AMERICAN THORACIC SOCIETY DOCUMENTS

Microbiological Laboratory Testing in the Diagnosis of Fungal Infections in Pulmonary and Critical Care Practice

An Official American Thoracic Society Clinical Practice Guideline

Chadi A. Hage, Eva M. Carmona, Oleg Epelbaum, Scott E. Evans, Luke M. Gabe, Qusay Haydour, Kenneth S. Knox, Jay K. Kolls, M. Hassan Murad, Nancy L. Wengenack, and Andrew H. Limper; on behalf of the American Thoracic Society Assembly on Pulmonary Infections and Tuberculosis

THIS OFFICIAL CLINICAL PRACTICE GUIDELINE OF THE AMERICAN THORACIC SOCIETY WAS APPROVED MAY 2019

Background: Fungal infections are of increasing incidence and importance in immunocompromised and immunocompetent patients. Timely diagnosis relies on appropriate use of laboratory testing in susceptible patients.

Methods: The relevant literature related to diagnosis of invasive pulmonary aspergillosis, invasive candidiasis, and the common endemic mycoses was systematically reviewed. Meta-analysis was performed when appropriate. Recommendations were developed using the Grading of Recommendations Assessment, Development, and Evaluation approach.

Results: This guideline includes specific recommendations on the use of galactomannan testing in serum and BAL and for the diagnosis of invasive pulmonary aspergillosis, the role of PCR in the diagnosis of invasive pulmonary aspergillosis, the role of β-D-glucan assays in the diagnosis of invasive candidiasis, and the application of serology and antigen testing in the diagnosis of the endemic mycoses.

Conclusions: Rapid, accurate diagnosis of fungal infections relies on appropriate application of laboratory testing, including antigen testing, serological testing, and PCR-based assays.

Keywords: fungal diagnosis; serology; PCR; antigen testing

Contents

Overview
Summary of Recommendations
Methods
- Committee Composition
- Confidentiality Agreement and Conflict-of-Interest Management
- Meetings
- Formulating Clinical Questions
- Literature Search and Study Selection
- Evidence Synthesis and the GRADE Approach
- Manuscript Preparation, Review, and Approval

Recommendations for Specific Diagnostic Questions
- Question 1
  - Background
  - Summary of evidence
  - Recommendations
  - Rationale
  - Implementation and limitations
  - Future research
- Question 2
  - Background
  - Summary of evidence
  - Recommendations
  - Rationale
  - Implementation and limitations
  - Future research
- Question 3
  - Background
  - Summary of evidence
  - Recommendation
  - Implementation and limitations
  - Future research
- Question 4
  - Histoplasmosis
    - Background
    - Summary of evidence
    - Recommendations

ORCID IDs: 0000-0002-0582-4362 (C.A.H.); 0000-0002-6920-0540 (O.E.); 0000-0003-4503-0644 (S.E.E.).

You may print one copy of this document at no charge. However, if you require more than one copy, you must place a reprint order. Domestic reprint orders: amy.schriver@sheridan.com; international reprint orders: louisa.mott@springer.com.

Correspondence and requests for reprints should be addressed to Andrew H. Limper, M.D., Pulmonary and Critical Care, Gonda 18, Mayo Clinic, Rochester MN 55905. E-mail: limper.andrew@mayo.edu.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Crit Care Med Vol 200, Iss 5, pp 535–550, Sep 1, 2019
Copyright © 2019 by the American Thoracic Society
DOI: 10.1164/rccm.201906-1185ST
Internet address: www.atsjournals.org

Rationale and implementation considerations
Future research
Blastomycosis
Background
Summary of evidence
Recommendations
Rationale
Implementation and limitations
Future research
Coccidioidomycosis
Background
Summary of evidence
Recommendations
Rationale
Implementation and limitations
Future research
Conclusions

Overview

The purpose of this guideline is to analyze evidence relevant to commonly available laboratory testing, including the use of specific antigen tests, serological assays (serum antibody detection), and PCR studies for diagnosis of fungal infections commonly encountered in pulmonary and critical care practice. These guidelines focus on the use of galactomannan (GM) antigen and PCR testing in the diagnosis of invasive pulmonary aspergillosis (IPA), (1→3)-β-D-glucan (BDG) assays for invasive candidiasis (IC), and the use of antigen and antibody testing in the diagnosis of endemic mycosis.

Summary of Recommendations

- In patients with severe immunocompromise, such as those with neutropenia or hematological malignancy or recipients of hematological stem cell or solid organ transplants presenting with unexplained lung infiltrates suspected of IPA, we recommend the use of serum GM testing (*strong recommendation, high-quality evidence*).
- In patients suspected of invasive fungal diseases, including those with a negative serum GM but strong risk factors for invasive aspergillosis or positive serum GM but confounding factors for false-positive GM results (e.g., those patients undergoing chemotherapy or at risk for mucositis, in which cross-reactive epitopes from other fungi or bacteria can penetrate the intestinal mucosa, causing positivity of the test), we recommend BAL testing with GM (*strong recommendation, high-quality evidence*).
- In patients with severe immunocompromise, such as those with hematological malignancy or recipients of hematological stem cell or solid organ transplants, who are suspected of having IPA, we recommend the use of blood or serum *Aspergillus* PCR testing (*strong recommendation, high-quality evidence*).
- In patients with severe immunocompromise, such as those with hematological malignancy or recipients of hematological stem cell or solid organ transplants who are suspected of having IPA, we recommend the inclusion of *Aspergillus* PCR in BAL testing as part of the evaluation (*strong recommendation, high-quality evidence*).
- In patients with severe immunocompromise, such as those with hematological malignancy or recipients of hematological stem cell or solid organ transplants who are strongly suspected of having IPA but in whom the result of PCR testing for *Aspergillus* is negative, we suggest consideration of biopsy and/or additional testing with or without additional PCR or GM testing (*conditional recommendation, low-quality evidence*).
- In critically ill patients in whom there is clinical concern for IC, we suggest not relying solely on results of serum BDG testing alone for diagnostic decision-making (*conditional recommendation, low-quality evidence*).
- We recommend the use of *Histoplasma* antigen in urine or serum for rapid diagnosis of suspected disseminated and acute pulmonary histoplasmosis when timely diagnosis and treatment are of paramount importance to outcome (*strong recommendation, high-quality evidence*).
- We suggest the use of *Histoplasma* serologies in immunocompetent patients with suspected pulmonary histoplasmosis. Adding *Histoplasma* antigen to serological testing might improve the diagnostic yield (*conditional recommendation, moderate-quality evidence*).
- In patients with appropriate geographic exposure and illness compatible with infection or pneumonia due to blastomycosis, we suggest using more than one diagnostic test, including direct visualization and culture of sputum BAL or other biopsy material, urine antigen testing, and serum antibody testing. The current evidence cannot support a single best test as being sensitive enough to be ordered in isolation from other testing. The approach should be tailored on the basis of severity of illness, the clinical context, and the availability of tests (*conditional recommendation, moderate-quality evidence*).
- In patients with suspected blastomycosis, we suggest that serum antibody testing specifically directed against the anti–BAD-1 (anti–*Blastomyces* adhesin 1) antigen for blastomycosis be used together with clinical and epidemiological data to establish the diagnosis (*conditional recommendation, low-quality evidence*).
- In patients with suspected blastomycosis, particularly in immunocompromised patients, we suggest that urinary antigen testing for blastomycosis be used together with clinical and epidemiological data to establish the diagnosis (*conditional recommendation, moderate-quality evidence*).
- In patients with appropriate geographic exposure and illness compatible with infection or pneumonia due to coccidioidomycosis, we suggest using more than one diagnostic test, including direct visualization and culture of sputum BAL or other biopsy material, urine and serum antigen testing, and serology (serum antibody testing). The current evidence cannot support a single best test. The approach should be tailored on the basis of severity of illness, the clinical context, and the availability of tests (*conditional recommendation, moderate-quality evidence*).
- In patients with suspected coccidioidomycosis, particularly in immunocompromised patients, we suggest performing urinary and serum antigen testing to aid in establishing the diagnosis (*conditional recommendation, moderate-quality evidence*).

- In patients with suspected community-acquired pneumonia (CAP) from the endemic area for coccidioidomycosis, we suggest initial serological testing with close clinical follow-up and serial testing (*conditional recommendation, moderate-quality evidence*).

The rising prevalence of fungal infections is likely related to several factors, including an ever-growing population of susceptible patients, such as immunocompromised individuals with malignancies, hematological and solid organ transplants, HIV, and inflammatory conditions treated with immunosuppressants. Furthermore, endemic mycoses continue to pose a threat to both immunocompetent and immunosuppressed individuals. In addition to heightened awareness, recent advances in laboratory diagnostics have evolved to assist the diagnosis of pulmonary fungal infections (1, 2). These methodologies include antigen testing in urine, blood, and BAL fluid; serological testing to detect antibodies to fungal components; and nucleic acid–based assays using PCR approaches. The purpose of this clinical practice guideline is to review available information for the diagnosis of selected fungal infections using these laboratory approaches.

Methods

Committee Composition

We convened a panel with broad expertise in the clinical aspects and diagnosis of fungal infections. Panel members with representative backgrounds from pulmonary medicine, critical care, and infectious diseases were included, in addition to those with expertise in adult and pediatric pulmonary medicine and invasive procedures. The committee membership included Chadi A. Hage, Eva M. Carmona, Oleg Epelbaum, Scott E. Evans, Luke M. Gabe, Kenneth S. Knox, Jay K. Kolls, Nancy L. Wengenack, and Andrew H. Limper. M. Hassan Murad and Qusay Haydour provided methodological expertise. The committee was co-chaired by Chadi A. Hage and Andrew H. Limper.

Confidentiality Agreement and Conflict-of-Interest Management

All committee members signed conflict-of-interest declarations at the outset of the project, and these were updated annually. The committee co-chair (A.H.L.) solicited updated conflict-of-interest declarations routinely at the start of each in-person meeting and conference call. The opinions and interests of the American Thoracic Society (ATS) were never permitted to exert influence on either the topics discussed or the final recommendations in this document.

Meetings

At the initial face-to-face meeting during the 2015 ATS International Conference in Denver, Colorado, the panel discussed the overall objectives and scope of the project and identified potential diagnostic areas and related clinical questions that could be addressed. After survey of the available literature, the questions were refined and finalized at the 2016 meeting in San Francisco. An ATS-designated methodologist (M.H.M.) presented the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach for guideline development to the panel, and a second methodologist (Q.H.) assisted the panel with literature review.

Formulating Clinical Questions

The panel chose four clinical questions that clinicians face when they care for patients with suspected fungal infections. By design, we focused on laboratory-based microbiological testing commonly available in clinical settings. These questions guided the systematic reviews of the literature.

Literature Search and Study Selection

A comprehensive search of the literature spanning from 1980 to April 14, 2016, was conducted and included MEDLINE In-Process and Other Non-Indexed Citations, MEDLINE, Embase, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Scopus. The search was limited to publications in English and was designed and executed by a librarian. Controlled vocabulary supplemented with keywords was used to search for studies of fungal diagnosis. The actual strategy is available in the online supplement and yielded 2,327 citations. The panel also assisted in identifying additional resources and monitored the literature for studies published after the search date. The methodologists and the panelists selected studies for inclusion. Data were extracted in duplicate.

Evidence Synthesis and the GRADE Approach

When appropriate, bivariate random effects meta-analysis was used to generate pooled sensitivity, specificity, and diagnostic likelihood ratios. When data were insufficient for meta-analysis, the panel narratively summarized data. The quality of evidence (certainty in the estimates) was graded as high, moderate, low, and very low following the GRADE approach for diagnostic studies.

