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REVIEW ARTICLE

An Accuracy-Based Approach to the Microbiologic Diagnosis of Pulmonary Infection: Part II

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Abstract

Introduction: Similar to that of bacterial infection, the performance of diagnostic tests for endemic fungal pneumonia and opportunistic fungal pneumonia are uncertain.

Methods: We undertook a literature search to assess the accuracy of diagnostic tests for pneumonia, identified through a search of MEDLINE-indexed journals. Sensitivity and specificity of diagnostic tests for pneumonia were calculated with respect to various reference standards.

Results: A combination of diagnostic testing is adequate to rule out most endemic fungal pneumonia, *Cryptococcus*, and *Candida.* Testing is inadequate to exclude *Pneumocystis*, *Aspergillus*, and *Mucorales*, and empiric treatment should be considered when there is clinical suspicion.

Conclusion: The accuracy of any single diagnostic test for either endemic fungal pneumonia or invasive fungal pneumonia is generally inadequate to rule out a pathogen. Multiple diagnostic methods are often needed to confidently establish a microbiologic diagnosis for most cases of invasive fungal infection.

Keywords

Pneumonia, Diagnosis, Accuracy

Introduction

Clinicians often face uncertainty with respect to the accuracy of diagnostic laboratory tests for common endemic fungi present in various regions of the world. Additionally, with the growing use of immunosuppressive medications and chemotherapy, clinicians must be familiar with the laboratory tests needed to diagnose disease caused by opportunistic invasive fungal infection (IFI).

In Part 2 of this review, we will assess the literature and provide the accuracy of diagnostic tests for infectious pneumonia caused by fungal pathogens.

Endemic Fungal Pneumonia

Coccidioides immitis

Coccidioides immitis is a large dimorphic fungus, endemic to the southwestern United States and parts of South America, transmitted through inhalation of fungal spores.

The sensitivity of respiratory secretions culture for *Coccidioides* has been reported at 80% [1] of PCR-positive cases, with a specificity of 100% [1]. The sensitivity of culture for cases that are diagnosed clinically or serologically is uncertain, as most diagnoses are made by culture itself.

BALF cytology sensitivity has been reported at 35% [2] of culture-positive cases, and is not known to provide additional yield to culture. Transbronchial biopsy sensitivity for this population has been reported at 100% [2], but similarly may not provide additional information while increasing the procedural risk.

Various antigens have been used to diagnose *Coccidioides*, including the cell wall polysaccharide

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galactomannan (GM). The *Coccidioides* GM EIA UAT sensitivity has been reported at 83% [3] of culture-positive cases, but reduces to 50% [4] and 68% [3] of cases diagnosed by either culture or ID serology, with a specificity of 98% [3]. The UAT cross-reacts with other endemic fungi such as *Histoplasma* and *Blastomyces*, limiting its value [3]. The UAT may detect *Coccidioides* in serologically-negative cases by ELISA, although it is not certain if this provides additional sensitivity to the serologic combination of ELISA, CF, and ID [3]. Antigenemia sensitivity among culture-positive or serologically detected cases has been reported at 73% [4], adding up to an additional 17% [4] sensitivity to that of the urine antigen alone. Conversely, the UAT adds little sensitivity to the serum antigen detection, and is thus not recommended.

Serology may complement IS or BALF culture. Among culture-positive or histopathology-proven cases, ELISA IgG and IgM serology sensitivity has been reported at 67% [5], but paradoxically in cases detected by either culture, histopathology, CF, or ID has been reported at 83% [6] and 84% [7]. The specificity has been reported at 90% [6] and 95% [7], but titers may remain elevated for months. CF sensitivity has been reported at 58% [8], 63% [3], and 67% [5] of cases diagnosed by either culture, serology, or histopathology, and is not certain to add cases to those detected by ELISA [9]. ID sensitivity has been reported at 53% [5] and 54% [3] of cases diagnosed by either culture, serology, or histopathology. The combination of ELISA, CF, and ID can increase sensitivity to as high as 84% [5] in immunocompromised patients, and as high as 95% [5] in those without an immunocompromising condition. ELISA, CF, and ID are all commercially available, and as the combination of serologic methods can increase sensitivity, at a minimum the ELISA and ID methods should be used concurrently.

Among culture-positive cases, the sensitivity of PCR in respiratory secretions has been reported at 100% [1], with a specificity of 98% to 100% [1]. PCR of fresh tissue sensitivity has been reported at 93% [1] and 94% [10] of culture-positive cases, with a specificity of 98% [10] and 100% [1]. The sensitivity reduces in paraffin-embedded tissue, however, to 73% [1]. The role of PCR is uncertain, but should be considered if culture and serology are negative with a high clinical suspicion.

Blastomyces dermatitidis

Blastomyces dermatitidis is an endemic dimorphic fungus of the midwestern and eastern United States, Africa, and India, transmitted through inhalation of spores.

BALF culture sensitivity has been reported at 67% [11] of cases with a positive culture by any means. This value is likely overestimated due to clinical under diagnoses by alternative methods such as serology or antigen detection. For example, among pathologically confirmed cases, culture sensitivity has been reported at only 57% [12], 66% [13], and 67% [13]. IS culture is less invasive than obtaining BALF, and may even have a higher sensitivity than BALF culture [11]. Colonization is not known to occur and thus any positive culture should be considered infectious with a specificity approaching 100% [11].

Transbronchial biopsy is diagnostic of *Blastomyces* in only 22% [11] of culture-positive cases, but can add up to 20% additional yield [14] to culture alone, although the procedure carries an increased risk over bronchoalveolar lavage.

The *Blastomyces* UAT has been detected in 90% [15] and 93% [16] of culture-positive or histologically-proven cases, with a specificity of 79% [16], since cross-reaction may occur with other endemic fungi such as *Histoplasma*, *Cryptococcus*, and *Paracoccidioidomycosis*. The specificity has been reported as high as 99% [15] in healthy individuals without alternative fungal infection. Antigen detection in either culture-positive or histology-proven pulmonary disease can be found in urine, serum, and BALF with reported sensitivities of 83% [17] and 100% [16] for urine, 64% [17] for serum, and 63% [17] for BALF. Skin testing is infrequently performed, although among clinically diagnosed cases sensitivity has been reported at 73% [18]. Similarly, lymphocyte transformation assays are infrequently used because the sensitivity has been reported at only 68% [19] of cases positive by either serology, culture, or skin testing. Theses assays do not appear to detect cases which are not already found by more traditional methods.

Serologic methods have been utilized to detect the A-antigen and surface protein *Blastomyces* adhesion (BAD-1). Among culture-positive cases of blastomycosis, EIA sensitivity has been reported at 77% [18], 80% [19], 83% [20], 88% [21,22]. The sensitivity has been reported higher in clinically suspected disease at 97% [18], but this likely overestimates the true sensitivity, which is presumably closer to the sensitivity estimated from culture-positive cases. Its specificity has been reported at 92% [18] and 94% [21], being reduced due to cross reaction with other endemic fungi [23]. ID appears to be less sensitive than EIA, and among clinically diagnosed cases has been reported at only 50% [18]. Similarly, among culture-positive cases, the sensitivity of ID has been reported at only 15% [21], 28% [18], 40% [11], and 65% [19], with a specificity approaching 100% [18]. CF sensitivity has been reported at only 15% [18] of clinically diagnosed cases, while among culture-positive cases the sensitivity has been reported as low as 9% [18], 16% [24], 40% [19], and 43% [22], although with specificity approaching 100% [18]. Serology by EIA, CF, and ID are all commercially available and should be used together to maximize diagnostic yield.

