. Histoplasmosis, an underdiagnosed disease affecting people living with HIV/AIDS in Brazil: results of a multicenter prospective cohort study using both classical mycology tests and histoplasma urine antigen detection. Open Forum Infect Dis 6:ofz073. https://doi.org/10.1093/ofid/ofz073.
100. Theel ES, Harring JA, Dababneh AS, Rollins LO, Bestrom JE, Jespersen DJ. 2015. Reevaluation of commercial reagents for detection of *Histoplasma capsulatum* antigen in urine. J Clin Microbiol 53:1198–1203. https://doi.org/10.1128/JCM.03175-14.
101. Davies SF. 1986. Serodiagnosis of histoplasmosis. Semin Respir Infect 1:9–15.
102. Fandino-Devia E, Rodriguez-Echeverri C, Cardona-Arias J, Gonzalez A. 2016. Antigen detection in the diagnosis of histoplasmosis: a meta-analysis of diagnostic performance. Mycopathologia 181:197–205. https://doi.org/10.1007/s11046-015-9965-3.
103. Swartzentruber S, Rhodes L, Kurkjian K, Zahn M, Brandt ME, Connolly P, Wheat LJ. 2009. Diagnosis of acute pulmonary histoplasmosis by antigen detection. Clin Infect Dis 49:1878–1882. https://doi.org/10.1086/648421.
104. Williams B, Fojtasek M, Connolly-Stringfield P, Wheat J. 1994. Diagnosis of histoplasmosis by antigen detection during an outbreak in Indianapolis, Ind. Arch Pathol Lab Med 118:1205–1208.
105. Libert D, Procop GW, Ansari MQ. 2018. Histoplasma urinary antigen testing obviates the need for coincident serum antigen testing. Am J Clin Pathol 149:362–368. https://doi.org/10.1093/ajcp/aqx169.
106. Baumgardner DJ. 2018. Use of urine antigen testing for *Blastomyces* in an integrated health system. J Patient Cent Res Rev 5:176–182. https://doi.org/10.17294/2330-0698.1452.
107. Frost HM, Novicki TJ. 2015. *Blastomyces* antigen detection for diagnosis and management of blastomycosis. J Clin Microbiol 53:3660–3662. https://doi.org/10.1128/JCM.02352-15.
108. Walkty A, Keynan Y, Karlowsky J, Dhaliwal P, Embil J. 2018. Central nervous system blastomycosis diagnosed using the MVista® *Blastomyces* quantitative antigen enzyme immunoassay test on cerebrospinal fluid: a case report and review of the literature. Diagn Microbiol Infect Dis 90:102–104. https://doi.org/10.1016/j.diagmicrobio.2017.10.015.
109. Bamberger DM, Pepito BS, Proia LA, Ostrosky-Zeichner L, Ashraf M, Marty F, Scully E, Wheat LJ. 2015. Cerebrospinal fluid *Coccidioides* antigen testing in the diagnosis and management of central nervous system coccidioidomycosis. Mycoses 58:598–602. https://doi.org/10.1111/myc.12366.
110. Durkin M, Connolly P, Kuberski T, Myers R, Kubak BM, Bruckner D, Pegues D, Wheat LJ. 2008. Diagnosis of coccidioidomycosis with use of the *Coccidioides* antigen enzyme immunoassay. Clin Infect Dis 47:e69-73. https://doi.org/10.1086/592073.
111. Kassis C, Zaidi S, Kuberski T, Moran A, Gonzalez O, Hussain S, Hartmann-Manrique C, Al-Jashaami L, Chebbo A, Myers RA, Wheat LJ. 2015. Role of *Coccidioides* antigen testing in the cerebrospinal fluid for the diagnosis of coccidioidal meningitis. Clin Infect Dis 61:1521–1526. https://doi.org/10.1093/cid/civ585.
112. Assi M, Lakkis IE, Wheat LJ. 2011. Cross-reactivity in the Histoplasma antigen enzyme immunoassay caused by sporotrichosis. Clin Vaccine Immunol 18:1781–1782. https://doi.org/10.1128/CVI.05017-11.
113. Tobar Vega P, Erramilli S, Lee E. 2019. *Talaromyces marneffei* laboratory cross reactivity with *Histoplasma* and *Blastomyces* urinary antigen. Int J Infect Dis 86:15–17. https://doi.org/10.1016/j.ijid.2019.06.018.