Manuscript Preparation, Review, and Approval

The writing committee (C.A.H., E.M.C., O.E., S.E.E., K.S.K., and A.H.L.) provided the initial draft of the guideline document for review and editing by the entire panel. The entire panel provided input to correct interpretive or factual errors. The final version was submitted to the ATS Documents Committee. The guideline underwent anonymous peer review by four content experts and one methodologist. After multiple cycles of review and revision, the guideline was reviewed and approved by a multidisciplinary board of directors. The guideline will be reviewed by the ATS 3 years after publication, and it will be determined if updating is necessary at that time.

Recommendations for Specific Diagnostic Questions

Question 1

Is serum and/or BAL GM testing sufficiently accurate to guide therapeutic decisions in place of histopathology and/or fungal culture in patients with impaired immunity suspected of having IPA?

Background. Patients with impaired immunity, including those with neutropenia, hematological malignancies, bone marrow or hematopoietic stem cell transplant, and lung and other solid organ transplants are at risk for IPA. In addition, IPA has been reported in intubated patients with chronic obstructive pulmonary disease, particularly those receiving corticosteroids, and after influenza. Pulmonary infiltrates are frequently present but are not specific; other times, patients with early disease or

extrapulmonary aspergillosis may even present with normal chest imaging studies. The “gold standard” for the diagnosis of invasive aspergillosis requires tissue histopathology confirmation of the invasion of hyphae or a positive culture for *Aspergillus* spp. in the appropriate clinical setting (3, 4). Unfortunately, in many of these patients, tissue biopsy cannot be obtained, owing to excessive bleeding risk or tenuous respiratory status. Furthermore, the sensitivity of the culture from BAL ranges from 30% to 60%. Initiation of appropriate therapy is particularly important in this often very tenuous population because invasive aspergillosis carries a high mortality and can be greater than 50% if not adequately treated in critically ill patients (5). An early and accurate diagnosis using less invasive techniques is therefore preferred, making serum and BAL GM a very attractive alternative test.

Summary of evidence. The diagnostic accuracy of serum and BAL GM for the diagnosis of IPA in immunocompromised adult patients with proven and probable disease was compared with that in those not having IPA, defined as both possible and no IPA by European Organization for Research and Treatment of Cancer criteria (6), as described in the accompanying technical summary (7). Fifty studies (45 studies included in the Leeflang and colleagues [8] meta-analysis and 5 additional studies) including an aggregate of 8,763 patients met our inclusion criteria (8–12). Several studies reported more than one cutoff point for the GM sandwich ELISA. Individual sensitivity and specificity analysis was performed on the basis of lower cutoff levels reported.

Among the 50 studies that tested serum GM, 29 used an assay cutoff index of 0.5, 7 studies used a cutoff of 1.0, and 14 studies used a cutoff of 1.5. For those studies that used a cutoff of 0.5, the combined sensitivity and specificity were 74% (95% confidence interval [CI], 64–82) and 85% (95% CI, 77–90), respectively; the positive and negative likelihood ratios were 4.8 (95% CI, 3.2–7.3) and 0.31 (95% CI, 0.22–0.43), respectively, with a diagnostic odds ratio of 16 (95% CI, 9–29). For those studies using a cutoff of 1, the sensitivity and specificity were 79% (95% CI, 60–91) and 88% (95% CI, 78–94) respectively; the positive and negative likelihood ratios were 6.6 (95% CI, 3.4–12.5) and 0.24 (95% CI, 0.11–0.5), respectively, with a diagnostic odds ratio of 28 (95% CI, 9–83). When the cutoff level was defined as 1.5, the sensitivity and specificity were 59% (95% CI, 44–72) and 95% (95% CI, 90–97), respectively; the positive and negative likelihood ratios were 10.8 (95% CI, 5.8–20.1) and 0.43 (95% CI, 0.3–0.62), respectively, with a diagnostic odds ratio of 25 (95% CI, 11–58).

Regarding the diagnostic accuracy of BAL GM, 13 studies included in the Zou and colleagues meta-analysis and 3 additional studies met our inclusion criteria and were included in the analysis (13–16). These studies included a total of 1,568 patients. Eleven studies used a cutoff index of 0.5, and five studies used a cutoff index of 1.0. For those studies with a cutoff of 0.5, the combined sensitivity and specificity were 79% (95% CI, 65–88) and 84% (95% CI, 74–91), respectively; the positive and negative likelihood ratios were 5 (95% CI, 3–8.3) and 0.25 (95% CI, 0.15–0.42), respectively, with a diagnostic odds ratio of 20 (95% CI, 9–44). For those using a cutoff of 1, the sensitivity and specificity were 90% (95% CI, 77% to 96%) and 94% (95% CI, 88–97), respectively; the positive and negative likelihood ratios were 14.3 (95% CI, 7.2–28.5) and 0.11 (95% CI, 0.04–0.26), respectively, with a diagnostic odds ratio of 134 (95% CI, 43–420). Additional details are included in the accompanying technical summary (7).

Recommendations. In patients with severe immunocompromise, such as those with neutropenia or hematological malignancy or recipients of hematological stem cell or solid organ transplants, who present with unexplained lung infiltrates suspected of IPA, we recommend the use of serum GM testing (*strong recommendation, high-quality evidence*).

- *Technical remark*: A serum GM index cutoff of 1.0 provides the best diagnostic performance characteristics. As noted, however, this is based on 7 studies using a cutoff 1.0 and 29 studies using a cutoff of 0.5.
- *Technical remark*: PCR approaches may provide another diagnostic approach as discussed in question 2 (*see below*).
- *Technical remark*: There is less data available on the use of serum GM testing in patients without neutropenia, such as those with solid organ transplant, than in hematological patients with neutropenia, but the test can still be applied in these settings and interpreted in the context of other clinical information.
- *Technical remark*: We suggest cautious interpretation of serum GM data in patients receiving antifungal therapy, owing to a multitude of factors, including the lack of definitions for threshold levels in this setting, the timing of testing in relation to initiation of treatment, and the scarcity of specific data to guide such interpretation.

In patients suspected of having invasive fungal diseases, including those with a negative serum GM but strong risk factors for IPA or positive serum GM but confounding factors for false-positive GM results (i.e., those patients undergoing chemotherapy or at risk for mucositis, in whom cross-reactive epitopes from other fungi or bacteria can penetrate the intestinal mucosa, causing positivity of the test result), we recommend BAL testing with GM (*strong recommendation, high-quality evidence*).

- *Technical remark*: If the result is negative but IPA is still suspected, we suggest biopsy with histopathology and culture and/or repeat BAL GM testing (*conditional recommendation, low-quality evidence*).
- *Technical remark*: We suggest cautious interpretation of BAL GM data in patients receiving antifungal therapy, owing to a multitude of factors, including the lack of definitions for threshold levels in this setting, the timing of testing in relation to initiation of treatment, and the scarcity of specific data to guide such interpretation.

Rationale. The strong recommendation for the use of serum GM in the diagnosis of IPA in immunosuppressed patients comes from the clinical need of having a test that can determine true positives while producing the lowest possible number of false-negative results, because this is often a fatal disease when left undiagnosed. If we assume a population of 1,000 and a disease prevalence of 10%, on the basis of data presented above for serum GM, the true positives (patients with disease) for the 0.5, 1, and 1.5 cutoff values were 74, 79, and 59, respectively, and the true negatives (patients free of disease) were 765, 792, and 855, respectively. False negatives (patients with disease who were

not diagnosed by the test) were 26, 21, and 41, respectively, and false positives (patients free of disease whom the test classified as having the disease) were 135, 108, and 45. Hence, a cutoff index of 1.0 provides the best operating performance for the test. The noninvasive nature and availability of the test in most centers, providing results in a timely manner, together with the relatively low cost of the test further strengthened the decision for a strong recommendation. However, the false-negative rate in the setting in which invasive aspergillosis is still suspected despite a negative test result illustrates the need to consider repeat testing or biopsy as we recommend for vulnerable patients at risk.

Likewise, BAL GM was strongly recommended on the basis of characteristics of this test similar to those of the serum GM. Assuming the same population size and pretest probability used above for the discussion of serum GM, the true positives and true negative results for the 0.5 and 1 cutoff values were 79 and 90 (true positives) and 756 and 846 (true-negative findings), respectively. False negatives were 21 and 10, and false positives were 144 and 54, respectively. The cost and availability of the test are similar to those of serum GM, but the increased risk of obtaining BAL samples in this population needs to be individually assessed, particularly for those patients with respiratory failure.

The panel considered other nonevidentiary factors from the GRADE Evidence to Decision framework. The panel judged these tests to be fairly inexpensive in this setting and highly feasible to implement. This approach will likely be acceptable to clinicians and patients, with minimal variation in values and preferences.

Implementation and limitations. Our synthesis of the literature evaluated studies that included immunosuppressed patients. Therefore, these results should be applied with caution for other populations. For instance, the study populations did not include other *Aspergillus*-related respiratory illnesses such as chronic necrotizing ("semiinvasive") pulmonary aspergillosis or allergic bronchopulmonary aspergillosis (ABPA). The overall role of GM testing in such clinical settings is not yet well defined.

Additional factors should also be taken into consideration when interpreting the results of GM testing in either serum or BAL when applied to an individual patient. For instance, the concomitant use of antifungal therapy in some patients with IPA may result in reduced sensitivity of the assay (8). Other factors have been associated with false-positive GM results. As noted above, patients undergoing chemotherapy or at risk for gastrointestinal mucositis may have false-positive serum GM assay results due to cross-reactive antigens from other fungi or bacteria that penetrate the intestinal mucosa (17). In addition, false-positive GM results have been described in patients receiving Plasma-Lyte intravenously or when Plasma-Lyte is used for BAL (18–20). The presence of sodium gluconate in Plasma-Lyte has been attributed to the false positivity of the results. This is due to the fact that gluconate is obtained by a fermentation process that involves *Aspergillus* spp., which are believed to release GM during fermentation that is carried through the manufacturing process of the medical grade gluconate used in some of the Plasma-Lyte solutions (21, 22) Similarly, positive results have also been described in individuals who ingested ice pops, which they may also contain sodium gluconate (23). Recent data, however, suggest that the contamination with GM is gluconate provider specific and that some providers use gluconate that is GM free (24). This needs to be taken into consideration, and although false positivity is possible, one should not routinely assume false positivity in patients exposed to gluconate, because some preparations are free of GM. Finally, it is also important to be aware that specimens containing *Histoplasma* spp. antigen and other fungal antigens may also cross-react in the *Aspergillus* spp. GM assay (25, 26). In the past, positive results have also been associated with the use of concomitant β-lactam antibiotics; however, recent studies suggest that the new preparations are less likely to cross-react with the assay (27, 28).

Future research. The widening use of GM testing both in serum and in BAL attests to its utility in the clinical management of immunocompromised patients with lung infiltrates that are suspicious for IPA, including patients with lung transplants. However, there remain a number of areas that will require additional investigations, such as ABPA, as well as semiinvasive aspergillosis and tracheobronchial aspergillosis that can occur in lung transplant recipients and in severely immunocompromised hosts such as those with advanced stages of AIDS. Furthermore, with the variety of available commercial tests and various cutoffs employed, it is in the best interest of the practitioners that they use (one or at most two such assays) and become familiar with test performance in their local clinical settings. Although beyond the scope of most available studies in the literature, research into diagnostic effectiveness with head-to-head comparisons across various commercial assays would ultimately be of significant benefit to the practitioner and would support greater uniformity of tests and cutoffs used across clinical practices. Additional studies will be needed to validate the use of BAL and potentially sputum GM for other forms of pulmonary aspergillosis, such as ABPA, semiinvasive aspergillosis, and tracheobronchial aspergillosis, that are often seen in lung transplant recipients and in severely immunocompromised hosts such as patients with advanced stages of AIDS. Finally, newer diagnostic modalities, including breath test assays and detection of volatile organic compounds, are also under investigation.