Among culture-positive cases, the sensitivity of *Blastomyces* PCR in tissue and respiratory secretions has been reported at 83% [25] and 86% [26], respectively, with a specificity reported at 93% [26] and 100% [25]. PCR is commercially available, although it is unclear if it provides additional benefit over the combination of culture and serology. Although promising as a complementary diagnostic test to serology, the role of PCR as a diagnostic tool is uncertain.

Histoplasma capsulatum

Histoplasma capsulatum is a small dimorphic fungus, most commonly found in midwestern United States, Central America, South America, southern Africa, and Southeast Asia, transmitted by inhalation of spores.

The sensitivity of respiratory culture varies by the duration of symptoms, and increases from acute to chronic stage of disease. The sensitivity is also higher in cavitary disease than in non-cavitary disease [27]. Culture sensitivity in the subacute phase of infection has been reported at 54% [28], 61% [29] and 78% [30] of UAT-positive cases, 83% [31] of clinically diagnosed cases, and 89% [29] of cases positive by histopathology, culture, or serology. It may also be positive in instances of a negative urine antigen, and is thus complementary. Fungal growth may require up to four weeks, however, limiting its usefulness in the acute or subacute phases of infection. Nevertheless, while BALF culture is frequently negative in the acute phase [27,28], its sensitivity has been reported to increase to 67% [28] in the chronic phase of cases diagnosed by either culture or serology. Sputum culture sensitivity may be slightly better than BALF in the acute phase and has been reported at 18% [27] of cases positive by either culture, cytology, histopathology, CF, or ID.

BALF cytology sensitivity has been reported at 55% [31] and 70% [32] of clinically diagnosed cases. The sensitivity reduces, however, to 19% [27] of cases detected by either culture, cytology, histopathology, CF, or ID. Tissue pathology is diagnostic in 67% [28] of culture-positive or serologically-diagnosed cases, but like culture is frequently negative in the acute phase [28].

The pooled sensitivity of antigenuria and antigenemia detection by ELISA and radioimmunoassay (RIA) methods among cases detected by either culture, serology, or PCR have been reported at 79% [33] and 82% [33], respectively, with serum and urine specificity of 97% [33] and 99% [33]. The *Histoplasma* UAT sensitivity varies by the stage of disease and can be reduced to as low as 39% [28] in the subacute phase before rising to over 80% [28] in cases of chronic infection found to be positive by either culture, cytology, histopathology, CF, or ID. BALF *Histoplasma* antigen sensitivity among culture-positive cases has been reported at 50% [30], 70% [32], and 84% [34], but has poor specificity due to cross-reactivity with *Blastomyces*.
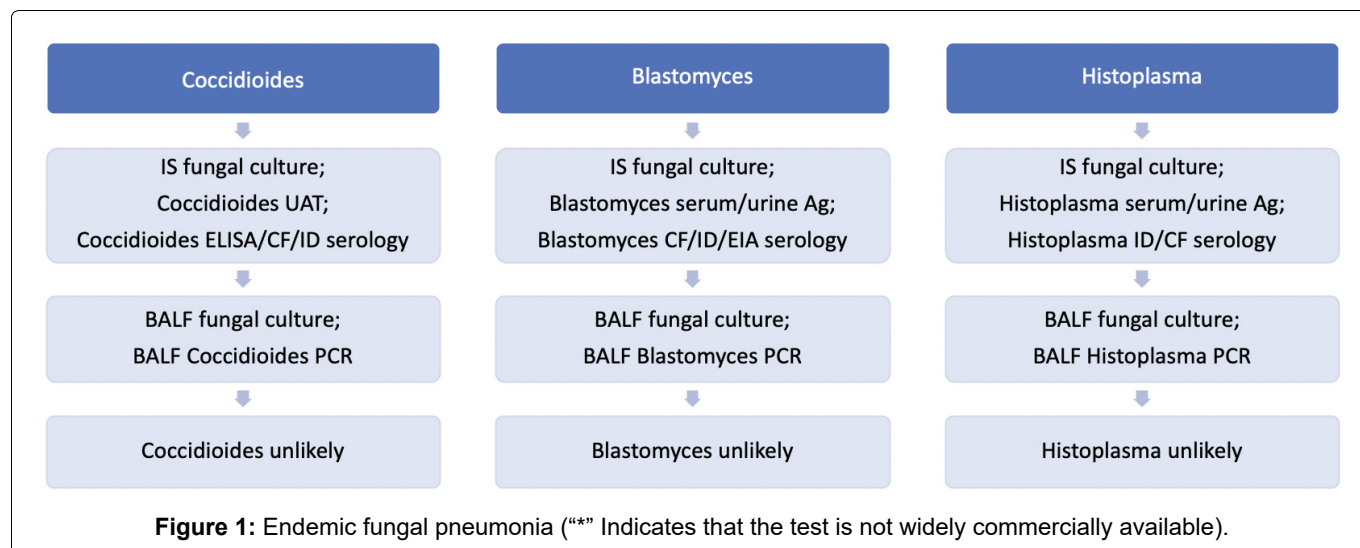
Serology can be used to detect the *Histoplasma* H and M antigens. ID serology has been reported positive in 17% [35] of cases diagnosed either clinically or by culture, and 25% [31], 55% [35], and 94% [36] of those with a confirmatory positive antigen, serology, or culture. ID sensitivity has been reported at 87% [27] of CF-positive cases, and appears to add minimal additional yield to CF alone [27], with a specificity of 96% [27] and 100% [31]. Among clinically diagnosed cases of histoplasmosis, CF sensitivity has been reported at 64% [35] and 91% [37], and among those diagnosed by either by culture or a positive antigen has been reported at 73% [38] and 87% [36]. Specificity is also poor due to cross-reaction with other endemic fungi [27], and titers may persist for months to years. CF and ID methods may be complementary. For example, the combination of CF and ID increases sensitivity to a reported 82% [28] of cases detected by either culture, cytology, or histopathology, and to 96% [27] of cases detected by any method of culture, cytology, histopathology, CF, or ID. Both CF and ID serology are widely commercially available. Although ID is not certain to provide additional sensitivity to CF, it may yield a result more rapidly than CF. Negative serology does not rule out infection, however, and PCR or tissue diagnosis should be considered if there is persistent clinical suspicion.

The sensitivity of PCR in respiratory secretions has been reported at 73% [26] of culture-positive cases, with a specificity of 100% [26]. The addition of culture to PCR can increase the sensitivity by up to an additional 30% [31], so multiple diagnostic modalities should be considered. Serum PCR sensitivity has been reported at 60% [31] and 77% [39] of culture-positive cases, with a specificity of 90% [31], while the sensitivity of bone marrow PCR in disseminated disease has been reported at 92% [40]. *Histoplasma* PCR is commercially available although uncommonly utilized. Although the diagnostic accuracy in BALF is uncertain, it should be considered whenever serology is negative.

LAMP is an alternative to PCR and among culture-positive cases the sensitivity has been reported in bone marrow and urine at 54% [40] and 67% [41], respectively. LAMP however, is neither widely commercially available nor well-studied in BALF specimens (Figure 1).

Opportunistic Fungal Pneumonia

The European Organization of the Research and Treatment of Cancer/Mycoses Study Group (EORTC) criteria are often used to support the diagnosis of invasive fungal infection (IFI). Proven invasive disease is defined by evidence of tissue damage with a positive culture. Probable disease is defined by the presence of underlying host factors, clinical criteria, and mycological criteria.

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Figure 1: Endemic fungal pneumonia ("*" Indicates that the test is not widely commercially available).

Cryptococcus (neoformans, gattii)

Cryptococcus species are encapsulated yeast that can lead to pneumonia following inhalation of fungal spores. Risk factors for infection include HIV infection, solid organ or bone marrow transplantation, diabetes mellitus, chronic glucocorticoid use, malignancy, and other immunosuppressive medication use [42].