114. Wheat J, Wheat H, Connolly P, Kleiman M, Supparatpinyo K, Nelson K, Bradsher R, Restrepo A. 1997. Cross-reactivity in *Histoplasma capsulatum* variety capsulatum antigen assays of urine samples from patients with endemic mycoses. Clin Infect Dis 24:1169–1171. https://doi.org/10.1086/513647.
115. Kozel TR, Wickes B. 2014. Fungal diagnostics. Cold Spring Harb Perspect Med 4:a019299. https://doi.org/10.1101/cshperspect.a019299.
116. Kauffman CA. 2007. Histoplasmosis: a clinical and laboratory update. Clin Microbiol Rev 20:115–132. https://doi.org/10.1128/CMR.00027-06.
117. Kuberski T, Herrig J, Pappagianis D. 2010. False-positive IgM serology in coccidioidomycosis. J Clin Microbiol 48:2047–2049. https://doi.org/10.1128/JCM.01843-09.
118. Guarner J, Brandt ME. 2011. Histopathologic diagnosis of fungal infections in the 21st century. Clin Microbiol Rev 24:247–280. https://doi.org/10.1128/CMR.00053-10.
119. Procop GW, Wilson M. 2001. Infectious disease pathology. Clin Infect Dis 32:1589–1601. https://doi.org/10.1086/320537.
120. Sears D, Schwartz BS. 2017. *Candida auris*: an emerging multidrug-resistant pathogen. Int J Infect Dis 63:95–98. https://doi.org/10.1016/j.ijid.2017.08.017.
121. Spivak ES, Hanson KE. 2018. *Candida auris*: an emerging fungal pathogen. J Clin Microbiol 56:e01588-17. https://doi.org/10.1128/JCM.01588-17.
122. Kordalewska M, Perlin DS. 2019. Molecular diagnostics in the times of surveillance for *Candida auris*. J Fungi (Basel) 5:77. https://doi.org/10.3390/jof5030077.
123. Kathuria S, Singh PK, Sharma C, Prakash A, Masih A, Kumar A, Meis JF, Chowdhary A. 2015. Multidrug-resistant *Candida auris* misidentified as *Candida haemulonii*: characterization by matrix-assisted laser desorption ionization-time of flight mass spectrometry and DNA sequencing and its antifungal susceptibility profile variability by Vitek 2, CLSI broth microdilution, and Etest method. J Clin Microbiol 53:1823–1830. https://doi.org/10.1128/JCM.00367-15.
124. Mizusawa M, Miller H, Green R, Lee R, Durante M, Perkins R, Hewitt C, Simner PJ, Carroll KC, Hayden RT, Zhang SX. 2017. Can multidrug-resistant *Candida auris* be reliably identified in clinical microbiology laboratories? J Clin Microbiol 55:638–640. https://doi.org/10.1128/JCM.02202-16.
125. Kordalewska M, Perlin DS. 2019. Identification of drug-resistant *Candida auris*. Front Microbiol 10:1918. https://doi.org/10.3389/fmicb.2019.01918.
126. Ding CH, Situ SF, Steven A, Razak MFA. 2019. The pitfall of utilizing a commercial biochemical yeast identification kit to detect *Candida auris*. Ann Clin Lab Sci 49:546–549.
127. Sexton DJ, Kordalewska M, Bentz ML, Welsh RM, Perlin DS, Litvintseva AP. 2018. Direct detection of emergent fungal pathogen *Candida auris* in clinical skin swabs by SYBR Green-based quantitative PCR assay. J Clin Microbiol 56:e01337-18. https://doi.org/10.1128/JCM.01337-18.
128. Leach L, Zhu Y, Chaturvedi S. 2017. Development and validation of a real-time PCR assay for rapid detection of *Candida auris* from surveillance samples. J Clin Microbiol 56:e01223-17. https://doi.org/10.1128/JCM.01223-17.
129. Ruiz-Gaitan AC, Fernandez-Pereira J, Valentin E, Tormo-Mas MA, Eraso E, Peman J, de Groot PWJ. 2018. Molecular identification of *Candida auris* by PCR amplification of species-specific GPI protein-encoding genes. Int J Med Microbiol 308:812–818. https://doi.org/10.1016/j.ijmm.2018.06.014.
130. Kordalewska M, Zhao Y, Lockhart SR, Chowdhary A, Berrio I, Perlin DS. 2017. Rapid and accurate molecular identification of the emerging multidrug-resistant pathogen *Candida auris*. J Clin Microbiol 55:2445–2452. https://doi.org/10.1128/JCM.00630-17.