Question 2

Should diagnosis of suspected *Aspergillus* infections in severely immunocompromised patients be based on the application of PCR?

Background. Because it is frequently used in the diagnosis of other infectious conditions, PCR-based detection of *Aspergillus* spp. has been proposed as a novel means to diagnose IPA. PCR-based strategies allow detection of very low copy number of target DNA; the oligonucleotide primers confer high specificity; and the use of quantitative real-time PCR (qPCR) facilitates quantification of pathogen burden and establishment of threshold values to define infections (29, 30). Several groups have recently investigated the performance of PCR of blood- and BAL-based samples in the diagnosis of IPA. However, these tests vary with respect to the extraction methods, primers, probes, gene targets, and amplification platforms used. Therefore, no particular PCR-based strategy is currently recommended for clinical diagnosis of suspected IPA.

Summary of evidence. The working group sought evidence to determine the

diagnostic accuracy of blood and BAL PCR for the diagnosis of IPA in immunocompromised adults with proven or probable disease as compared with patients not having IPA, defined as possible or no IPA by European Organization for Research and Treatment of Cancer criteria. As characterized in the accompanying technical summary (7), six recent moderate- to high-quality meta-analyses that met these definitions were identified, three addressing blood-based samples and three addressing BAL-acquired samples.

Three meta-analyses (31–33) investigating the performance of *Aspergillus* PCR in blood-derived specimens reported results from a total of 37 nonredundant published studies, representing data from 3,955 unique patients. The meta-analysis by Cruciani and colleagues (32) analyzed studies of PCR diagnostic performance from whole blood or serum, reporting a mean sensitivity of 80.5% (95% CI, 73.0–86.3) and a mean specificity of 78.5% (95% CI, 67.8–86.4) when a single positive PCR test result is used to diagnose IPA. When two consecutive positive test results are required to define disease, the authors find a mean sensitivity of 58.0% (95% CI, 36.5–76.8) and a mean specificity of 96.2% (95% CI, 89.6–98.6). In their meta-analysis, Arvanitis and colleagues (31) included studies assessing PCR performance in blood or serum, and they reported a mean sensitivity of 84% (95% CI, 75–91) and a mean specificity of 76% (95% CI, 65–84). Separate analysis of studies that required at least two positive PCR test results to define disease demonstrated a mean sensitivity of 64% (95% CI, 38–84) and a mean specificity of 95% (95% CI, 88–98). In another meta-analysis, Mengoli and colleagues (33) reported the diagnostic performance of PCR in studies from whole blood, serum, or plasma. If only a single positive sample was required, sensitivity was 88% (95% CI, 75–94) and specificity was 75% (95% CI, 63–84). Sensitivity and specificity of PCR for two consecutive positive samples were 75% (95% CI, 54–88) and 87% (95% CI, 78–93), respectively.

Three meta-analyses (34–36) of BAL-derived samples reported results from 26 nonredundant studies, incorporating data from 1,872 unique patients. The meta-analysis by Avni and colleagues (34) analyzed data from studies assessing the performance of BAL *Aspergillus* PCR relative to BAL GM, reporting PCR specificity to be uniformly high with a mean of 96.4% (95% CI, 93.3–98.1), though the sensitivity revealed greater variability between studies with a mean of 90.2% (95% CI, 77.2–96.1). The meta-analysis by Sun and colleagues (35) found that the mean sensitivity of BAL PCR was 91% (95% CI, 79–96) and the mean specificity was 92% (95% CI, 87–96). The meta-analysis by Tuon (36) also reported uniformly high BAL PCR specificity (mean, 94%) with lower and more variable sensitivity (mean, 79%).

Recommendations. In patients with severe immunocompromise, such as those with hematological malignancy or recipients of hematological stem cell or solid organ transplants who are suspected of having IPA, we recommend the use of blood or serum *Aspergillus* PCR testing (*strong recommendation, high-quality evidence*).

- *Technical remark*: Use of a single positive PCR result as the criterion for the diagnosis of IPA provides moderately high sensitivity to exclude disease, whereas requiring two consecutive positive test results to define disease provides very high specificity.
- *Technical remark*: The studies supporting the recommendation to use of blood and serum *Aspergillus* PCR testing address patients suspected of having IPA. In patients with low pretest probability for IPA who are found to have a positive blood or serum *Aspergillus* PCR result, additional testing (e.g., BAL) may be warranted.

In patients with severe immunocompromise, such as those with hematological malignancy or recipients of hematological stem cell or solid organ transplants who are suspected of having IPA, we recommend the inclusion of *Aspergillus* PCR in BAL testing as part of the evaluation (*strong recommendation, high-quality evidence*).

In patients with severe immunocompromise, such as those with hematological malignancy or recipients of hematological stem cell or solid organ transplants who are strongly suspected of having IPA but in whom the result of PCR testing for *Aspergillus* is negative, we recommend consideration of biopsy and/or additional testing with or without additional PCR or GM testing (*conditional recommendation, low-quality evidence*).

Rationale. The two strong recommendations for the use of *Aspergillus* PCR testing (blood and BAL) arise from the clinical need to rapidly and accurately identify patients with a potentially fatal infection in whom the gold standard test of lung biopsy is precluded by other clinical characteristics. These strong recommendations are also supported by the robust performance of these tests in multiple recent clinical trials.

Using a single positive blood PCR test result to define IPA disease in a hypothetical population of 1,000 patients with a disease prevalence of 10%, if we consider the data from the trials reported above in aggregate, results in identification of 84 true positives (patients with disease) and 689 true-negative findings (patients free of disease). This is also expected to result in 212 false positives (the PCR test is positive, but the patient is free of disease) and 16 false-negative findings (the PCR test is negative, but the patient has disease). These hypothetical outcomes reflect positive and negative predictive values of 28% and 50%, respectively, resulting in positive and negative likelihood ratios of 3.58 and 0.21, respectively. Requiring two consecutive positive blood test results in the same population is expected to result in 66 true positives, 835 true-negative findings, 65 false positives, and 35 false-negative findings. These hypothetical outcomes reflect positive and negative predictive values both of 50%, resulting in positive and negative likelihood ratios of 9.04 and 0.37, respectively. Thus, the blood *Aspergillus* PCR test is noninvasive, addresses a clinically important concern, and is readily available in most hospitals, and testing criteria can be modified to fit the clinical scenario.

The diagnostic performance of BAL *Aspergillus* PCR is also robust. Serial BAL samples are not feasible in most patients; thus, only one BAL PCR test per patient is considered in this recommendation. Taking the above studies in aggregate, in a hypothetical population of 1,000 patients with an IPA disease prevalence of 10%, this test would be expected to yield 66 true positives, 835 true negatives, 34 false negatives, and 65 false positives. These hypothetical outcomes reflect positive and negative predictive values of 62% and 50%, respectively, resulting in positive and negative likelihood ratios of 14.78 and 0.14, respectively. Therefore, the BAL *Aspergillus* PCR test is viewed as providing actionable clinical information.

Implementation and limitations. The patients studied in the trials that contributed

to these recommendations were predominantly patients with hematological malignancies or hematological stem cell transplant recipients. Application of these results to other immunocompromised populations should be approached with caution.

These recommendations address only adult patients, though a small number of contributing studies allowed inclusion of pediatric and adolescent patients. The effect of these inclusions is not clear.

These recommendations address only patients suspected of having IPA, and test performance has not been assessed as a screening tool. Patients in whom the result of a serum or blood *Aspergillus* PCR test is positive but in whom other findings suggest diagnoses besides IPA may warrant additional testing (e.g., BAL).

Although ongoing use of antifungal therapy was clearly stated in many contributing studies, it is not described in others. Prophylactic antifungal therapy is generally recommended for immunocompromised patients during periods of peak vulnerability, but it is not clearly established how this impacts PCR test performance, because PCR can detect very low copy numbers.

Relatedly, whereas active antifungal therapy reduces the sensitivity of GM testing for IPA, the ability of PCR to detect low copy numbers likely makes it an attractive option for assessing patients receiving active antifungal therapy. Alternately, this high sensitivity may not allow reliable differentiation of *Aspergillus* colonization and IPA in BAL PCR testing.

The trials that contributed to these recommendations used nonuniform molecular techniques with variations in the methods used to disrupt the fungal cell wall, the DNA extraction methods, the primer target genes, and the PCR cycle number thresholds to determine negative test results. The effect of these variations is unclear. In addition, the presence of endogenous PCR inhibitors that can occur in biological samples can also act to reduce the sensitivity of PCR testing.

For both the blood and BAL sections, some primary studies were included in more than one meta-analysis. This may result in certain primary studies exerting disproportionate influence on the recommendations. However, the fact that such studies met inclusion criteria for two or more high-quality meta-analyses likely suggests methodological rigor, potentially offsetting the concern of overrepresentation.

Future research. Additional studies in IPA-susceptible populations beyond patients with hematological malignancies or recipients of hematological stem cell transplants are needed to clarify the generalizability of the results described above to other groups. Investigations to determine the optimal methods to disrupt the fungal cell wall, to extract DNA, to target fungal genes, and to set PCR cycle number thresholds to determine negative test results are warranted, as are studies to determine the effects of active antifungal therapy on test performance. Outcomes research is needed to determine the effectiveness of PCR-based testing to improve critical endpoints, such as survival. These studies may further clarify the impact of requiring one or two positive blood test results. Future studies may also be warranted to determine whether the concomitant use of PCR- and GM-based tests should become a new gold standard for patients with thrombocytopenia, in whom biopsy is often precluded.

Question 3

In critically ill patients with suspected IC, is the BDG assay alone sufficient for diagnostic decision-making?

Background. IC, of which candidemia is one manifestation, is the most common deep-seated mycosis in critically ill patients and is associated with a crude mortality rate exceeding 50% in some ICU settings (37). That number can rise further in cases of septic shock (38). Although IC is especially prevalent among those with neutropenia, profound immunocompromise is not required for infection. Other predisposing factors, including mucosal disruption and use of broad-spectrum antibacterial agents, are commonplace among critically ill adults. The definitive diagnosis of IC is established when *Candida* spp. are identified in tissue specimens from normally sterile body sites or if culture of a normally sterile fluid yields the organism. In the critically ill population, invasive diagnostic methods are often infeasible, and the delays inherent in awaiting culture results can deprive patients of prompt antifungal therapy. Delayed treatment has been associated with adverse outcomes in IC (39), which has generated interest in biomarker-guided empirical therapy of suspected but unproven infection. Perhaps the most commonly used blood assay for this purpose is the detection of BDG, a cell wall product expressed by most fungal pathogens, with the notable exception of the *Mucormycetes* and *Cryptococcus* spp. The U.S. Food and Drug Administration added BDG (Fungitell; Associates of Cape Cod) to its register of approved serological tests in 2004 (40), and since then, use of this assay has steadily evolved despite multiple causes of false positivity, among them particular antibiotics, blood transfusions, and infusion of intravenous immunoglobulin (41), and despite uncertainty about its ability to differentiate infection from colonization in the critically ill population (42).