The pooled sensitivity of BALF culture has been reported at 60% [43] of histologically-proven or cytology-proven cases. Of these histologically proven cases, tissue culture has been reported positive in a wide range at 0% [44] and 71% [45]. BALF culture sensitivity has been reported at 93% [46] of cases in which the serum *Cryptococcus* antigen (CryAg) is detected, but is reduced to 63% [44] when compared to cases found to be positive by either the CryAg or biopsy. Colonization with *Cryptococcus* is common in the absence of pneumonia, and thus specificity for infection is low.

The sensitivity of cytology has been reported at only 47% [47] of culture-positive cases, but cytologic examination of BALF may be positive in up to 20% [44] of culture-negative clinically diagnosed cases. Transbronchial biopsies have been reported positive in 75% [44] of culture-positive cases, but can also detect up to 20% [44] of culture-negative infections. Therefore, tissue sampling may be considered concurrently with BALF culture. Any added benefit of transbronchial biopsies over the combination of culture, CryAg, and PCR, however, is uncertain.

BALF CryAg can be detected using latex agglutination (LAT), EIA, or lateral flow assay (LFA) methods. The sensitivity of BALF CryAg in clinically diagnosed cases has been reported at 93% [48]. Among culture-positive cases, BALF CryAg sensitivity has been reported at 71% [49], 80% [50], 83% [51], and 100% [52,53], with a specificity of 97% [54] and 99% [49,50], illustrating its ability to detect cases missed by culture alone. The CryAg can also be detected in tissue with a reported sensitivity of 100% [54] of cases positive by either histopathology

or culture. Serum CryAg sensitivity is similar to that of BALF and has been reported at 56% [55], 74% [51], 75% [48], 93% [52], and 96% [45] of cases positive by either histopathology or culture, with a specificity of 99% [51]. Serum CryAg should be considered concurrently with IS or BALF culture. The pooled urine CryAg sensitivity is similar to serum at 85% [56], but infrequently utilized and not generally recommended as it is uncertain whether it adds additional cases to serum alone.

Serology is neither commercially available nor well-studied. IF sensitivity has been reported at 20% [57] of culture-positive cases, but with such low sensitivity lacks a role in diagnosis.

BALF PCR sensitivity has been reported at 80% [58] and 100% [59,60] of culture-positive cases. It is also uncertain whether PCR provides additional sensitivity to that of BALF culture and CryAg alone. All the same, the addition of PCR may be considered if there is high clinical suspicion.

Aspergillus (fumigatus, flavus, terreus, niger, and others)

Aspergillus species are narrow septate hyphae molds that lead to pneumonia in patients with immunocompromising conditions. Invasive aspergillosis (IA) is a form of severe opportunistic pneumonia distinct from chronic necrotizing pulmonary aspergillosis (CPA), aspergilloma, or allergic bronchopulmonary aspergillosis (ABPA). Risk factors for invasive aspergillosis include neutropenia, hematologic malignancy, allogeneic stem cell transplant, solid organ transplantation, prolonged glucocorticoid use, immunosuppressive medication use, and inherited immunodeficiency syndromes [61].

Although *Aspergillus* grows rapidly, the sensitivity of BALF culture for IA is poor, and has been reported in 10% [62], 25% [63], 30% [64], 33% [65], 36% [66], 50% [67], and 56% [68] of cases meeting proven or probable EORTC criteria. Among patients with histopathologically-proven IA, the sensitivity remains low at 35% [69], 37%

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[69], 40% [70] and 64% [71]. The yield of sputum may be up to twice that of BALF owing to the presence of *Aspergillus* in tracheal secretions [62,64], and procuring culture by IS is less invasive than by bronchoscopy. Specificity of culture is poor due to frequent colonization in non-infected individuals, and a diagnosis of IA should only be made with support of clinical, radiologic, and serologic findings [69,71].

Aspergillus ELISA IgA sensitivity has been reported at 17% [72] and 29% [73] of cases meeting EORTC criteria for IA. ELISA IgG sensitivity has been reported at 50% [74], 80% [75], 83% [72], and 74% to 88% [76] of cases meeting EORTC criteria for CPA, although specificity appears to be low, consistent with that of invasive disease [77]. ID sensitivity has been reported at 38% [78], 70% [79], and 88% [79] of histopathologic-proven cases, and 56% [80] of clinically diagnosed cases. Serology can provide additional cases to GM alone, but due to the long processing time and an inability to distinguish between invasive aspergillosis, aspergilloma, or allergic bronchopulmonary aspergillosis (ABPA), serology is generally not recommended.

Serum galactomannan (GM), a cell wall component of *Aspergillus*, has a pooled sensitivity reported at 71% [81] of cases with proven EORTC criteria and 61% [81] of cases with either proven or probable EORTC criteria, with a specificity of 89% [81] for proven IA and 93% [81] for proven or probable IA. The sensitivity is reduced in solid-organ transplant recipients [81], but high in immunocompromised patients with a pooled sensitivity reported at 78% [82] of those cases meeting EORTC criteria, and a specificity of 85% [82]. The sensitivity is slightly lower and specificity slightly higher when using an ODI threshold of 1 instead of 0.5 [82], but nevertheless is only a modestly sensitive test, so a negative culture or GM should not preclude further diagnostic testing.

The pooled sensitivity of BALF GM exceeds that of serum, having been reported at 82% [83], 87% [83], and 90% [84] of cases meeting proven or probable EORTC criteria, with a specificity of 89% [83] and 92% [85]. The diagnostic accuracy is similar when using ODI cutoff values of either 0.5 or 1.0. The specificity might be reduced for those on beta-lactam antibiotics, but this controversy has not been fully resolved [85]. The pooled sensitivity for cases with proven EORTC criteria is even better, having been reported positive in 94% [85] and 100% [48] of cases, with a specificity of 72% [84]. Although the sensitivity of BALF appears to exceed that of serum by a reported 10% [86] and 40% [87], either source may be independently positive and thus in cases with negative serum GM, BALF GM should be obtained [46].

Serum β-D-glucan (BDG) pooled sensitivity has been reported at 77% [88] with a specificity of 83% [88]. When both BDG and GM are positive, the specificity improves to 98% [89,90] and a diagnosis may confidently be made. The sensitivity, however, is reduced to 49% [90] and 55% [89]. Thus, BDG and serum GM are excellent initial studies, and they may be diagnostic of IA if both are positive when used in conjunction with clinical findings.

BALF *Aspergillus* PCR pooled sensitivity has been reported at 90% [91] of cases meeting proven or possible EORTC criteria, with a specificity of 96% [91]. The addition of BALF GM to PCR has been reported to improve the sensitivity to 84% [85] and 97% [91], with a specificity of 98% [91]. Serum PCR pooled sensitivity has been reported at 79% [92], 84% [93], and 88% [94] of cases meeting proven or possible EORTC criteria, with a specificity of 75% [93] and 80% [92]. As is the case with BALF, the addition of GM to serum PCR has been reported to improve the sensitivity up to 99% [93]. PCR may also improve the detection of Aspergillus, but should be used in conjunction with culture and fungal cell wall component testing.

Pneumocystis jirovecii

Pneumocystis jirovecii is an ascomycetous fungus that can lead to pneumonia in immunocompromised patients. Risk factors for pneumonia (PJP) include lymphopenia, the use of immunosuppressive medications excluding mycophenolate, chronic glucocorticoid use, HIV infection, and solid organ transplantation [61].