131. Leach L, Russell A, Zhu Y, Chaturvedi S, Chaturvedi V. 2019. 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 57:e00630-19. https://doi.org/10.1128/JCM.00630-19.
132. Lima A, Widen R, Vestal G, Uy D, Silbert S. 2019. 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 57:e01604-18. https://doi.org/10.1128/JCM.01604-18.
133. Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O, European Confederation of Medical Mycology. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare

invasive yeast infections. Clin Microbiol Infect 20(Suppl 3):76–98. https://doi.org/10.1111/1469-0691.12360.
134. Chagas-Neto TC, Chaves GM, Melo AS, Colombo AL. 2009. Bloodstream infections due to *Trichosporon* spp.: species distribution, *Trichosporon asahii* genotypes determined on the basis of ribosomal DNA intergenic spacer 1 sequencing, and antifungal susceptibility testing. J Clin Microbiol 47:1074–1081. https://doi.org/10.1128/JCM.01614-08.
135. De Almeida GM, Costa SF, Melhem M, Motta AL, Szeszs MW, Miyashita F, Pierrotti LC, Rossi F, Burattini MN. 2008. *Rhodotorula* spp. isolated from blood cultures: clinical and microbiological aspects. Med Mycol 46:547–556. https://doi.org/10.1080/13693780801972490.
136. Rhimi W, Theelen B, Boekhout T, Otranto D, Cafarchia C. 2020. *Malassezia* spp. yeasts of emerging concern in fungemia. Front Cell Infect Microbiol 10:370. https://doi.org/10.3389/fcimb.2020.00370.
137. Buchta V, Bolehovská R, Hovorková E, Cornely OA, Seidel D, Žák P. 2019. *Saprochaete clavata* invasive infections: a new threat to hematological-oncological patients. Front Microbiol 10:2196. https://doi.org/10.3389/fmicb.2019.02196.
138. Bidart M, Bonnet I, Hennebique A, Kherraf ZE, Pelloux H, Berger F, Cornet M, Bailly S, Maubon D. 2015. An in-house assay is superior to Sepsityper for direct matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry identification of yeast species in blood cultures. J Clin Microbiol 53:1761–1764. https://doi.org/10.1128/JCM.03600-14.
139. Tortorano AM, Richardson M, Roilides E, van Diepeningen A, Caira M, Munoz P, Johnson E, Meletiadis J, Pana ZD, Lackner M, Verweij P, Freiberger T, Cornely OA, Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, Lanternier F, Pagano L, Skiada A, Akova M, Arendrup MC, Boekhout T, Chowdhary A, Cuenca-Estrella M, Guinea J, Guarro J, de Hoog S, Hope W, Kathuria S, Lortholary O, Meis JF, Ullmann AJ, Petrikkos G, Lass-Flörl C. 2014. ESCMID and ECMM joint guidelines on diagnosis and management of hyalohyphomycosis: *Fusarium* spp., *Scedosporium* spp. and others. Clin Microbiol Infect 20(Suppl 3):27–46. https://doi.org/10.1111/1469-0691.12465.
140. Nucci F, Nouér SA, Capone D, Nucci M. 2018. Invasive mould disease in haematologic patients: comparison between fusariosis and aspergillosis. Clin Microbiol Infect 24:1105.e1–1105.e4. https://doi.org/10.1016/j.cmi.2018.05.006.
141. Nucci M, Carlesse F, Cappellano P, Varon AG, Seber A, Garnica M, Nouér SA, Colombo AL. 2014. Earlier diagnosis of invasive fusariosis with *Aspergillus* serum galactomannan testing. PLoS One 9:e87784. https://doi.org/10.1371/journal.pone.0087784.
142. Sanguinetti M, Posteraro B. 2017. Identification of molds by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 55:369–379. https://doi.org/10.1128/JCM.01640-16.
143. Triest D, Stubbe D, De Cremer K, Piérard D, Normand AC, Piarroux R, Detandt M, Hendrickx M. 2015. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of molds of the *Fusarium* genus. J Clin Microbiol 53:465–476. https://doi.org/10.1128/JCM.02213-14.
144. CLSI. 2018. Interpretive criteria for identification of bacteria and fungi by targeted DNA sequencing. MM18, 2nd ed. Clinical and Laboratory Standards Institute, Wayne, PA.