Summary of evidence. Performance of a focused literature search yielded 87 potentially relevant studies, 32 of which were deemed sufficiently applicable to be subjected to full-text review. Ultimately, a total of 10 ICU-based studies encompassing 1,510 subjects were pooled to determine the performance characteristics of BDG in the critically ill population (43–52). Eight of these 10 studies used the manufacturer's recommended positivity criterion of 80 pg/ml, whereas two (44, 50) used different cutoffs, so a separate pooled analysis including data only from the studies with the 80 pg/ml threshold was conducted, which amounted to a total of 1,218 patients.

When the results of all 10 studies reporting, on a per-patient basis, the performance characteristics of BDG in potential cases of IC in the ICU were pooled, the cumulative sensitivity was 0.81 (95% CI, 0.74–0.86) and the cumulative specificity was 0.60 (95% CI, 0.49–0.71). The corresponding positive likelihood ratio was 2.00 (95% CI, 1.5–2.8), and the corresponding negative likelihood ratio was 0.32 (95% CI, 0.21–0.49). The resultant diagnostic odds ratio was 6.00 (95% CI, 3–13). Limiting the pooled analysis to the eight studies that used a BDG positivity threshold of 80 pg/ml led to very little difference in the overall performance characteristics: sensitivity, 0.81 (95% CI, 0.73–0.87); specificity, 0.61 (95% CI, 0.46–0.75); positive likelihood ratio, 2.10 (95% CI, 1.40–3.10); negative likelihood ratio, 0.32 (95% CI, 0.19–0.51); and diagnostic odds ratio, 7.00 (95% CI, 3.00–16.0).

Recommendation. In critically ill patients in whom there is clinical concern for IC, we suggest against reliance solely on results of serum BDG testing alone for

diagnostic decision-making (*conditional recommendation, low-quality evidence*).

- *Technical remark*: Although the committee did not endorse the use of BDG as a stand-alone test, it allows that BDG may nevertheless have utility when combined with clinical risk determination and microbiological data in identifying patients at risk for IC.

Implementation and limitations. IC is a prevalent infection in the critically ill population that can pose a grave threat to their survival. Delays associated with diagnosing this potentially lethal mycosis using traditional methods place high value on biomarkers such as BDG for early identification and thereby prompt treatment of IC cases. Echinocandins, the currently recommended first-line agents for the treatment of IC in the ICU, are generally well-tolerated antifungal medications, so avoidance of drug toxicity from overdiagnosis is not a highly valued outcome in this context relative to the timely detection of IC. Therefore, the most essential characteristic of BDG should be a very high sensitivity for IC in the critically ill population. The issuance of a conditional recommendation against the use of BDG as an isolated criterion for diagnosis was based primarily on its low sensitivity of 0.81 for such a life-threatening infection.

In a hypothetical population of 1,000 random ICU patients in which the prevalence of IC is assumed to be 10% (i.e., 100 cases of the disease), the pooled performance characteristics of BDG reported above based on the eight studies that used a positivity threshold of 80 pg/ml would yield 81 true-positive results, 19 false-negative results, 351 false-positive results, and 549 true-negative results. The corresponding positive predictive value and negative predictive value would be 0.19 and 0.50, respectively. This means that for every 1,000 patients who undergo BDG testing, 81 would be correctly identified as having IC, 19 cases of IC would be missed, and 351 patients would be incorrectly identified as having IC. Applying the obtained likelihood ratios to a hypothetical patient with 50% pretest probability of having IC, an elevated BDG would result in a posttest probability of 68%, whereas a negative BDG would yield a posttest probability of 24%. Thus, in equivocal cases, in which BDG testing would be most useful, the performance characteristics of BDG in isolation do not allow the clinician to confidently diagnose or exclude IC.

The low to moderate confidence in the pooled results of the included studies stems partially from their heterogeneity and the methodological flaws from which several of them suffer. Notably, all of the studies with the exception of the one by Posteraro and colleagues investigated the performance characteristics of BDG as a surveillance tool for detection of occult IC in at-risk patients, whereas the question posed herein is about the role of BDG as a diagnostic tool in the setting of clinically suspected IC (45). Nine of the 10 included studies collected serum samples for BDG at prespecified time points that were independent of the concurrent clinical suspicion for IC in any given subject. The use of BDG in that fashion is to assist in screening a vulnerable population such as the critically ill and to potentially initiate so-called preemptive therapy in positive cases. Therefore, from an application standpoint, the preponderance of the evidence on which this recommendation is based is indirect. Encouragingly, in the Posteraro and colleagues study of BDG measurement at sepsis onset on Day 5 or later of ICU stay (45), as opposed to scheduled measurements employed in the other studies, the performance characteristics of BDG were substantially better than our overall pooled results: sensitivity, 0.93 (95% CI, 0.66–1.00); specificity, 0.94 (95% CI, 0.47–0.90); positive likelihood ratio, 14.74 (95% CI, 4.65–47.5); and negative likelihood ratio, 0.07 (95% CI, 0.02–0.39).

It should also be noted that BDG testing is not specific for *Candida*, such that *Aspergillus*, *Pneumocystis*, and other fungal infections can likewise yield positive results. Accordingly, the committee views BDG as a general indicator of potential invasive fungal infection (41) and proposes the concept of BDG acting akin to a fungal "sedimentation rate." It is in that application that BDG may have value, especially in combination with host factors and other laboratory results, for its negative predictive value to help assess a reduced likelihood of invasive fungal infection.

Because the incorporation of BDG into the evaluation of suspected IC is inextricably linked to decisions regarding empirical therapy, limitations related to the administration of such treatment are worth noting. A large 2015 prospective study failed to demonstrate improved survival with empirical therapy for IC using a variety of antifungal agents in a mechanically ventilated ICU cohort (53). The next year, EMPIRICUS (Empirical Antifungal Treatment in ICUs), a placebo-controlled randomized clinical trial of micafungin as empirical therapy for IC, likewise showed no survival benefit in mechanically ventilated ICU patients (54). Importantly, benefit of empirical treatment was absent regardless of predefined stratification by BDG level greater than or less than 80 pg/ml at enrollment.

Future research. The uncertain role of biomarkers such as BDG for the early identification of IC in suspected ICU cases is primarily due to two unanswered questions. The first is the biomarkers' ability to detect IC and differentiate infection from colonization. Other blood tests, among them antibodies against *Candida albicans* germ tube and *Candida* PCR, appear to share BDG's problems with specificity in the often heavily colonized ICU population. The NOBICS clinical trial (Novel Biomarker in Invasive Candidiasis/Candida Sepsis; www.clinicaltrials.gov identifier NCT02801682) being conducted in Austria aims to evaluate the ability of various cytokine and noncytokine serological assays to detect early IC in the ICU while minimizing false positivity in colonized patients. In addition to the development of novel biomarkers, future research should include understanding and overcoming the specificity limitations of currently available serological tests as well as investigation into possible combined approaches that might consist of multiple biomarkers with or without a clinical prediction rule. The other open question is whether BDG-triggered empirical antifungal therapy improves clinically relevant outcomes in the critically ill population. Multiple German centers are currently collaborating in a clinical trial of culture-based antifungal therapy versus BDG-based treatment of IC in patients with sepsis or septic shock (CandiSep [(1,3)-β-D-Glucan Based Diagnosis of Invasive *Candida* Infection in Sepsis], www.clinicaltrials.gov identifier NCT02734550). The primary endpoint of this trial is 28-day mortality. Additional laboratory research for newer testing

modalities for candidiasis are also currently underway.

Question 4

Should diagnosis of the common endemic mycoses (i.e., histoplasmosis, blastomycosis, and coccidioidomycosis) be based on serology and antigen testing?

Histoplasmosis. BACKGROUND. Histoplasmosis is the most prevalent endemic mycosis in North America. With the continually expanding at-risk population and widening areas of endemicity (55, 56), the need to better understand the optimal diagnostic methods for histoplasmosis is pressing. The mortality in histoplasmosis is estimated to be 5% in children and 8% in adults (57). Fungal cultures are the gold standard test to confirm the diagnosis. However, they can be technically challenging and are time consuming. Rapid diagnosis is essential in patients with severe infections such as disseminated and severe acute pulmonary histoplasmosis to allow for early initiation of appropriate antifungal therapy, potentially improving outcomes.

Antigen and antibody detection are currently the most widely accepted non–culture-based methods to diagnose histoplasmosis. Their diagnostic yields depend on the clinical presentations, infectious burden, immune status, and timing in relation to the infection. The laboratory methods used to detect *Histoplasma* antigen and antibodies have been updated in the last decade to include quantitative methods and lower thresholds of detection that have improved their sensitivity and allowed for comparison of titers and monitoring response to treatment. Cross-reactivity with other endemic fungal pathogens is problematic (58, 59). *Histoplasma* antigen was studied mostly in patients with disseminated histoplasmosis, whereas studies of antibodies examined patients with predominantly pulmonary histoplasmosis.

SUMMARY OF EVIDENCE. Histoplasma *antigens.* A recent meta-analysis examined the diagnostic yield of *Histoplasma* antigen (60). A total of nine studies were analyzed, including seven on urine antigen and six on serum antigen with a total of 1,029 cases and 2,195 controls. Disseminated histoplasmosis was the predominant diagnosis, followed by pulmonary histoplasmosis. All patients with the disseminated infection were immunocompromised. Overall, the sensitivity of *Histoplasma* antigen detection was 81.4% (95% CI, 79.1–83.5%), and the specificity was 98.3% (95% CI, 97.7–98.7%). The overall sensitivity was slightly higher for antigenemia (83.9%; 95% CI, 80.5–87%) than for antigenuria (79.5%; 95% CI, 76.3–82.4%). Specificities were comparable in serum and urine. On the basis of a positive likelihood ratio of 43.2 and a negative likelihood ratio of 0.18, *Histoplasma* antigen was determined to be an excellent discriminator between healthy and ill individuals, with an odds ratio of 321 (95% CI, 118–875) and an area under the curve of 0.98.

Two additional studies of *Histoplasma* antigen met our inclusion criteria but were not included in this meta-analysis (61, 62). Both studies examined patients with acute pulmonary histoplasmosis as part of outbreak investigations with significant overlap in their patient populations. Sensitivity of *Histoplasma* antigen was lower in these studies (64–68%) than in the meta-analysis. However, combining urine and serum antigen testing increased the sensitivity significantly to 93%. Similarly, combining antigen and antibody testing further increased the sensitivity to 96.3% in acute pulmonary histoplasmosis (61).

Histoplasma *serologies (antibody detection).* Fewer studies examining the use of *Histoplasma* serologies met our inclusion criteria. The yield of *Histoplasma* serology depends on the immune status of the patient and the timing of testing in relation to the infection (63). The sensitivity of serology in immunocompetent patients with pulmonary histoplasmosis ranges between 80% and 95%, in contrast to the unacceptably low sensitivity in recipients of organ transplants (18%) (64) and those with HIV–AIDS (45%) (65, 66). The specificity of *Histoplasma* serology is excellent, given the low levels of background false positivity ($<$5%) in residents of endemic areas outside periods of outbreaks (67).

Although complement fixation (CF) and gel diffusions are the predominantly used methods for *Histoplasma* serology, more recent enzyme immunoassay (EIA)-based quantitative assays are currently available to measure serum levels of IgG and IgM, with sensitivity of 87% for IgG and 67% for IgM and specificity of 95% and 96%, respectively (61).

RECOMMENDATIONS. We recommend the use of *Histoplasma* antigen in urine or serum for rapid diagnosis of suspected disseminated and acute pulmonary histoplasmosis, in which timely diagnosis and treatment are paramount to outcome (*strong recommendation, high-quality evidence*).