Culture is not performed as the fungus fails to routinely grow on standard medium, although culture can be performed successfully under special conditions [95]. The sensitivity is therefore uncertain. Instead, cytology, DFA, and PCR are the means most commonly used to diagnose PJP. Grocott's methanamine silver stain (GMS) is the preferred cytologic method with a sensitivity having been reported at 77% [96] of cases with two confirmatory stains, with a specificity of 99% [96].

BALF direct fluorescent antibody (DFA) is an alternative to conventional staining methods, with a sensitivity having been reported at 90% [97] and 99% [98] of cases positive by conventional stains, with a specificity of 86% [97] and 94% [99]. Although DFA is commonly used as an initial diagnostic test, its sensitivity is poor when compared against PCR. Among PCR-positive specimens, the sensitivity has been reported at only 33% [100], 47% [101] and 93% [102], while IF sensitivity has been reported at 71% [103]. IS and expectorated sputum DFA can be obtained in place of BALF as an initial diagnostic test, with sensitivity in clinically diagnosed cases having been reported at 48% [99] and 55% [104], respectively.

BDG is commonly elevated in *Pneumocystis* infection. Specificity is poor, however, due to cross reactions with other opportunistic fungi as previously discussed. The pooled sensitivity of serum BDG has been reported at 95% [105] and 96% [88] of patients positive by either

DFA, PCR, or histopathology, with a specificity of 84% [88] and 86% [105]. BDG can be detected in BALF, but is less sensitive than serum BDG and is not certain to provide additional sensitivity [106].

BALF *Pneumocystis* PCR sensitivity has been reported at 83% [107] cytology-proven cases, and pooled 98% [108] of fluorescent-positive cases, with a specificity of 91% [108]. The sensitivity in clinically suspected cases, interestingly, reduces to approximately 44% [108]. This may underestimate the true sensitivity as a result of misdiagnosis of alternative processes such as CMV, while the pooled 98% figure may be attributed to an overestimation of cases undiagnosed by either PCR or DFA. The positive correlation between BDG and PCR positivity, however, indicates that the sensitivity of PCR is likely very high. IS sensitivity may even exceed that of BALF [103]. A combination of DFA, BDG, and PCR should be considered in diagnosis of PJP, however none of these has adequate sensitivity to rule out infection, so empiric treatment should be considered when there is high clinical suspicion.

Candida (albicans, tropicalis, parapsilosis, glabrata, auris, and others)

Candida species are ubiquitous environmental yeasts that often colonize airways, but can lead to pneumonia in a patient with an immunocompromising condition. Risk factors for infection include neutropenia, hematologic malignancy, allogeneic stem cell transplant, solid organ transplant recipient, prolonged corticosteroids, T-cell suppressing medication use, inherited severe immunodeficiency, and acute graft-versus-host disease [61].

BALF culture sensitivity among histopathologically-confirmed cases of invasive *Candida* pneumonia has been reported at 70% [109], 83% [110], 90% [111], and 100% [112]. Despite its excellent sensitivity, specificity of culture for invasive disease is poor, reported at 0% [24], 20% [112], and 57% [113], owing to a high frequency of non-pathologic colonization. The accuracy of culture by IS appears to be similar to that of BALF [113], but IS sample collection is less invasive. IS or BALF culture is recommended with the understanding that a positive culture should not be considered diagnostic of invasive pneumonia without pathologic evidence of tissue invasion.

Serum or BALF BDG detection supports a diagnosis of invasive fungal disease. The pooled sensitivity of serum BDG for invasive candidiasis, including infection outside of the respiratory tract, has been reported at 81% [88] with a specificity of 81% [88]. BALF and IS BDG sensitivity among clinically suspected cases of *Candida* pneumonia have been reported at 89% [114] and 67% [114], respectively, with a specificity of 86% [114] and 82% [114]. As previously noted, BDG can be elevated in other invasive fungal diseases such as IA and PJP.

BALF PCR sensitivity for *Candida* pneumonia has been reported at 89% [115] of culture-positive cases. The sensitivity of PCR of cases confirmed by histopathology, however, is unknown. Likewise, the specificity of PCR is also unknown but should be consistent with the low specificity of culture in general. Among clinically diagnosed invasive candidiasis (IC) the pooled sensitivity of serum PCR has been reported at 95% [116] with a specificity of 92% [116]. While PCR positivity may supplement clinical suspicion, it has insufficient specificity to permit a trustworthy diagnosis of invasive pneumonia without pathologic confirmation.

Mucorales (Mucor, Rhizopus, Absidia, Rhizomucor, and others)

Mucorales are an opportunistic group of fungi that can lead to pneumonia through the release of inhaled spores. Risk factors for pneumonia include chronic glucocorticoid use, diabetes mellitus, neutropenia, hematologic malignancy, hematopoietic cell transplantation, solid organ transplantation, and AIDS [61].
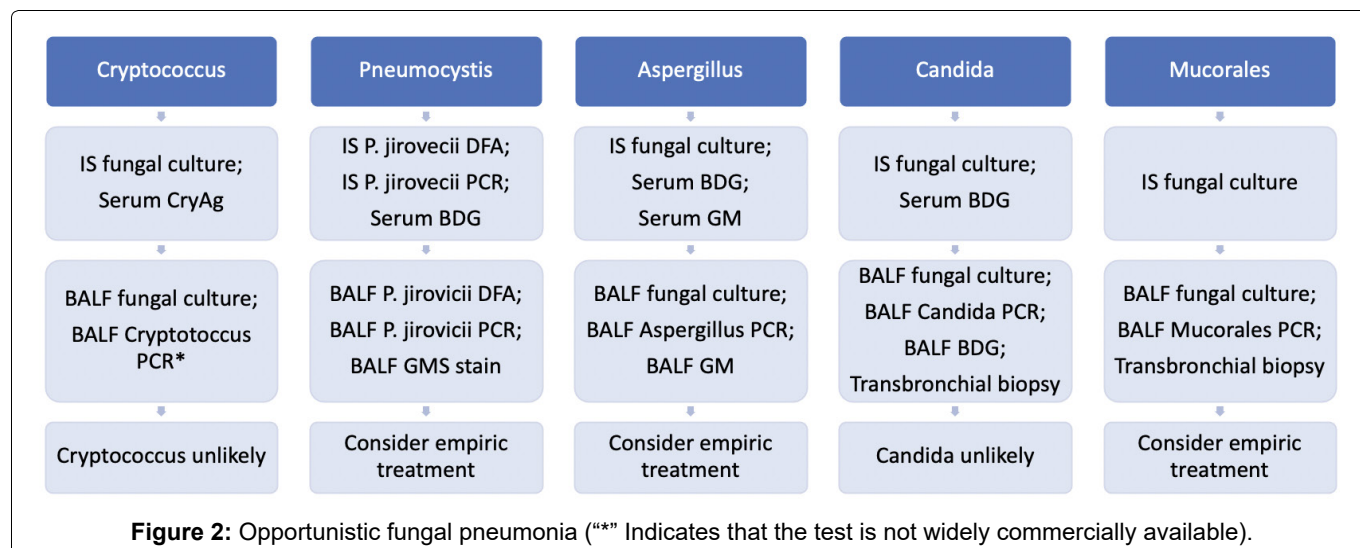
BALF culture sensitivity has been reported at 45% [117] and 66% [113] of those with diagnostic histopathology, and pooled at 43% [118] of those with proven or possible EORTC criteria, with a specificity that approaches 100% [113]. Although the sensitivity of culture has been reported at only 20% [118] and 42% [116] of PCR-positive cases, it can provide an additional sensitivity of up to 5% [118] to that of PCR alone. Colonization may occur, but a positive culture in combination with clinical suspicion should be considered pathogenic.