145. Cortez KJ, Roilides E, Quiroz-Telles F, Meletiadis J, Antachopoulos C, Knudsen T, Buchanan W, Milanovich J, Sutton DA, Fothergill A, Rinaldi MG, Shea YR, Zaoutis T, Kottilil S, Walsh TJ. 2008. Infections caused by *Scedosporium* spp. Clin Microbiol Rev 21:157–197. https://doi.org/10.1128/CMR.00039-07.
146. Sangoi AR, Rogers WM, Longacre TA, Montoya JG, Baron EJ, Banaei N. 2009. Challenges and pitfalls of morphologic identification of fungal infections in histologic and cytologic specimens: a ten-year retrospective review at a single institution. Am J Clin Pathol 131:364–375. https://doi.org/10.1309/AJCP99OOOZSNISCZ.
147. Gomez CA, Budvytiene I, Zemek AJ, Banaei N. 2017. Performance of targeted fungal sequencing for culture-independent diagnosis of invasive fungal disease. Clin Infect Dis 65:2035–2041. https://doi.org/10.1093/cid/cix728.
148. Wiederhold NP. 2017. Antifungal resistance: current trends and future strategies to combat. Infect Drug Resist 10:249–259. https://doi.org/10.2147/IDR.S124918.
149. Guevara-Suarez M, Sutton DA, Cano-Lira JF, García D, Martin-Vicente A, Wiederhold N, Guarro J, Gené J. 2016. Identification and antifungal susceptibility of penicillium-like fungi from clinical samples in the United States. J Clin Microbiol 54:2155–2161. https://doi.org/10.1128/JCM.00960-16.
150. Jacobs SE, Wengenack NL, Walsh TJ. 2020. Non-aspergillus hyaline molds: emerging causes of sino-pulmonary fungal infections and other invasive mycoses. Semin Respir Crit Care Med 41:115–130. https://doi.org/10.1055/s-0039-3401989.
151. Revankar SG, Sutton DA. 2010. Melanized fungi in human disease. Clin Microbiol Rev 23:884–928. https://doi.org/10.1128/CMR.00019-10.
152. Weitzman I, Summerbell RC. 1995. The dermatophytes. Clin Microbiol Rev 8:240–259. https://doi.org/10.1128/CMR.8.2.240-259.1995.
153. Saccente M, Woods GL. 2010. Clinical and laboratory update on blastomycosis. Clin Microbiol Rev 23:367–381. https://doi.org/10.1128/CMR.00056-09.
154. Chen SC, Meyer W, Sorrell TC. 2014. *Cryptococcus gattii* infections. Clin Microbiol Rev 27:980–1024. https://doi.org/10.1128/CMR.00126-13.
155. Berkow EL, Nunnally NS, Bandea A, Kuykendall R, Beer K, Lockhart SR. 2018. Detection of TR(34)/L98H *CYP51A* mutation through passive surveillance for azole-resistant *Aspergillus fumigatus* in the United States from 2015 to 2017. Antimicrob Agents Chemother 62:e02240-17. https://doi.org/10.1128/AAC.02240-17.
156. Novak-Frazer L, Anees-Hill SP, Hassan D, Masania R, Moore CB, Richardson MD, Denning DW, Rautemaa-Richardson R. 2020. Deciphering *Aspergillus fumigatus CYP51A*-mediated triazole resistance by pyrosequencing of respiratory specimens. J Antimicrob Chemother 75:3501–3509. https://doi.org/10.1093/jac/dkaa357.
157. Muñoz-Cadavid C, Rudd S, Zaki SR, Patel M, Moser SA, Brandt ME, Gómez BL. 2010. Improving molecular detection of fungal DNA in formalin-fixed paraffin-embedded tissues: comparison of five tissue DNA extraction methods using panfungal PCR. J Clin Microbiol 48:2147–2153. https://doi.org/10.1128/JCM.00459-10.
158. Raja HA, Miller AN, Pearce CJ, Oberlies NH. 2017. Fungal identification using molecular tools: a primer for the natural products research community. J Nat Prod 80:756–770. https://doi.org/10.1021/acs.jnatprod.6b01085.