- *Technical remark*: The systematic review which we cited showed that the urine and serum antigen testing results were comparable. There are more data for the use of *Histoplasma* antigen in urine, but at this time, we would not limit the testing to either sample source. Therefore, we prefer to state urine or serum. To date, there are no convincing data for combining both.

We suggest the use of *Histoplasma* serologies in immunocompetent patients with suspected pulmonary histoplasmosis. Adding *Histoplasma* antigen to serological testing might improve the diagnostic yield (*conditional recommendation, moderate-quality evidence*).

RATIONALE AND IMPLEMENTATION CONSIDERATIONS. *Histoplasma* antigen appears to be most useful in patients with high fungal burden (i.e., those with disseminated or acute pulmonary histoplasmosis, in whom antibody detection is not as reliable, especially early after the exposure and in those unable to mount an antibody response due to immunosuppression). *Histoplasma* antigen testing is available in specialized centers or through mail-in reference laboratories. Combining antibody and antigen testing further improves the diagnostic yield in pulmonary histoplasmosis (61). In addition to serum and urine, *Histoplasma* antigen can be detected in cerebrospinal fluid (68) in *Histoplasma* meningitis and in BAL (69) in pulmonary histoplasmosis.

In a hypothetical population of 1,000 random patients who reside in an endemic area with a prevalence of 10%, testing for *Histoplasma* antigen would yield 81 true-positive results, 19 false-negative results, 15 false-positive results, and 885 true-negative results, with a positive predictive value of 0.84 and a negative predictive value of 0.50. During a period of outbreak when the prevalence in the at-risk population can be as high as 25%, the yield would be 204 true positives, 46 false positives, 13 false positives, and 737 true negatives, with a positive predictive value of 0.94 and a negative predictive value of 0.50. However, a positive *Histoplasma* antigen in an

immunocompromised patient with suspected histoplasmosis (0.5 pretest probability) would result in a posttest probability of 0.98, given the positive likelihood ratio of 43.2.

In addition to its excellent diagnostic yield, *Histoplasma* antigen can also be used to gauge the severity of the infection because its level correlates with severity of illness; in one study, the positive predictive value of an antigen level greater than 16 pg/ml was 88% for moderate to severe histoplasmosis (64), for which patients need to be treated with amphotericin B on an inpatient basis (70). Antigen levels can also be used to monitor patients' progress while receiving antifungal therapy because levels decrease with effective therapy (59), and their increase predicts relapse (71).

The two major limitations of *Histoplasma* antigen are the cross-reactivity with other endemic mycoses and the availability of testing being restricted to reference laboratories in the United States. *Histoplasma* antigen is detected in the majority of patients with other endemic fungal infections, including blastomycosis, coccidioidomycosis, paracoccidioidomycosis, and talaromycosis (58). The limited commercial availability of the test is a major limitation, especially in endemic areas outside the United States, such as Latin America and Africa. Currently, the test is offered by few reference laboratories, which may delay testing and results reporting, which in turn limits its utility for rapid diagnosis in severe infections.

FUTURE RESEARCH. Large studies of simultaneous testing of antigens and antibodies in various forms of histoplasmosis are needed to better define their respective roles in the diagnosis and monitoring of the disease while treatment is administered. Furthermore, the newer serological testing of IgG and IgM will need to be tested in larger and more heterogeneous groups of patients with histoplasmosis to better define its diagnostic role. Molecular methods have been developed for the identification of *Histoplasma* in culture and in biological samples, but they have not been well validated in large clinical cohorts of histoplasmosis. Such testing might prove very useful, especially if made available for local laboratory testing.

The ability to perform antigen testing at local laboratories using prepackaged testing kits or newer testing platforms will have an impact on the rapid diagnosis of histoplasmosis in endemic areas that are not in close proximity to reference laboratories, especially in resource-limited areas.

Blastomycosis. BACKGROUND. Blastomycosis is caused by *Blastomyces dermatitidis*, a dimorphic fungus endemic to the central and southeastern United States, resulting in acute, subacute, and chronic lung infections and much less commonly in the adult respiratory distress syndrome or severe diffuse pneumonia (72). Most presentations are far less severe, such as lobar pneumonia, masslike consolidations, nodules, or chronic fibrocavitary disease. Dissemination from the lung is uncommon, but spread to skin, bone, genitourinary tract, and uncommonly the central nervous system can occur. In immunosuppressed patients, the disease is accelerated and can be life threatening. Accurate diagnosis of blastomycosis can be challenging, and successful diagnosis may employ cytopathological visualization of organisms from respiratory secretions and BAL, culture, serum antibody testing, antigen testing approaches, and more recently PCR strategies. Effective diagnosis is essential for implementing appropriate therapeutic agents. Comprehensive review of the literature revealed relatively few studies addressing this topic. In particular, studies directly comparing various traditional diagnostic modalities, such as visualization of *B. dermatitidis* and culture, with recent diagnostic strategies, particularly antigen testing, were not found. In addition, literature reviews of antibody testing included a variety of test strategies and assays. Accordingly, our approach for these guideline recommendations employs a narrative of our review of the relevant studies of the major testing strategies that met our criteria for inclusion in this review.

SUMMARY OF EVIDENCE. *Blastomycosis urinary antigen.* The diagnostic accuracy of blastomycosis antigen testing in urine, serum, and BAL has been studied. A retrospective analysis of blastomycosis urinary antigen testing in 42 confirmed cases of infection revealed an overall sensitivity of 92.9% and a specificity of 79.3% compared with healthy controls (73). These cases included both patients with localized pulmonary disease and patients with disseminated infection. It should be noted, however, that blastomycosis urinary antigen testing was observed to have high rates of cross-reactivity with histoplasmosis, paracoccidioidomycosis, and talaromycosis. The assay did not seem to cross-react with candidiasis or coccidioidomycosis. Rare cross-reactivity occurred with aspergillosis (73). An additional study of 67 patients with confirmed blastomycosis also evaluated antigen testing in urine, serum, and BAL (74). The overall reported sensitivities of antigen testing were 76.3% in urine, 55.6% in serum, and 62.5% in BAL. Assay specificity characteristics were not included in the study. Hence, urinary antigen testing may be a useful component to consider in the diagnostic evaluation of suspected blastomycosis. However, overall sensitivity and specificity characteristics of these assays are not yet optimal, and the assay has significant cross-reactivity with other systemic mycoses, particularly histoplasmosis.

Serum antibody testing. Serum antibody testing was analyzed in one study meeting our inclusion criteria (75). In that analysis, 41 cases of blastomycosis (39 proven, 2 probable), 50 cases of histoplasmosis, and 124 normal or clinical controls were analyzed using an EIA recognizing the *B. dermatitidis* surface protein BAD-1. The overall sensitivity of serum antibody testing in patients with proven or probable blastomycosis was 87.8%. Positive serum antibody testing was observed in 6% of the cases of histoplasmosis and in 2% of normal patients living in an endemic region. There were no observations of positive blastomycosis serum antibody testing results from controls without fungal disease or from normal healthy control individuals who did not live in endemic regions. Earlier investigations using immunodiffusion (ID) serum antibody testing recognizing the A antigen of *B. dermatitidis* exhibited sensitivities ranging from of 28% to 64% (76–78). Although serological reactivity to *B. dermatitidis* may diminish over time, the exact time course has not been well established.

Microscopic visualization. Visualization of typical blastomycosis organisms in freshly prepared respiratory secretions or in histopathology can rapidly confirm the diagnosis of infection. The diagnostic yield of visualized organisms on KOH preparations of respiratory secretions including BAL was reported as 36% when a single sample was studied and 46% in confirmed cases with multiple specimens (79). The diagnostic yield of culture

was up to 86% across multiple specimens from the same patient and 75% from a single specimen. However, culture positivity could take up to 5 weeks for completion (79).

RECOMMENDATIONS. In patients with appropriate geographic exposure and illness compatible with infection or pneumonia due to blastomycosis, we suggest using more than one diagnostic test, including direct visualization and culture of sputum BAL or other biopsy material, urine antigen testing, and serum antibody testing. The current evidence cannot support a single best test as being sensitive enough to be ordered in isolation of other testing. The approach should be tailored on the basis of severity of illness, the clinical context, and the availability of tests (*conditional recommendation, moderate-quality evidence*).

- *Technical remark*: Although microscopic visualization and culture to identify blastomycosis organisms are quite specific, they lack sensitivity, and hence the addition of serum antibody testing and urine antigen testing increases the overall diagnostic yield.

In patients with suspected blastomycosis, we suggest that serum antibody testing specifically directed against the anti–BAD-1 antigen for blastomycosis be used together with clinical and epidemiological data to establish the diagnosis (*conditional recommendation, low-quality evidence*).

- *Technical remark*: Serum antibody assays can have significant cross-reactivity to other endemic mycoses, particularly histoplasmosis.

In patients with suspected blastomycosis, particularly in immunocompromised patients, we suggest that urinary antigen testing for blastomycosis be used together with clinical and epidemiological data to establish the diagnosis (*conditional recommendation, moderate-quality evidence*).

- *Technical remark*: Although the assay has reasonable sensitivity and good specificity in patients without infection, the assay does have substantial cross-reactivity to other mycoses, including histoplasmosis, paracoccidioidomycosis, and talaromycosis.

RATIONALE. None of the widely available diagnostic approaches for the diagnosis of blastomycosis exhibit consistently high performance characteristics to recommend using a single test in isolation from other approaches. For instance, direct visualization of blastomycosis organisms, though specific and timely, exhibits suboptimal sensitivity and yield. Culture of respiratory secretions, including BAL, has reasonably high sensitivity; yet, the diagnosis may be delayed for up to 5 weeks with this approach. Antigen testing, particularly as applied to urine, can be a useful diagnostic adjunct with good sensitivity. However, antigen assays do exhibit significant cross-reactivity to other endemic mycoses. Serum antibody testing has been variably reported to exhibit low to moderate sensitivity, but again it carries the risk of cross-reactivity with other fungal infections. PCR approaches are emerging but are not yet widely available in clinical settings and have not been analyzed in large clinical studies. The clinician confronted with a case of possible blastomycosis should consider using a variety of assays to optimize the overall diagnostic yield and to prevent overdiagnosis of blastomycosis occurring through cross-reactivity of assays with other fungi, particularly histoplasmosis.

IMPLEMENTATION AND LIMITATIONS. Direct visualization of blastomycosis organisms, such as by KOH-treated preparation of respiratory secretions and BAL, can rapidly and specifically confirm the diagnosis, but it requires local expertise. Culture can also be performed at local or regional referral laboratories using standardized protocols. Unfortunately, fungal cultures can take a number of weeks. *Blastomyces* urinary antigen testing is commercially available through mail-in reference laboratories. In a similar fashion, serum antibody testing can also be performed either locally or through commercial laboratories. Emerging PCR essays for blastomycosis are available in some tertiary referral centers and may also be accessed through reference laboratories.

FUTURE RESEARCH. The diagnosis of endemic mycoses such as blastomycosis remains a clinical challenge that clearly will benefit from further investigation. Comparative analysis of the various diagnostic modalities is required.

Coccidioidomycosis. BACKGROUND. Coccidioidomycosis is an endemic mycosis caused by the fungi *Coccidioides immitis* and *Coccidioides posadasii*, causing its highest incidence in the San Joaquin Valley of California, in south-central Arizona, and in northwestern Mexico (80). The disease can range from a subclinical, self-resolving illness in most cases to life-threatening pulmonary or disseminated disease requiring admission to the ICU. Immunosuppression is a major risk factor for dissemination and severe disease, similar to its risk for other endemic mycoses. Unlike other endemic mycoses, however, ethnic (i.e., Filipino and African American) predisposition to dissemination is well characterized. Coccidioidomycosis has emerged as a common cause of CAP, with estimates that 15–30% of CAP reported in endemic areas being caused by *Coccidioides* (81, 82). The number of coccidioidomycosis cases has been increasing over the last decade among residents of and visitors to endemic areas (83, 84).