Serology has not been adequately evaluated in *Mucorales* infection. ID sensitivity has been reported at 73% [119] of culture-positive cases, with a specificity that approaches 100% [119]. Serology is not commercially available, however, and its role is uncertain as it is not known to provide additional sensitivity to culture alone.

The pooled sensitivity of PCR of either tissue, serum, or BALF specimens has been reported at 88% [118] of cases meeting either proven or probable EORTC criteria, and when PCR is combined with culture, the sensitivity can increase to 93% [118]. BALF PCR has been reported positive in 40% [20] and 100% [120] of culture-positive cases, but has also been reported to provide up to an additional 80% [120] sensitivity to culture. Serum PCR, however, does not add additional cases [120,121], and is not generally recommended. As *Mucorales* culture grows poorly, the addition of PCR may improve detection, thus both PCR and culture should be used concurrently to optimize the yield of diagnosis (Figure 2).

Conclusion

Changes in delivery of medical education as well

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Figure 2: Opportunistic fungal pneumonia ("*" Indicates that the test is not widely commercially available).

as altered societal expectations have led to increased emphasis on laboratory testing over that of clinical expertise. Clinicians themselves increasingly rely on a laboratory or radiological finding more than they do their own clinical diagnosis. This approach may be acceptable so long as there is well-defined guidance and when there is acceptance and knowledge of the frequency of false-negative testing. Most endemic fungi such as *Histoplasma, Blastomyces,* and *Coccidioides,* as well as opportunistic *Cryptococcus* and *Candida* can be correctly identified with current laboratory techniques, however infection caused by *Pneumocystis, Aspergillus,* and *Mucorales* cannot be ruled out with current testing. Laboratory investigations should complement, rather than supersede, either clinical judgment or classic radiological findings.

Contribution

All authors have contributed equally.

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Conflicts of Interest

There are no conflicts of interest to report by any author.

References

1. Binnicker MJ, Bukwalter SP, Eisberner JJ, Stewart RA, McCullough AE, et al. (2007) Detection of *Coccidioides* species in clinical specimens by real-time PCR. J Clin Microbiol 45: 173-178.

2. DiTomasso JP, Ampel NM, Sobonya RE, Bloom JW (1994) Bronchoscopy diagnosis of pulmonary coccidioidomycosis comparison of cytology, culture, and transbronchial biopsy. Diagn Microbiol Infect Dis 18: 83-87.

3. Durkin M, Connolly P, Kuberski T, Myers R, Kubak BM, et al. (2008) Diagnosis of coccidioidomycosis with use of the *Coccidioides* antigen enzyme immunoassay. Clin Infect Dis 47: e69-e73.

4. Durkin M, Estok L, Hospenthal D, Crum-Cianflone N, Swartzentruber S, et al. (2009) Detection of *Coccidioides* antigenemia following dissociation of immune complexes. Clin Vaccine Immunol 16: 1453-1456.

5. Blair JE, Coakley B, Santelli AC, Hentz JG, Wengenack NL (2006) Serologic testing for symptomatic coccidioidomycosis in immunocompetent and immunosuppressed hosts. Mycopathologia 162: 317-324.

6. Malo J, Holbrook E, Zangeneh T, Strawter C, Oren E, et al. (2017) Enhanced antibody detection and diagnosis of coccidioidomycosis with the MiraVista IgG and IgM detection enzyme immunoassay. J Clin Microbiol 55: 893-901.

7. Grys TE, Brighton A, Chang YH, Liesman R, LaSalle CB, et al. (2019) Comparison of two FDA-cleared EIA assays for the detection of *Coccidioides* antibodies against a composite clinical standard. Med Mycol 57: 595-600.

8. Wallace JM, Catanzaro A, Moser KM, Harrell JH (1981) Flexible fiberoptic bronchoscopy for diagnosing pulmonary coccidioidomycosis. Am Rev Respir Dis 123: 286-290.

9. Kaufman L, Sekhon AS, Moledina N, Jalbert M, Pappagianis D (1995) Comparative evaluation of commercial premier EIA and microimmunodiffusion and complement fixation tests for *Coccidioides immitis* antibodies. J Clin Microbiol 33: 618-619.

10. Hayden RT, Qian X, Roberts GD, Lloyd RV (2001) In situ hybridization for the identification of yeast like organisms in tissue section. Diagn Mol Pathol 10: 15-23.

11. Marynowicz MA, Prakash UB (2002) Pulmonary blastomycosis: An appraisal of diagnostic techniques. Chest 121: 768-773.

12. Taxy JB (2007) Blastomycosis: Contributions of morphology to diagnosis: A surgical pathology, cytopathology, and autopsy pathology study. Am J Surg Pathol 31: 615-623.

13. Lemos LB, Guo M, Baliga M (2000) Blastomycosis: Organ involvement and etiologic diagnosis. A review of 123 patients from Mississippi. Ann Diagn Pathol 4: 391-406.

14. Patel AJ, Gattuso P, Reddy VB (2010) Diagnosis of blastomycosis in surgical pathology and cytopathology: Correlation with microbiologic culture. Am J Surg Pathol 34: 256-261.

15. Connolly P, Hage CA, Bariola JR, Bensadoun E, Rodgers M, et al. (2012) *Blastomyces dermatitidis* antigen detection by quantitative enzyme immunoassay. Clin Vaccine Immunol 19: 53-56.

Ferguson et al. J Infect Dis Epidemiol 2021, 7:239

• Page 7 of 11 •

16. Durkin M, Witt J, Lemonte A, Wheat B, Connolly P (2004) Antigen assay with the potential to aid in diagnosis of blastomycosis. J Clin Microbiol 42: 4873-4875.

17. Frost HM, Novicki TJ (2015) *Blastomyces* antigen detection for diagnosis and management of blastomycosis. J Clin Microbiol 53: 3660-3662.

18. Klein BS, Kuritsky JN, Chappell WA, Kaufman L, Green J, et al. (1986) Comparison of the enzyme immunoassay, immunodiffusion, and complement fixation tests in detecting antibody in human serum to the A antigen of *Blastomyces dermatitidis.* Am Rev Respir Dis 133: 144-148.

19. Turner S, Kaufman L, Jalbert M (1986) Diagnostic assessment of an enzyme-linked immunosorbent assay for human and canine blastomycosis. J Clin Microbiol 23: 294-297.

20. Guinea J, Escribano P, Vena A, Muñoz P, Martínez-Jiménez MdC, et al. (2017) Increasing incidence of mucormycosis in a large Spanish hospital from 2007 to 2015: Epidemiology and microbiological characterization of the isolates. PLoS One 12: e0179136.

21. Richer SM, Smedema ML, Durkin MM, Brandhorst TT, Hage CA, et al. (2014) Development of a highly sensitive and specific blastomycosis antibody enzyme immunoassay using *Blastomyces dermatitidis* surface protein BAD-1. Clin Vaccine Immunol 21: 143-146.

22. Lo CY, Notenboom RH (1990) A new enzyme immunoassay specific for blastomycosis. Am Rev Respir Dis 141: 84-88.

23. Bradsher RW, Pappas PG (1995) Detection of specific antibodies in human blastomycosis by enzyme immunoassay. South Med J 88: 1256-1259.

24. Meersseman W, Lagrou K, Spriet I, Maertens J, Verbeken E, et al. (2009) Significance of the isolation of *Candida* species from airway samples in critically ill patients: A prospective, autopsy study. Intensive Care Med 25: 1526-1531.

25. Sidamonidze K, Peck MK, Perez M, Baumgardner D, Smith G, et al. (2012) Real-time PCR assay for identification of *Blastomyces dermatitidis* in culture and in tissue. J Clin Microbiol 50: 1783-1786.