159. Larkin PMK, Lawson KL, Contreras DA, Le CQ, Trejo M, Realegeno S, Hilt EE, Chandrasekaran S, Garner OB, Fishbein GA, Yang S. 2020. Amplicon-based next-generation sequencing for detection of fungi in formalin-fixed, paraffin-embedded tissues: correlation with histopathology and clinical applications. J Mol Diagn 22:1287–1293. https://doi.org/10.1016/j.jmoldx.2020.06.017.
160. Lau AF, Walchak RC, Miller HB, Slechta ES, Kamboj K, Riebe K, Robertson AE, Gilbreath JJ, Mitchell KF, Wallace MA, Bryson AL, Balada-Llasat JM, Bulman A, Buchan BW, Burnham CD, Butler-Wu S, Desai U, Doern CD, Hanson KE, Henderson CM, Kostrzewa M, Ledeboer NA, Maier T, Pancholi P, Schuetz AN, Shi G, Wengenack NL, Zhang SX, Zelazny AM, Frank KM. 2019. Multicenter study demonstrates standardization requirements for mold identification by MALDI-TOF MS. Front Microbiol 10:2098. https://doi.org/10.3389/fmicb.2019.02098.
161. Wilkendorf LS, Bowles E, Buil JB, van der Lee HAL, Posteraro B, Sanguinetti M, Verweij PE. 2020. Update on matrix-assisted laser desorption ionization–time of flight mass spectrometry identification of filamentous fungi. J Clin Microbiol 58:e01263-20. https://doi.org/10.1128/JCM.01263-20.
162. Rychert J, Slechta ES, Barker AP, Miranda E, Babady NE, Tang YW, Gibas C, Wiederhold N, Sutton D, Hanson KE. 2017. Multicenter evaluation of the Vitek MS v3.0 system for the identification of filamentous fungi. J Clin Microbiol 56:e01353-17. https://doi.org/10.1128/JCM.01353-17.
163. McMullen AR, Wallace MA, Pincus DH, Wilkey K, Burnham CA. 2016. Evaluation of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of clinically relevant filamentous fungi. J Clin Microbiol 54:2068–2073. https://doi.org/10.1128/JCM.00825-16.
164. Lau AF, Drake SK, Calhoun LB, Henderson CM, Zelazny AM. 2013. Development of a clinically comprehensive database and a simple procedure for identification of molds from solid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 51:828–834. https://doi.org/10.1128/JCM.02852-12.
165. Becker PT, de Bel A, Martiny D, Ranque S, Piarroux R, Cassagne C, Detandt M, Hendrickx M. 2014. Identification of filamentous fungi isolates by MALDI-TOF mass spectrometry: clinical evaluation of an extended reference spectra library. Med Mycol 52:826–834. https://doi.org/10.1093/mmy/myu064.
166. Gautier M, Ranque S, Normand AC, Becker P, Packeu A, Cassagne C, L'Ollivier C, Hendrickx M, Piarroux R. 2014. Matrix-assisted laser

desorption ionization time-of-flight mass spectrometry: revolutionizing clinical laboratory diagnosis of mould infections. Clin Microbiol Infect 20:1366–1371. https://doi.org/10.1111/1469-0691.12750.
167. Lionakis MS, Bodey GP, Tarrand JJ, Raad II, Kontoyiannis DP. 2004. The significance of blood cultures positive for emerging saprophytic moulds in cancer patients. Clin Microbiol Infect 10:922–925. https://doi.org/10.1111/j.1469-0691.2004.00933.x.
168. Nucci M, Anaissie E. 2007. Fusarium infections in immunocompromised patients. Clin Microbiol Rev 20:695–704. https://doi.org/10.1128/CMR.00014-07.
169. Azar MM, Hage CA. 2017. Laboratory diagnostics for histoplasmosis. J Clin Microbiol 55:1612–1620. https://doi.org/10.1128/JCM.02430-16.
170. Vetter E, Torgerson C, Feuker A, Hughes J, Harmsen S, Schleck C, Horstmeier C, Roberts G, Cockerill F, III. 2001. Comparison of the BACTEC MYCO/F lytic bottle to the isolator tube, BACTEC Plus Aerobic F/bottle, and BACTEC Anaerobic Lytic/10 bottle and comparison of the BACTEC Plus Aerobic F/bottle to the Isolator tube for recovery of bacteria, mycobacteria, and fungi from blood. J Clin Microbiol 39:4380–4386. https://doi.org/10.1128/JCM.39.12.4380-4386.2001.