Diagnosis is clinically challenging. Culture is the gold standard for diagnosis, but the result is positive in only about half of the cases in immunocompromised patients, and culture usually does not provide the initial basis for diagnosis. In addition, results of cytopathology or histopathology are positive in only 20–30% of immunocompromised patients (85–89). In the outpatient setting, obtaining cultures for *Coccidioides* is uncommon, but eosinophilia can be a clue (90, 91). Studies often do not specify if diagnosis was made in the context of CAP. Serological methods, including ID, CF, and EIA, provide the laboratory basis for diagnosis in most cases (92), but they may provide false-negative findings in immunocompromised patients (88, 89, 93) as well as during the first few months after acute infection (94, 95). Serological testing for CAP in *Coccidioides* endemic areas is surprisingly uncommon (93). EIA often is used as an initial diagnostic test, followed by ID and/or CF if the EIA result is positive. It may also be used as a screening test for subclinical or past infection before the initiation of immunosuppression (85, 92, 93).

SUMMARY OF EVIDENCE. Comprehensive review of the literature revealed relatively few studies addressing this important topic. In particular, studies directly comparing gold standard diagnostic modalities such as visualization of organisms and culture with newer diagnostic strategies

were sparse. Our review of the literature revealed that antigen detection studies often were conducted in immunosuppressed populations in which cultures were frequently obtained. Serological antibody testing, however, often relied on clinical diagnosis as the standard and included a variety of test platforms and assays.

One study met the criteria for analysis with comparison of antigen with culture/cytology/histopathology. An analysis of *Coccidioides* polysaccharide urine antigen testing in 24 confirmed cases revealed an overall sensitivity of 70.8% and a specificity of 97.8% compared with all healthy and disease control subjects (96). These cases represented a group of patients that included many with severe and disseminated disease, and 79.2% were immunosuppressed. *Coccidioides* urinary antigen testing was observed to have cross-reactivity with other endemic fungal pathogens in approximately 10% of cases.

In a subsequent laboratory-based study by the same group, cases were categorized as definite (positive result of culture or cytology; $n = 9$) or probable (positive serology result or clinical diagnosis; $n = 19$). The characteristics of serum testing were similar to those of urine testing in the original study, with a sensitivity of 73.1% (97). Serum and antigen testing appear complementary, with some samples being positive in either serum or urine but not both.

Serum antibody testing has a rich history and clinical utility in the diagnosis of coccidioidomycosis. However, studies evaluating serology are hampered by changing platforms and methodologies. In addition, studies infrequently have culture or cytology data as the gold standard comparator.

In a laboratory-based study of 47 samples from subjects with proven coccidioidomycosis, EIA was 100% sensitive and 96% specific, and ID was 100% specific (98). In a comprehensive descriptive study in which confirmed cases were defined as having either a confirmatory positive CF result or positive culture/histology result, 273 positive coccidioidomycosis EIA test results yielded a sensitivity of 81.6% (99).

A retrospective chart review study of 360 subjects (31% with definite coccidioidomycosis, 83% immunocompetent, 88% with disease confined to thorax) showed varying sensitivity. Analysis included multiple serological tests, and serological positivity was used to define the probable group. Sensitivity was highest for the EIA (67% for immunosuppressed group, 87% for healthy group). When positivity for any serological method was taken into consideration, the sensitivity of serology in immunosuppressed subjects was 84% compared with 95% in healthy, immunocompetent subjects (93).

Finally, a retrospective chart review in 27 solid organ transplant recipients with newly diagnosed coccidioidomycosis showed sensitivities that ranged from 21% for IgM ID to 56% for IgG EIA. When multiple and serial serologies were employed, sensitivity increased incrementally to 92% (100).

Recommendations. In patients with appropriate geographic exposure and illness compatible with infection or pneumonia due to coccidioidomycosis, we suggest using more than one diagnostic test, including direct visualization and culture of sputum, BAL, or other biopsy material; urine and serum antigen testing; and serology (serum antibody testing). The current evidence cannot support a single best test. The approach should be tailored on the basis of severity of illness, the clinical context, and the availability of tests (*conditional recommendation, moderate-quality evidence*).

- *Technical remark*: Although microbiological culture or organism visualization is quite specific to identify *Coccidioides* organisms, it lacks overall sensitivity, and hence the addition of serum antibody testing and urine and serum antigen testing increases the overall diagnostic yield.

In patients with suspected coccidioidomycosis, particularly in immunocompromised patients, we suggest performing urinary and serum antigen testing to aid in establishing the diagnosis (*conditional recommendation, moderate-quality evidence*).

- *Technical remark*: *Coccidioides*-specific antigen testing is performed at a few reference laboratories. False positives and false negatives occur. *Coccidioides* infection also can result in a positive BDG test result.

In patients with suspected CAP from endemic areas, we suggest initial serological testing with close clinical follow-up and serial testing (*conditional recommendation, moderate-quality evidence*).

- *Technical remark*: EIA panels, compared with ID and CF methods, offer the advantage of local availability with rapid turnaround and higher sensitivity, although they are less specific.

Rationale. Direct visualization and culture of *Coccidioides* organisms has low sensitivity and thus cannot be recommended as a single test despite its high specificity. *Coccidioides* antigen testing is less sensitive than *Histoplasma* urine and serum antigen testing but can be useful, particularly in immunocompromised patients. Serum antibody testing has been variably reported, and different assays have different test characteristics. Algorithms for serological testing have been published (92) and endorse initial EIA testing followed by serial or confirmatory ID or CF. Cross-reactivity with other fungal infections does occur, and the clinician should use care in interpreting both positive and negative results in the context of symptomatology and radiological findings.

Implementation and limitations. Antigen testing is available through reference laboratories. Cross-reactivity with other endemic fungal pathogens is a limitation, and nonspecific antigen detection assays, including BDG, can be confusing (85, 96, 97). EIA is able to be performed locally and is highly sensitive and semiquantitative, depending on the assay. Traditional ID and CF testing is available at reference laboratories and useful to confirm IgG positivity. ID and CF are commonly performed to obtain a titer, which can be followed. There is controversy about EIA IgM positivity in isolation, and confirmatory and serial testing is recommended (99–102). Although commercially available EIA kits have comparable testing characteristics, particularly when IgM and IgG are both considered in a clinical context (103), new assays with different capture antigens and quantitation are available at reference laboratories (104). It should also be noted that serological testing for coccidioidomycosis can wane over time, and results might become negative after successful treatment of infection.

Future research. The diagnosis of coccidioidomycosis remains a clinical

challenge. Additional studies evaluating the role of skin testing (105), immunodiagnosis (106), and molecular diagnosis (107) are emerging. Comparative analyses of the new serological assays will also be important to refine clinicians' thinking and define best practices in coccidioidomycosis diagnosis.

Conclusions

With the rising incidence of invasive fungal infections in both immunocompromised and immunocompetent patients, the clinician should become familiar with the application of relevant laboratory testing outlined in this guideline to confirm the diagnosis of these important infections.

This official clinical practice guideline was prepared by an *ad hoc* subcommittee of the ATS Assembly on Pulmonary Infections and Tuberculosis. ■

This official clinical practice guideline was prepared by an *ad hoc* subcommittee of the ATS Assembly on Pulmonary Infections and Tuberculosis.

Members of the subcommittee are as follows:

Andrew H. Limper, M.D.[1] (*Chair*)
Chadi A. Hage, M.D.[2] (*Co-Chair*)
Eva M. Carmona, M.D. Ph.D.[1]
Oleg Epelbaum, M.D.[3]
Scott E. Evans, M.D.[4]
Luke M. Gabe, M.D.[5]
Qusay Haydour, M.D.[6]
Kenneth S. Knox, M.D.[7]
Jay K. Kolls, M.D.[8]
M. Hassan Murad, M.D.[1]
Nancy L. Wengenack, Ph.D.[1]

[1]Mayo Clinic, Rochester, Minnesota; [2]Indiana University School of Medicine, Indianapolis, Indiana; [3]Westchester Medical Center, Valhalla, New York; [4]University of Texas MD Anderson Cancer Center, University of Texas, Houston, Texas; [5]Christus St. Vincent Health, Santa Fe, New Mexico; [6]Akron General, Akron, Ohio; [7]University of Arizona College of Medicine, Tucson, Arizona; and [8]Tulane School of Medicine, New Orleans, Louisiana

Author Disclosures: S.E.E. owns stocks or options in Pulmotect; and has U.S. and international patents for PUL-042: Techniques to stimulate innate immunity against pneumonia. J.K.K. served on an advisory committee for Boehringer Ingelheim; served as a consultant for Boehringer Ingelheim and Vitae Pharma; and is a cofounder of MiniVax. A.H.L., C.A.H., E.M.C., O.E., L.M.G., Q.H., K.S.K., M.H.M., and N.L.W. report no relationships with relevant commercial interests.