26. Babady NE, Buckwalter SP, Hall L, Febre KML, Binnicker MJ, et al. (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.

27. Wheat J, French ML, Kohler RB, Zimmerman SE, Smith WR, et al. (1982) The diagnostic laboratory tests for histoplasmosis: Analysis of experience in a large urban outbreak. Ann Intern Med 97: 680-685.

28. Hage CA, Ribes JA, Wengenack NL, Baddour LM, Assi M, et al. (2011) A multicenter evaluation of tests for diagnosis of histoplasmosis. Clin Infect Dis 53: 448-454.

29. Wheat LJ, Wass J, Norton J, Kohler RB, French ML (1984) Cavitary histoplasmosis occurring during two large urban outbreaks. Analysis of clinical, epidemiologic, roentgenographic, and laboratory features. Medicine (Baltimore) 63: 201-209.

30. Freifeld AG, Wheat LJ, Kaul DR (2009) Histoplasmosis in solid organ transplant recipients: Early diagnosis and treatment. Curr Opin Organ Transplant 14: 601-605.

31. Dantas KC, Freitas RS, da Silva MV, Criado PR, do Carmo Luiz O, et al. (2018) Comparison of diagnostic methods to detect *Histoplasma capsulatum* in serum and blood samples from AIDS patients. PLoS One 13: e0190408.

32. Wheat LJ, Connolly-Stringfield P, Williams B, Connolly K, Blair R, et al. (1992) Diagnosis of histoplasmosis in patients with the acquired immunodeficiency syndrome by detection of *Histoplasma capsulatum* polysaccharide antigen in bronchoalveolar lavage fluid. Am Rev Respir Dis 145: 1421-1424.

33. 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.

34. Hage CA, Davis TE, Egan L (2007) Diagnosis of pulmonary histoplasmosis and blastomycosis by detection of antigen in bronchoalveolar lavage fluid using an improved second-generation enzyme linked immunoassay. Respir Med 101: 43-47.

35. Swartzentruber S, Rhodes L, Kurkjian K, Zahn M, Brandt ME, et al. (2009) Diagnosis of acute pulmonary histoplasmosis by antigen detection. Clin Infect Dis 49: 1878-1882.

36. Arango-Bustamante K, Restrepo A, Cano LE, Tobón AM, González A (2013) Diagnostic value of culture and serological tests in the diagnosis of histoplasmosis in HIV and non-HIV Colombian patients. Am J Trop Med Hyg 89: 937-942.

37. Gerber JD, Riley RE, Jones RD (1972) Evaluation of a microtiter latex agglutination test for histoplasmosis. Appl Microbiol 24: 191-197.

38. Richer SM, Smedema ML, Durkin MM, Herman KM, Hage CA, et al. (2016) Improved diagnosis of acute pulmonary histoplasmosis by combining antigen and antibody detection. Clin Infect Dis 62: 896-902.

39. Muraosa Y, Toyotome T, Yahiro M, Watanabe A, Shikanai-Yasuda MA, et al. (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.

40. da Silva Zatii M, Arantes TD, Fernandes JAL, Bay MB, Milan EP, et al. (2019) Loop-mediated isothermal amplification and nested PCR of the internal transcribed spacer (ITS) for *Histoplasma capsulatum* detection. PLoS Negl Trop Dis 13: e0007692.

41. Scheel CM, Zhou Y, Theodoro RC, Abrams B, Balajee SA, et al. (2014) Development of a loop-mediated isothermal amplification method for detection of *Histoplasma capsulatum* DNA in clinical symptoms. J Clin Microbiol 52: 483-488.

42. Setianingrum F, Rautemaa-Richardson R, Denning DW (2019) Pulmonary cryptococcosis: A review of pathobiology and clinical aspects. Med Mycol 57: 133-150.

43. Fisher JF, Valencia-Rey PA, Davis WB (2016) Pulmonary cryptococcosis in the immunocompetent patient - Many questions, some answers. Open Forum Infect Dis 3: ofw167.

44. Malabonga VM, Basti J, Kamholz SL (1991) Utility of bronchoscopy sampling techniques for cryptococcal disease in AIDS. Chest 99: 370-372.

45. Zhou Y, Lin PC, Ye JR, Su S-S, Dong L, et al. (2018) The performance of serum cryptococcal capsular polysaccharide antigen test, histopathology and culture of the lung tissue for diagnosis of pulmonary cryptococcosis in patients without HIV infection. Infect Drug Resist 11: 2483-2490.

46. Meyohas MC, Roux P, Bollens D, Chouaid C, Rozenbaum W, et al. (1995) Pulmonary cryptococcosis: Localized and

Ferguson et al. J Infect Dis Epidemiol 2021, 7:239

• Page 8 of 11 •

disseminated infections in 27 patients with AIDS. Clin Infect Dis 21: 628-633.

47. Yoo SD, Worodria W, Davis JL, Cattamanchi A, den Boon S, et al. (2010) The prevalence and clinical course of HIV-associated pulmonary cryptococcosis in Uganda. J Acquir Immune Defic Syndr 54: 269-274.

48. Zeng HQ, Zhang XB, Cai XY, Yang DY, Lin L, et al. (2021) Diagnostic value of bronchoalveolar lavage fluid cryptococcal antigen-lateral flow immunochromatographic assay for pulmonary cryptococcosis in non-HIV patients. Diagn Microbiol Infect Dis 99: 115276.

49. Kralovic SM, Rhodes JC (1998) Utility of routine testing of bronchoalveolar lavage fluid for cryptococcal antigen. J Clin Microbiol 36: 3088-3089.

50. Senghor Y, Guitard J, Angoulvant A, Hennequin C (2018) Cryptococcal antigen detection in broncho-alveolar lavage fluid. Med Mycol 56: 774-777.

51. Oshima K, Takozono T, Saijo T, Tashiro M, Kurihara S, et al. (2018) Examination of cryptococcal glucuronoxylomannan antigen in bronchoalveolar lavage fluid for diagnosing pulmonary cryptococcosis in HIV-negative patients. Med Mycol 56: 88-94.

52. Baughman RP, Rhodes JC, Dohn MN, Henderson H, Frame PT (1992) Detection of cryptococcal antigen in bronchoalveolar lavage fluid: A prospective study of diagnostic utility. Am Rev Respir Dis 145: 1226-1229.

53. Rivet-Danon D, Guitard J, Grenouillet F, Gay F, Ait-Ammar N, et al. (2015) Rapid diagnosis of cryptococcosis using an antigen detection immunochromatographic test. J Infect 70: 499-503.

54. Liaw YS, Yang PC, Yu CJ, Chang DB, Wang HJ, et al. (1995) Direct determination of cryptococcal antigen in transthoracic needle aspirate for diagnosis of pulmonary cryptococcosis. J Clin Microbiol 33: 1588-1591.

55. Pappas PG, Perfect JR, Cloud GA, Larsen RA, Pankey GA, et al. (2001) Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. Clin Infect Dis 33: 690-699.

56. Huang HR, Fan LC, Rajbanshi B, Xu JF (2015) Evaluation of a new cryptococcal antigen lateral flow immunoassay in serum, cerebrospinal fluid and urine for the diagnosis of cryptococcosis: A meta-analysis and systematic review. PLoS One 10: e0127117.

57. Kaufman L, Blumer S (1968) Value and interpretation of serological tests for the diagnosis of Cryptococcus. Appl Microbiol 16: 1907-1912.

58. Tanaka K, Miyazaki T, Maesaki S, Mitsutake K, Kakeya H, et al. (1996) Detection of Cryptococcus neoformans gene in patients with pulmonary cryptococcosis. J Clin Microbiol 34: 2826-2828.