171. Campigotto A, Richardson SE, Sebert M, McElvania TeKippe E, Chakravarty A, Doern CD. 2016. Low utility of pediatric isolator blood culture system for detection of fungemia in children: a 10-year review. J Clin Microbiol 54:2284–2287. https://doi.org/10.1128/JCM.00578-16.
172. Armstrong AE, Rossoff J, Hollemon D, Hong DK, Muller WJ, Chaudhury S. 2019. Cell-free DNA next-generation sequencing successfully detects infectious pathogens in pediatric oncology and hematopoietic stem cell transplant patients at risk for invasive fungal disease. Pediatr Blood Cancer 66:e27734. https://doi.org/10.1002/pbc.27734.
173. Hong DK, Blauwkamp TA, Kertesz M, Bercovici S, Truong C, Banaei N. 2018. Liquid biopsy for infectious diseases: sequencing of cell-free plasma to detect pathogen DNA in patients with invasive fungal disease. Diagn Microbiol Infect Dis 92:210–213. https://doi.org/10.1016/j.diagmicrobio.2018.06.009.
174. Jarvis JN, Percival A, Bauman S, Pelfrey J, Meintjes G, Williams GN, Longley N, Harrison TS, Kozel TR. 2011. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. Clin Infect Dis 53:1019–1023. https://doi.org/10.1093/cid/cir613.
175. Kabanda T, Siedner MJ, Klausner JD, Muzoora C, Boulware DR. 2014. Point-of-care diagnosis and prognostication of cryptococcal meningitis with the cryptococcal antigen lateral flow assay on cerebrospinal fluid. Clin Infect Dis 58:113–116. https://doi.org/10.1093/cid/cit641.
176. Mercier T, Dunbar A, de Kort E, Schauwvlieghe A, Reynders M, Guldentops E, Blijlevens NMA, Vonk AG, Rijnders B, Verweij PE, Lagrou K, Maertens J. 2020. Lateral flow assays for diagnosing invasive pulmonary aspergillosis in adult hematology patients: a comparative multicenter study. Med Mycol 58:444–452. https://doi.org/10.1093/mmy/myz079.
177. Jenks JD, Prattes J, Frank J, Spiess B, Mehta SR, Boch T, Buchheidt D, Hoenigl M. 2020. Performance of the bronchoalveolar lavage fluid aspergillus galactomannan lateral flow assay with cube reader for diagnosis of invasive pulmonary aspergillosis: a multicenter cohort study. Clin Infect Dis https://doi.org/10.1093/cid/ciaa1281.
178. Cáceres DH, Gómez BL, Tobón AM, Chiller TM, Lindsley MD. 2020. Evaluation of a Histoplasma antigen lateral flow assay for the rapid diagnosis of progressive disseminated histoplasmosis in Colombian patients with AIDS. Mycoses 63:139–144. https://doi.org/10.1111/myc.13023.
179. Donovan FM, Ramadan FA, Khan SA, Bhaskara A, Lainhart WD, Narang AT, Mosier JM, Ellingson KD, Bedrick EJ, Saubolle MA, Galgiani JN. 2020. Comparison of a novel rapid lateral flow assay to enzyme immunoassay results for early diagnosis of coccidioidomycosis. Clin Infect Dis https://doi.org/10.1093/cid/ciaa1205.
180. Tracy MC, Moss RB. 2018. The myriad challenges of respiratory fungal infection in cystic fibrosis. Pediatr Pulmonol 53:S75–S85. https://doi.org/10.1002/ppul.24126.
181. CFF. 2019. 2019 patient registry snapshot. Cystic Fibrosis Foundation, Bethesda, MD. https://www.cff.org/Research/Researcher-Resources/Patient-Registry/2019-Cystic-Fibrosis-Foundation-Patient-Registry-Snapshot/.
182. Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, Howenstine M, McColley SA, Rock M, Rosenfeld M, Sermet-Gaudelus I, Southern KW, Marshall BC, Sosnay PR. 2017. Diagnosis of cystic fibrosis: consensus guidelines from the cystic fibrosis foundation. J Pediatr 181S:S4–S15. https://doi.org/10.1016/j.jpeds.2016.09.064.
183. Hong G, Alby K, Ng SCW, Fleck V, Kubrak C, Rubenstein RC, Dorgan DJ, Kawut SM, Hadjiliadis D. 2020. The presence of *Aspergillus fumigatus* is associated with worse respiratory quality of life in cystic fibrosis. J Cyst Fibros 19:125–130. https://doi.org/10.1016/j.jcf.2019.08.008.