References

1. Limper AH. The changing spectrum of fungal infections in pulmonary and critical care practice: clinical approach to diagnosis. *Proc Am Thorac Soc* 2010;7:163–168.
2. Limper AH, Adenis A, Le T, Harrison TS. Fungal infections in HIV/AIDS. *Lancet Infect Dis* 2017;17:e334–e343.
3. Hope WW, Walsh TJ, Denning DW. The invasive and saprophytic syndromes due to *Aspergillus* spp. *Med Mycol* 2005;43(Suppl 1): S207–S238.
4. Singh N, Paterson DL. *Aspergillus* infections in transplant recipients. *Clin Microbiol Rev* 2005;18:44–69.
5. Nivoix Y, Velten M, Letscher-Bru V, Moghaddam A, Natarajan-Amé S, Fohrer C, *et al*. Factors associated with overall and attributable mortality in invasive aspergillosis. *Clin Infect Dis* 2008;47: 1176–1184.
6. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, *et al*.; European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) consensus group. *Clin Infect Dis* 2008;46:1813–1821.
7. Haydour Q, Hage CA, Carmona EM, Epelbaum O, Evans SE, Gabe LM, *et al*. Diagnosis of fungal infections: a systematic review and meta-analysis supporting ATS clinical practice guideline. *Ann Am Thorac Soc* [online ahead of print] 20 Jun 2019; DOI: 10.1513/AnnalsATS.201811-766OC.
8. Leeflang MM, Debets-Ossenkopp YJ, Wang J, Visser CE, Scholten RJ, Hooft L, *et al*. Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev* 2015; (12):CD007394.
9. Bölük G, Kazak E, Özkalemkaş F, Ener B, Akalin H, Ağca H, *et al*. Comparison of galactomannan, β-D-glucan, and *Aspergillus* DNA in sera of high-risk adult patients with hematological malignancies for the diagnosis of invasive aspergillosis. *Turk J Med Sci* 2016;46:335–342.
10. Paholcsek M, Fidler G, Konya J, Rejto L, Mehes G, Bukta E, *et al*. Combining standard clinical methods with PCR showed improved diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies and prolonged neutropenia. *BMC Infect Dis* 2015;15:251.
11. Imbert S, Gauthier L, Joly I, Brossas JY, Uzunov M, Touafek F, *et al*. *Aspergillus* PCR in serum for the diagnosis, follow-up and prognosis of invasive aspergillosis in neutropenic and nonneutropenic patients. *Clin Microbiol Infect* 2016;22:562, e1–e8.
12. Bellanger AP, Millon L, Berceanu A, Grenouillet F, Grenouillet FE, Larosa F, *et al*. Combining *Aspergillus* mitochondrial and ribosomal QPCR, in addition to galactomannan assay, for early diagnosis of invasive aspergillosis in hematology patients. *Med Mycol* 2015;53:760–764.
13. Zou M, Tang L, Zhao S, Zhao Z, Chen L, Chen P, *et al*. Systematic review and meta-analysis of detecting galactomannan in bronchoalveolar lavage fluid for diagnosing invasive aspergillosis. *PLoS One* 2012;7:e43347.
14. Reinwald M, Spiess B, Heinz WJ, Vehreschild JJ, Lass-Flörl C, Kiehl M, *et al*. Diagnosing pulmonary aspergillosis in patients with hematological malignancies: a multicenter prospective evaluation of an *Aspergillus* PCR assay and a galactomannan ELISA in bronchoalveolar lavage samples. *Eur J Haematol* 2012;89: 120–127.
15. Heng SC, Chen SC, Morrissey CO, Thursky K, Manser RL, De Silva HD, *et al*. Clinical utility of *Aspergillus* galactomannan and PCR in bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in patients with haematological malignancies. *Diagn Microbiol Infect Dis* 2014;79:322–327.
16. Rose SR, Vallabhajosyula S, Velez MG, Fedorko DP, VanRaden MJ, Gea-Banacloche JC, *et al*. The utility of bronchoalveolar lavage β-D-glucan testing for the diagnosis of invasive fungal infections. *J Infect* 2014;69:278–283.
17. Ansorg R, van den Boom R, Rath PM. Detection of *Aspergillus* galactomannan antigen in foods and antibiotics. *Mycoses* 1997;40: 353–357.
18. Hage CA, Reynolds JM, Durkin M, Wheat LJ, Knox KS. Plasmalyte as a cause of false-positive results for *Aspergillus* galactomannan in bronchoalveolar lavage fluid. *J Clin Microbiol* 2007;45:676–677.
19. Surmont I, Stockman W. Gluconate-containing intravenous solutions: another cause of false-positive galactomannan assay reactivity. *J Clin Microbiol* 2007;45:1373.
20. Racil Z, Kocmanova I, Lengerova M, Winterova J, Mayer J. Intravenous PLASMA-LYTE as a major cause of false-positive results of Platelia *Aspergillus* test for galactomannan detection in serum. *J Clin Microbiol* 2007;45:3141–3142.

21. Dowdells C, Jones RL, Mattey M, Bencina M, Legisa M, Mousdale DM. Gluconic acid production by *Aspergillus terreus*. *Lett Appl Microbiol* 2010;51:252–257.
22. Singh OV, Kumar R. Biotechnological production of gluconic acid: future implications. *Appl Microbiol Biotechnol* 2007;75:713–722.
23. Guigue N, Menotti J, Ribaud P. False positive galactomannan test after ice-pop ingestion. *N Engl J Med* 2013;369:97–98.
24. Spriet I, Lagrou K, Maertens J, Willems L, Wilmer A, Wauters J. Plasmalyte: no longer a culprit in causing false-positive galactomannan test results. *J Clin Microbiol* 2016;54:795–797.
25. Xavier MO, Pasqualotto AC, Cardoso IC, Severo LC. Cross-reactivity of *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, and *Cryptococcus* species in the commercial Platelia *Aspergillus* enzyme immunoassay. *Clin Vaccine Immunol* 2009;16:132–133.
26. Vergidis P, Walker RC, Kaul DR, Kauffman CA, Freifeld AG, Slagle DC, *et al*. False-positive *Aspergillus* galactomannan assay in solid organ transplant recipients with histoplasmosis. *Transpl Infect Dis* 2012;14: 213–217.
27. Adam O, Aupérin A, Wilquin F, Bourhis JH, Gachot B, Chachaty E. Treatment with piperacillin-tazobactam and false-positive *Aspergillus* galactomannan antigen test results for patients with hematological malignancies. *Clin Infect Dis* 2004;38:917–920.
28. Viscoli C, Machetti M, Cappellano P, Bucci B, Bruzzi P, Van Lint MT, *et al*. False-positive galactomannan Platelia *Aspergillus* test results for patients receiving piperacillin-tazobactam. *Clin Infect Dis* 2004; 38:913–916.
29. Kralik P, Ricchi M. A basic guide to real time PCR in microbial diagnostics: definitions, parameters, and everything. *Front Microbiol* 2017;8:108.
30. Kourkoumpetis TK, Fuchs BB, Coleman JJ, Desalermos A, Mylonakis E. Polymerase chain reaction-based assays for the diagnosis of invasive fungal infections. *Clin Infect Dis* 2012;54:1322–1331.
31. Arvanitis M, Ziakas PD, Zacharioudakis IM, Zervou FN, Caliendo AM, Mylonakis E. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. *J Clin Microbiol* 2014;52: 3731–3742.
32. Cruciani M, Mengoli C, Loeffler J, Donnelly P, Barnes R, Jones BL, *et al*. Polymerase chain reaction blood tests for the diagnosis of invasive aspergillosis in immunocompromised people. *Cochrane Database Syst Rev* 2015;(9):CD009551.
33. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. *Lancet Infect Dis* 2009;9:89–96.
34. Avni T, Levy I, Sprecher H, Yahav D, Leibovici L, Paul M. Diagnostic accuracy of PCR alone compared to galactomannan in bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis: a systematic review. *J Clin Microbiol* 2012;50:3652–3658.
35. Sun W, Wang K, Gao W, Su X, Qian Q, Lu X, *et al*. Evaluation of PCR on bronchoalveolar lavage fluid for diagnosis of invasive aspergillosis: a bivariate metaanalysis and systematic review. *PLoS One* 2011;6: e28467.
36. Tuon FF. A systematic literature review on the diagnosis of invasive aspergillosis using polymerase chain reaction (PCR) from bronchoalveolar lavage clinical samples. *Rev Iberoam Micol* 2007;24: 89–94.
37. Lortholary O, Renaudat C, Sitbon K, Madec Y, Denoeud-Ndam L, Wolff M, *et al*.; French Mycosis Study Group. Worrisome trends in incidence and mortality of candidemia in intensive care units (Paris area, 2002-2010). *Intensive Care Med* 2014;40:1303–1312.
38. Guzman JA, Tchokonte R, Sobel JD. Septic shock due to candidemia: outcomes and predictors of shock development. *J Clin Med Res* 2011;3:65–71.
39. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. *Antimicrob Agents Chemother* 2005;49:3640–3645.
40. Odabasi Z, Mattiuzzi G, Estey E, Kantarjian H, Saeki F, Ridge R, *et al*. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004;39:199–205.
41. Tran T, Beal SG. Application of the 1,3-β-D-glucan (Fungitell) assay in the diagnosis of invasive fungal infections. *Arch Pathol Lab Med* 2016;140:181–185.
42. Epelbaum O, Chasan R. Candidemia in the intensive care unit. *Clin Chest Med* 2017;38:493–509.
43. León C, Ruiz-Santana S, Saavedra P, Castro C, Úbeda A, Loza A, *et al*. Value of β-D-glucan and *Candida albicans* germ tube antibody for discriminating between *Candida* colonization and invasive candidiasis in patients with severe abdominal conditions. *Intensive Care Med* 2012;38:1315–1325.
44. León C, Ruiz-Santana S, Saavedra P, Galván B, Blanco A, Castro C, *et al*.; Cava Study Group. Usefulness of the "*Candida* score" for discriminating between *Candida* colonization and invasive candidiasis in non-neutropenic critically ill patients: a prospective multicenter study. *Crit Care Med* 2009;37:1624–1633.
45. Posteraro B, De Pascale G, Tumbarello M, Torelli R, Pennisi MA, Bello G, *et al*. Early diagnosis of candidemia in intensive care unit patients with sepsis: a prospective comparison of (1→3)-β-D-glucan assay, *Candida* score, and colonization index. *Crit Care* 2011;15:R249.
46. Hanson KE, Pfeiffer CD, Lease ED, Balch AH, Zaas AK, Perfect JR, *et al*. β-D-glucan surveillance with preemptive anidulafungin for invasive candidiasis in intensive care unit patients: a randomized pilot study. *PLoS One* 2012;7:e42282.
47. Azoulay E, Guigue N, Darmon M, Mokart D, Lemiale V, Kouatchet A, *et al*. (1, 3)-β-D-Glucan assay for diagnosing invasive fungal infections in critically ill patients with hematological malignancies. *Oncotarget* 2016;7:21484–21495.
48. Liew YX, Teo J, Too IAL, Ngan CCL, Tan AL, Chlebicki MP, *et al*. *Candida* surveillance in surgical intensive care unit (SICU) in a tertiary institution. *BMC Infect Dis* 2015;15:256.
49. Martín-Mazuelos E, Loza A, Castro C, Macías D, Zakariya I, Saavedra P, *et al*. β-D-Glucan and *Candida albicans* germ tube antibody in ICU patients with invasive candidiasis. *Intensive Care Med* 2015;41: 1424–1432.
50. Levesque E, El Anbassi S, Sitterle E, Foulet F, Merle JC, Botterel F. Contribution of (1,3)-β-D-glucan to diagnosis of invasive candidiasis after liver transplantation. *J Clin Microbiol* 2015;53:771–776.
51. Tissot F, Lamoth F, Hauser PM, Orasch C, Flückiger U, Siegemund M, *et al*.; Fungal Infection Network of Switzerland (FUNGINOS). β-glucan antigenemia anticipates diagnosis of blood culture-negative intraabdominal candidiasis. *Am J Respir Crit Care Med* 2013;188:1100–1109.
52. Fortún J, Meije Y, Buitrago MJ, Gago S, Bernal-Martinez L, Pemán J, *et al*. Clinical validation of a multiplex real-time PCR assay for detection of invasive candidiasis in intensive care unit patients. *J Antimicrob Chemother* 2014;69:3134–3141.
53. Bailly S, Bouadma L, Azoulay E, Orgeas MG, Adrie C, Souweine B, *et al*. Failure of empirical systemic antifungal therapy in mechanically ventilated critically ill patients. *Am J Respir Crit Care Med* 2015;191: 1139–1146.
54. Timsit JF, Azoulay E, Schwebel C, Charles PE, Cornet M, Souweine B, *et al*.; EMPIRICUS Trial Group. Empirical micafungin treatment and survival without invasive fungal infection in adults with ICU-acquired sepsis, *Candida* colonization, and multiple organ failure: the EMPIRICUS randomized clinical trial. *JAMA* 2016;316:1555–1564.
55. Benedict K, Derado G, Mody RK. Histoplasmosis-associated hospitalizations in the United States, 2001-2012. *Open Forum Infect Dis* 2016;3:ofv219.
56. Benedict K, Mody RK. Epidemiology of histoplasmosis outbreaks, United States, 1938-2013. *Emerg Infect Dis* 2016;22:370–378.
57. Chu JH, Feudtner C, Heydon K, Walsh TJ, Zaoutis TE. Hospitalizations for endemic mycoses: a population-based national study. *Clin Infect Dis* 2006;42:822–825.
58. Connolly PA, Durkin MM, Lemonte AM, Hackett EJ, Wheat LJ. Detection of histoplasma antigen by a quantitative enzyme immunoassay. *Clin Vaccine Immunol* 2007;14:1587–1591.
59. Hage CA, Kirsch EJ, Stump TE, Kauffman CA, Goldman M, Connolly P, *et al*. Histoplasma antigen clearance during treatment of histoplasmosis in patients with AIDS determined by a quantitative antigen enzyme immunoassay. *Clin Vaccine Immunol* 2011;18: 661–666.