59. Gago S, Esteban C, Valero C, Zaragoza O, de la Bellacasa JP, et al. (2014) A multiplex real-time PCR assay for identification of Pneumocystis jirovecii, Histoplasma capsulatum, and Cryptococcus neoformans/Cryptococcus gattii in samples from AIDS patients with opportunistic pneumonia. J Clin Microbiol 52: 1168-1176.

60. Rivera V, Gaviria M, Muñoz-Cadavid C, Cano L, Naranjo T (2015) Validation and clinical application of a molecular method for the identification of Cryptococcus neoformans/Cryptococcus gattii complex DNA in human clinical specimens. Braz J Infect Dis 19: 563-570.

61. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ,

Baddley JW, 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.

62. Zhou W, Li H, Zhang Y, Huang M, He Q, et al. (2017) Diagnostic value of galactomannan antigen test in serum and bronchoalveolar lavage fluid samples from patients with nonneutropenic invasive pulmonary aspergillosis. J Clin Microbiol 55: 2153-2161.

63. Sanguinetti M, Posteraro B, Pagano L, Pagliari G, Fianchi L, et al. (2003) Comparison of real-time PCR, conventional PCR, and galactomannan antigen detection by enzyme-linked immunosorbent assay using bronchoalveolar lavage fluid samples from hematology patients for diagnosis of invasive pulmonary aspergillosis. J Clin Microbiol 41: 3922-3925.

64. Bergeron A, Belle A, Sulahian A, Lacroix C, Chevret S, et al. (2010) Contribution of galactomannan antigen detection in BAL to the diagnosis of invasive pulmonary aspergillosis in patients with hematologic malignancies. Chest 137: 410-415.

65. Verweij PE, Latge JP, Rijs AJ, Melchers WJ, De Pauw BE, et al. (1995) Comparison of antigen detection and PCR assay using bronchoalveolar lavage fluid for diagnosing invasive pulmonary aspergillosis in patients receiving treatment for hematological malignances. J Clin Microbiol 33: 3150-3153.

66. Hohenthal U, Itala M, Salonen J, Sipilä J, Rantakokko-Jalava K, et al. (2005) Bronchoalveolar lavage in immunocompromised patients with haematological malignancy-value of new microbiological methods. Eur J Haematol 74: 203-211.

67. Maertens J, Maertens V, Theunissen K, Meersseman W, Meersseman P, et al. (2009) Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. Clin Infect Dis 49: 1688-1693.

68. Musher B, Fredricks D, Leisenring W, Balajee SA, Smith C, et al. (2004) Aspergillus galactomannan enzyme immunoassay and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. J Clin Microbiol 42: 5517-5522.

69. Tarrand JJ, Lichterfeld M, Warraich I, Luna M, Han XY, et al. (2003) Diagnosis of invasive septate mold infections. A correlation of microbiological culture and histologic or cytologic examination. Am J Clin Pathol 119: 854-858.

70. Levy H, Horak DA, Tegtmeier BR, Yokota SB, Forman SJ, et al. (1992) The value of bronchoalveolar lavage and bronchial washings in the diagnosis of invasive pulmonary aspergillosis. Respir Med 86: 243-248.

71. Cahill BC, Hibbs JR, Savik K, Juni BA, Dosland BM, et al. (1997) Aspergillus airway colonization and invasive disease after lung transplantation. Chest 112: 1160-1164.

72. Centeno-Lima S, de Lacerda JM, do Carmo JA, Abecasis M, Casimiro C, et al. (2002) Follow-up of anti-Aspergillus IgG and IgA antibodies in bone marrow transplanted patients with invasive aspergillosis. J Clin Lab Anal 16: 156-162.

73. Kappe R, Schulze-Berge A, Sonntag HG (1996) Evaluation of eight antibody tests and one antigen test for the diagnosis of invasive aspergillosis. Mycoses 39: 13-23.

74. Mishra SK, Falkenberg S, Masihi KN (1983) Efficacy of enzyme-linked immunosorbent assay in serodiagnosis of aspergillosis. J Clin Microbiol 17: 708-710.

75. Holmberg K, Berdischewsky M, Young LS (1980) Serologic immunodiagnosis of invasive aspergillosis. J Infect Dis 141: 656-664.

76. Page ID, Richardson MD, Denning DW (2016) Comparison of six *Aspergillus*-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis (CPA). J Infect 72: 240-249.

77. Erdmann JH, Graf B, Blau IW, Fischer F, Timm G, et al. (2016) Anti-*Aspergillus* immunoglobulin-G testing in serum of hematopoietic stem cell transplant recipients. Transpl Infect Dis18: 354-360.

78. Schaefer JC, Yu B, Armstrong D (1976) An aspergillus immunodiffusion test in the early diagnosis of aspergillosis in adult leukemia patients. Am Rev Respir Dis 113: 325-329.

79. Coleman RM, Kaufman L (1972) Use of the immunodiffusion test in the serodiagnosis of aspergillosis. Appl Microbiol 23: 301-308.

80. Manso E, Montillo M, De Sio G, D'Amico S, Discepoli G, et al. (1994) Value of antigen and antibody detection in the serological diagnosis of invasive aspergillosis in patients with hematological malignancies. Eur J Clin Microbiol Infect Dis 13: 756-760.

81. Pfeiffer CD, Fine JP, Safdar N (2006) Diagnosis of invasive aspergillosis using a galactomannan assay: A meta-analysis. Clin Infect Dis 42: 1417-1427.

82. Leeflang MMG, Debets-Ossenkopp YJ, Wang J, Visser CE, Scholten RJPM, et al. (2015) Galactomannan detection for invasive aspergillosis in immunocompromised patients. Cochrane Database Syst Rev 2015: CD007394.

83. Zou M, Tang L, Zhao S, Zhao Z, Chen L, et al. (2012) Systematic review and meta-analysis of detecting galactomannan in bronchoalveolar lavage fluid for diagnosing invasive aspergillosis. PLoS One 7: e43347.

84. Guo YL, Chen YQ, Want K, Qin SM, Wu C, et al. (2010) Accuracy of BAL galactomannan in diagnosing invasive aspergillosis: A bivariate metaanalysis and systematic review. Chest 138: 817-824.

85. Heng SC, Morrissey O, Chen SCA, Thursky K, Manser RL, et al. (2015) Utility of bronchoalveolar lavage fluid galactomannan alone or in combination with PCR for the diagnosis of invasive aspergillosis in adult hematology patients: A systematic review and meta-analysis. Crit Rev Microbiol 41: 124-134.

86. Husain S, Clancy CJ, Nguyen MH, Swartzentruber S, Leather H, et al. (2008) Performance characteristics of the platelia *Aspergillus* enzyme immunoassay for detection of *Aspergillus* galactomannan antigen in bronchoalveolar lavage fluid. Clin Vaccine Immunol 15: 1760-1763.

87. Meersseman W, Lagrou K, Maertens J, Wilmer A, Hermans G, et al. (2008) Galactomannan in bronchoalveolar lavage fluid: A tool for diagnosing aspergillosis in intensive care unit patients. Am J Respir Crit Care Med 177: 27-34.

88. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, et al. (2012) Diagnostic accuracy of serum 1,3-B-D-glucan for *Pneumocystis jiroveci* pneumonia, invasive candidiasis, and invasive aspergillosis: Systematic review and meta-analysis. J Clin Microbiol 50: 7-15.

89. Lu Y, Chen YQ, Guo YL, Qin SM, Wu C, et al. (2011) Diagnosis of invasive fungal disease using serum (1->3)-B-D-glucan: A bivariate meta-analysis. Intern Med 50: 2783-2791.