184. Engel TGP, Slabbers L, de Jong C, Melchers WJG, Hagen F, Verweij PE, Merkus P, Meis JF, Dutch Cystic Fibrosis Fungal Collection Consortium. 2019. Prevalence and diversity of filamentous fungi in the airways of cystic fibrosis patients: a Dutch, multicentre study. J Cyst Fibros 18:221–226. https://doi.org/10.1016/j.jcf.2018.11.012.
185. Blanchard AC, Waters VJ. 2019. Microbiology of cystic fibrosis airway disease. Semin Respir Crit Care Med 40:727–736. https://doi.org/10.1055/s-0039-1698464.
186. Hong G, Miller HB, Allgood S, Lee R, Lechtzin N, Zhang SX. 2017. Use of selective fungal culture media increases rates of detection of fungi in the respiratory tract of cystic fibrosis patients. J Clin Microbiol 55:1122–1130. https://doi.org/10.1128/JCM.02182-16.
187. Engel TGP, Tehupeiory-Kooreman M, Melchers WJG, Reijers MH, Merkus P, Verweij PE. 2020. Evaluation of a new culture protocol for enhancing fungal detection rates in respiratory samples of cystic fibrosis patients. J Fungi (Basel) 6:82. https://doi.org/10.3390/jof6020082.
188. Delhaes L, Touati K, Faure-Cognet O, Cornet M, Botterel F, Dannaoui E, Morio F, Le Pape P, Grenouillet F, Favennec L, Le Gal S, Nevez G, Duhamel A, Borman A, Saegeman V, Lagrou K, Gomez E, Carro ML, Canton R, Campana S, Buzina W, Chen S, Meyer W, Roilides E, Simitsopoulou M, Manso E, Cariani L, Biffi A, Fiscarelli E, Ricciotti G, Pihet M, Bouchara JP. 2019. Prevalence, geographic risk factor, and development of a standardized protocol for fungal isolation in cystic fibrosis: results from the international prospective study "MFIP." J Cyst Fibros 18:212–220. https://doi.org/10.1016/j.jcf.2018.10.001.
189. Schwarz C, Vandeputte P, Rougeron A, Giraud S, Dugé de Bernonville T, Duvaux L, Gastebois A, Alastruey-Izquierdo A, Martín-Gomez MT, Mazuelos EM, Sole A, Cano J, Pemán J, Quindos G, Botterel F, Bougnoux ME, Chen S, Delhaès L, Favennec L, Ranque S, Sedlacek L, Steinmann J, Vazquez J, Williams C, Meyer W, Le Gal S, Nevez G, Fleury M, Papon N, Symoens F, Bouchara JP, ECMM/ISHAM Working Group Fungal Respiratory Infections in Cystic Fibrosis (Fri-CF). 2018. Developing collaborative works for faster progress on fungal respiratory infections in cystic fibrosis. Med Mycol 56:42–59. https://doi.org/10.1093/mmy/myx106.
190. Hendrickson JA, Hu C, Aitken SL, Beyda N. 2019. Antifungal resistance: a concerning trend for the present and future. Curr Infect Dis Rep 21:47. https://doi.org/10.1007/s11908-019-0702-9.
191. CLSI. 2020. Performance standards for antifungal susceptibility testing of filamentous fungi. Approved guideline; CLSI document M61Ed2. Clinical and Laboratory Standards Institute, Wayne, PA.
192. CLSI. 2020. Performance standards for antifungal susceptibility testing of yeasts. Approved guideline; CLSI document M60ed2. Clinical and Laboratory Standards Institute, Wayne, PA.
193. Lockhart SR. 2019. *Candida auris* and multidrug resistance: defining the new normal. Fungal Genet Biol 131:103243. https://doi.org/10.1016/j.fgb.2019.103243.
194. CLSI. 2018. Epidemiologic cutoff values for antifungal susceptibility testing. Approved guideline; CLSI document M59Ed2. Clinical and Laboratory Standards Institute, Wayne, PA.
195. Berkow EL, Lockhart SR, Ostrosky-Zeichner L. 2020. Antifungal susceptibility testing: current approaches. Clin Microbiol Rev 33:e00069-19.
196. Perfect JR. 2017. The antifungal pipeline: a reality check. Nat Rev Drug Discov 16:603–616. https://doi.org/10.1038/nrd.2017.46.