60. Fandiño-Devia E, Rodríguez-Echeverri C, Cardona-Arias J, Gonzalez A. Antigen detection in the diagnosis of histoplasmosis: a meta-analysis of diagnostic performance. *Mycopathologia* 2016;181:197–205.
61. Richer SM, Smedema ML, Durkin MM, Herman KM, Hage CA, Fuller D, *et al*. Improved diagnosis of acute pulmonary histoplasmosis by combining antigen and antibody detection. *Clin Infect Dis* 2016;62: 896–902.
62. Swartzentruber S, Rhodes L, Kurkjian K, Zahn M, Brandt ME, Connolly P, *et al*. Diagnosis of acute pulmonary histoplasmosis by antigen detection. *Clin Infect Dis* 2009;49:1878–1882.
63. Davies SF. Serodiagnosis of histoplasmosis. *Semin Respir Infect* 1986; 1:9–15.
64. Hage CA, Ribes JA, Wengenack NL, Baddour LM, Assi M, McKinsey DS, *et al*. A multicenter evaluation of tests for diagnosis of histoplasmosis. *Clin Infect Dis* 2011;53:448–454.
65. Arango-Bustamante K, Restrepo A, Cano LE, de Bedout C, Tobón AM, González A. Diagnostic value of culture and serological tests in the diagnosis of histoplasmosis in HIV and non-HIV Colombian patients. *Am J Trop Med Hyg* 2013;89:937–942.
66. Tobón AM, Agudelo CA, Rosero DS, Ochoa JE, De Bedout C, Zuluaga A, *et al*. Disseminated histoplasmosis: a comparative study between patients with acquired immunodeficiency syndrome and non-human immunodeficiency virus-infected individuals. *Am J Trop Med Hyg* 2005;73:576–582.
67. Wheat J, French ML, Kohler RB, Zimmerman SE, Smith WR, Norton JA, *et al*. The diagnostic laboratory tests for histoplasmosis: analysis of experience in a large urban outbreak. *Ann Intern Med* 1982;97:680–685.
68. Bloch KC, Myint T, Raymond-Guillen L, Hage CA, Davis TE, Wright PW, *et al*. Improvement in diagnosis of histoplasma meningitis by combined testing for histoplasma antigen and immunoglobulin G and immunoglobulin M anti-histoplasma antibody in cerebrospinal fluid. *Clin Infect Dis* 2018;66:89–94.
69. Hage CA, Davis TE, Fuller D, Egan L, Witt JR III, Wheat LJ, *et al*. Diagnosis of histoplasmosis by antigen detection in BAL fluid. *Chest* 2010;137:623–628.
70. Limper AH, Knox KS, Sarosi GA, Ampel NM, Bennett JE, Catanzaro A, *et al*.; American Thoracic Society Fungal Working Group. An official American Thoracic Society statement: treatment of fungal infections in adult pulmonary and critical care patients. *Am J Respir Crit Care Med* 2011;183:96–128.
71. Wheat LJ, Connolly-Stringfield P, Blair R, Connolly K, Garringer T, Katz BP. Histoplasmosis relapse in patients with AIDS: detection using *Histoplasma capsulatum* variety *capsulatum* antigen levels. *Ann Intern Med* 1991;115:936–941.
72. Limper AH. Clinical approach and management for selected fungal infections in pulmonary and critical care patients. *Chest* 2014;146: 1658–1666.
73. Durkin M, Witt J, Lemonte A, Wheat B, Connolly P. Antigen assay with the potential to aid in diagnosis of blastomycosis. *J Clin Microbiol* 2004;42:4873–4875.
74. Frost HM, Novicki TJ. Blastomyces antigen detection for diagnosis and management of blastomycosis. *J Clin Microbiol* 2015;53:3660–3662.
75. Richer SM, Smedema ML, Durkin MM, Brandhorst TT, Hage CA, Connolly PA, *et al*. Development of a highly sensitive and specific blastomycosis antibody enzyme immunoassay using *Blastomyces dermatitidis* surface protein BAD-1. *Clin Vaccine Immunol* 2014;21:143–146.
76. Klein BS, Vergeront JM, Weeks RJ, Kumar UN, Mathai G, Varkey B, *et al*. Isolation of *Blastomyces dermatitidis* in soil associated with a large outbreak of blastomycosis in Wisconsin. *N Engl J Med* 1986; 314:529–534.
77. Klein BS, Vergeront JM, DiSalvo AF, Kaufman L, Davis JP. Two outbreaks of blastomycosis along rivers in Wisconsin: isolation of *Blastomyces dermatitidis* from riverbank soil and evidence of its transmission along waterways. *Am Rev Respir Dis* 1987;136:1333–1338.
78. Klein BS, Vergeront JM, Kaufman L, Bradsher RW, Kumar UN, Mathai G, *et al*. Serological tests for blastomycosis: assessments during a large point-source outbreak in Wisconsin. *J Infect Dis* 1987;155: 262–268.
79. Martynowicz MA, Prakash UB. Pulmonary blastomycosis: an appraisal of diagnostic techniques. *Chest* 2002;121:768–773.
80. Fisher MC, Koenig GL, White TJ, Taylor JW. Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. *Mycologia* 2002;94:73–84.
81. Valdivia L, Nix D, Wright M, Lindberg E, Fagan T, Lieberman D, *et al*. Coccidioidomycosis as a common cause of community-acquired pneumonia. *Emerg Infect Dis* 2006;12:958–962.
82. Chang DC, Anderson S, Wannemuehler K, Engelthaler DM, Erhart L, Sunenshine RH, *et al*. Testing for coccidioidomycosis among patients with community-acquired pneumonia. *Emerg Infect Dis* 2008;14:1053–1059.
83. Stockamp NW, Thompson GR III. Coccidioidomycosis. *Infect Dis Clin North Am* 2016;30:229–246.
84. Hector RF, Rutherford GW, Tsang CA, Erhart LM, McCotter O, Anderson SM, *et al*. The public health impact of coccidioidomycosis in Arizona and California. *Int J Environ Res Public Health* 2011;8: 1150–1173.
85. Malo J, Luraschi-Monjagatta C, Wolk DM, Thompson R, Hage CA, Knox KS. Update on the diagnosis of pulmonary coccidioidomycosis. *Ann Am Thorac Soc* 2014;11:243–253.
86. Woods CW, McRill C, Plikaytis BD, Rosenstein NE, Mosley D, Boyd D, *et al*. Coccidioidomycosis in human immunodeficiency virus-infected persons in Arizona, 1994-1997: incidence, risk factors, and prevention. *J Infect Dis* 2000;181:1428–1434.
87. Bergstrom L, Yocum DE, Ampel NM, Villanueva I, Lisse J, Gluck O, *et al*. Increased risk of coccidioidomycosis in patients treated with tumor necrosis factor α antagonists. *Arthritis Rheum* 2004;50:1959–1966.
88. Mendoza N, Noel P, Blair JE. Diagnosis, treatment, and outcomes of coccidioidomycosis in allogeneic stem cell transplantation. *Transpl Infect Dis* 2015;17:380–388.
89. Mendoza N, Blair JE. The utility of diagnostic testing for active coccidioidomycosis in solid organ transplant recipients. *Am J Transplant* 2013;13:1034–1039.
90. Galgiani JN. Coccidioidomycosis. *West J Med* 1993;159:153–171.
91. Lombard CM, Tazelaar HD, Krasne DL. Pulmonary eosinophilia in coccidioidal infections. *Chest* 1987;91:734–736.
92. Gabe LM, Malo J, Knox KS. Diagnosis and management of coccidioidomycosis. *Clin Chest Med* 2017;38:417–433.
93. Blair JE, Coakley B, Santelli AC, Hentz JG, Wengenack NL. Serologic testing for symptomatic coccidioidomycosis in immunocompetent and immunosuppressed hosts. *Mycopathologia* 2006;162:317–324.
94. Smith CE, Saito MT, Simons SA. Pattern of 39,500 serologic tests in coccidioidomycosis. *J Am Med Assoc* 1956;160:546–552.
95. Blair JE, Chang YHH, Cheng MR, Vaszar LT, Vikram HR, Orenstein R, *et al*. Characteristics of patients with mild to moderate primary pulmonary coccidioidomycosis. *Emerg Infect Dis* 2014;20:983–990.
96. Durkin M, Connolly P, Kuberski T, Myers R, Kubak BM, Bruckner D, *et al*. Diagnosis of coccidioidomycosis with use of the *Coccidioides* antigen enzyme immunoassay. *Clin Infect Dis* 2008;47:e69–e73.
97. Durkin M, Estok L, Hospenthal D, Crum-Cianflone N, Swartzentruber S, Hackett E, *et al*. Detection of *Coccidioides* antigenemia following dissociation of immune complexes. *Clin Vaccine Immunol* 2009;16: 1453–1456.
98. Kaufman L, Sekhon AS, Moledina N, Jalbert M, Pappagianis D. Comparative evaluation of commercial Premier EIA and microimmunodiffusion and complement fixation tests for *Coccidioides immitis* antibodies. *J Clin Microbiol* 1995;33:618–619.
99. Crum NF, Lederman ER, Stafford CM, Parrish JS, Wallace MR. Coccidioidomycosis: a descriptive survey of a reemerging disease. Clinical characteristics and current controversies. *Medicine (Baltimore)* 2004;83:149–175.
100. Blair JE, Mendoza N, Force S, Chang YHH, Grys TE. Clinical specificity of the enzyme immunoassay test for coccidioidomycosis varies according to the reason for its performance. *Clin Vaccine Immunol* 2013;20:95–98.
101. Kuberski T, Herrig J, Pappagianis D. False-positive IgM serology in coccidioidomycosis. *J Clin Microbiol* 2010;48:2047–2049.
102. Blair JE, Currier JT. Significance of isolated positive IgM serologic results by enzyme immunoassay for coccidioidomycosis. *Mycopathologia* 2008;166:77–82.

103. Lindsley MD, Ahn Y, McCotter O, Gade L, Hurst SF, Brandt ME, *et al*. Evaluation of the specificity of two enzyme immunoassays for coccidioidomycosis by using sera from a region of endemicity and a region of nonendemicity. *Clin Vaccine Immunol* 2015;22:1090–1095.
104. Malo J, Holbrook E, Zangeneh T, Strawter C, Oren E, Robey I, *et al*. Enhanced antibody detection and diagnosis of coccidioidomycosis with the MiraVista IgG and IgM detection enzyme immunoassay. *J Clin Microbiol* 2017;55:893–901.
105. Wack EE, Ampel NM, Sunenshine RH, Galgiani JN. The return of delayed-type hypersensitivity skin testing for coccidioidomycosis. *Clin Infect Dis* 2015;61:787–791.
106. Ampel NM, Robey I, Nguyen CT, Roller B, August J, Knox KS, *et al*. *Ex vivo* cytokine release, determined by a multiplex cytokine assay, in response to coccidioidal antigen stimulation of whole blood among subjects with recently diagnosed primary pulmonary coccidioidomycosis. *mSphere* 2018;3:e00065-18.
107. Saubolle MA, Wojack BR, Wertheimer AM, Fuayagem AZ, Young S, Koeneman BA. Multicenter clinical validation of a cartridge-based real-time PCR system for detection of *Coccidioides* spp. in lower respiratory specimens. *J Clin Microbiol* 2018;56:e01277-17.