90. Zhang Li, Guo Z, Xie S, Zhou J, Chen G, et al. (2019) The performance of galactomannan in combination with 1,3-B-D-glucan or aspergillus-lateral flow device for the diagnosis of invasive aspergillosis: Evidences from 13 studies. Diagn Microbiol Infect Dis 93: 44-53.

91. Avni T, Levy I, Sprecher H, Yahav D, Leibovici L, et al. (2012) Diagnostic accuracy of PCR alone compared to galactomannan in bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis: A systematic review. J Clin Microbiol 50: 3652-3658.

92. Cruciani M, Mengoli C, Barnes R, Donnelly JP, Loeffler J, et al. (2019) Polymerase chain reaction blood tests for the diagnosis of invasive aspergillosis in immunocompromised people. Cochrane Database Syst Rev 9: CD009551.

93. Arvanitis M, Anagnostou T, Mylonakis E (2015) Galactomannan and polymerase chain reaction-based screening for invasive aspergillosis among high-risk hematology patients: A diagnostic meta-analysis. Clin Infect Dis 61: 1263-1272.

94. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP, et al. (2009) Use of PCR for diagnosis of invasive aspergillosis: Systematic review and meta-analysis. Lancet Infect Dis 2: 89-96.

95. Liu Y, Fahle GA, Kovacs JA (2018) Inability to culture *Pneumocystis jirovecii.* mBio 9: e00939-e01018.

96. Procop GW, Haddad S, Quinn J, Wilson ML, Henshaw NG, et al. (2004) Detection of *Pneumocystis jiroveci* in respiratory specimens by four staining methods. J Clin Microbiol 42: 3333-3335.

97. Cregan P, Yamamoto A, Lum A, VanDerHeide T, MacDonald M, et al. (1990) Comparison of four methods for rapid detection of *Pneumocystis carinii* in respiratory specimens. J Clin Microbiol 28: 2432-2436.

98. Wolfson JS, Waldron MA, Sierra LS (1990) Blinded comparison of a direct immunofluorescent monoclonal antibody staining method and a Giemsa staining method for identification of *Pneumocystis carinii* in induced sputum and bronchoalveolar lavage specimens of patients infected with human immunodeficiency virus. J Clin Microbiol 28: 2136-2138.

99. Lim SK, Eveland WC, Porter RJ (1974) Direct fluorescent-antibody method for the diagnosis of *Pneumocystis carinii* pneumonitis from sputa or tracheal aspirates from humans. Appl Microbiol 27: 144-149.

100. Tekinsen FF, Koc AN (2013) Investigation of *Pneumocystis jirovecii* in clinical specimens by different methods. Mikrobiyol Bul 47: 658-667.

101. Fillaux J, Malvy S, Alvarez M, Fabre R, Cassaing S, et al. (2008) Accuracy of a routine real-time PCR assay for the diagnosis of *Pneumocystis jirovecii* pneumonia. J Microbiol Methods 75: 258-261.

102. Hauser PM, Bille J, Lass-Florl C, Geltner C, Feldmesser M, et al. (2011) Multicenter, prospective clinical evaluation of respiratory samples from subjects at risk for *Pneumocystis jirovecii* infection by use of a commercial real-time PCR assay. J Clin Microbiol 49: 1872-1878.

103. Azoulay E, Bergeron A, Chevret S, Bele N, Schlemmer B, et al. (2009) Polymerase chain reaction for diagnosing *Pneumocystis* pneumonia in non-HIV immunocompromised patients with pulmonary infiltrates. Chest 135: 655-661.

104. Choe PG, Kang YM, Kim G, Park WB, Park SW, et al. (2014) Diagnostic value of direct fluorescence

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• Page 10 of 11 •

antibody staining for detecting *Pneumocystis jirovecii* in expectorated sputum from patients with HIV infection. Med Mycol 52: 326-330.

105. Karageorgopoulos DE, Qu JM, Kobila IP, Zhu Y-G, Vasileiou VA, et al. (2013) Accuracy of B-D-glucan for the diagnosis of *Pneumocystis jirovecii* pneumonia: A meta-analysis. Clin Microbiol Infect 19: 39-49.

106. Salerno D, Mushatt D, Myers L, Zhuang Y, de la Rua N, et al. (2014) Serum and BAL beta-D-glucan for the diagnosis of *Pneumocystis* pneumonia in HIV-positive patients. Respir Med 108: 1688-1695.

107. Leibovitz E, Pollack H, Moore T, Papellas J, Gallo L, et al. (1995) Comparison of PCR and standard cytological staining for detection of *Pneumocystis carinii* from respiratory specimens from patients with or at high risk for infection by human immunodeficiency virus. J Clin Microbiol 33: 3004-3007.

108. Fan LC, Lu HW, Cheng KB, Li HP, Xu JF, et al. (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.

109. Mohsenifar Z, Chopra SK, Johnson BL, Simmons DH (1979) *Candida* pneumonia: Experience with 20 patients. West J Med 131: 196-200.

110. Kontoyiannis DP, Reddy BT, Torres HA, Luna M, Lewis RE, et al. (2002) Pulmonary candidiasis in patients with cancer: An autopsy study. CID 34: 400-403.

111. Haron E, Vartivarian S, Anaissie E, Dekmezian R, Bodey GP, et al. (1993) Primary *Candida* pneumonia. Experience at a large cancer center and review of the literature. Medicine 72: 137-142.

112. el-Ebiary M, Torres A, Faregas N, de la Bellacasa JP, González J, et al. (1997) Significance of the isolation of *Candida* species from respiratory samples in critically ill, non-neutropenic patients. An immediate postmortem histologic study. Am J Resp Crit Care 152: 583-590.

113. Kontoyiannis DP, Wessel VC, Bodey GP, Rolston KV (2000) Zygomycosis in the 1990s in a tertiary-care cancer center. Clin Infect Dis 30: 851-856.

114. Su KC, Chou KT, Hsiao YH, Tseng CM, Su VYF (2017) Measuring (1,3)-B-D-glucan in tracheal aspirate, bronchoalveolar lavage fluid, and serum for detection of suspected *Candida* pneumonia in immunocompromised and critically ill patients: A prospective observational study. BMC Infect Dis 17: 252.

115. Khorvash F, Abbasi S, Yaran M, Abdi F, Ataei B, et al. (2014) Molecular detection of *Candida* spp. and *Aspergillus fumigatus* in bronchoalveolar lavage fluid of patients with ventilator-associated pneumonia. J Res Med Sci 19: S46-S50.

116. Avni T, Leibovici L, Paul M (2011) PCR diagnosis of invasive candidiasis: Systematic review and meta-analysis. J Clin Microbiol 49: 665-670.

117. Hammond SP, Bishkek R, Milner DA, Petschnigg EM, Baden LR, et al. (2011) Molecular methods to improve diagnosis and identification of mucormycosis. J Clin Microbiol 49: 2151-2153.

118. Shadrivova OV, Burygina EV, Klimko NN (2019) Molecular diagnostics of mucormycosis in hematological patients: A literature review. J Fungi (Basel) 5: 112.

119. Jones KW, Kaufman L (1978) Development and evaluation of an immunodiffusion test for diagnosis of systemic zygomycosis (mucormycosis): Preliminary report. J Clin Microbiol 7: 97-103.

120. Scherer E, Iriart X, Bellanger AP, Dupont D, Guitard J, et al. (2018) Quantitative PCR (qPCR) detection of Mucorales DNA in bronchoalveolar lavage fluid to diagnose pulmonary mucormycosis. J Clin Microbiol 56: e00289-e00318.

121. Millon L, Herbrecht R, Grenouillet F, Morio F, Alanio A, et al. (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.e1-810.e8.