5 Diagnostic Immunology

Christopher D. Pfeiffer and Brian Wong

Introduction

The frequency of invasive fungal infections has risen dramatically in recent decades, mostly because of a larger population of at-risk patients who are immunocompromised, neutropenic, or critically ill. For clinicians evaluating these patients, it has become increasingly important to make the diagnosis early so that timely antifungal therapy can be instituted. Although culture of body fluids or tissue for the causative fungus continues to be the gold standard for definitive diagnosis, this process can sometimes take several weeks for results and often lacks sufficient sensitivity. For example, blood cultures are positive in only ~50% of the cases of invasive candidiasis (IC) and very rarely in the cases of invasive aspergillosis (IA) [1, 2]. A presumptive diagnosis can also be made on the basis of characteristic histopathology and special tissue stains. However, obtaining adequate samples from protected anatomical sites are often not feasible in the populations at highest risk for such infections. Nonculture-based diagnostic tests are classified into four groups according to what component of the invading pathogen or host immune response they target. These include detection of host antibody, fungal antigen, fungal metabolites, or fungal nucleic acid. Overall, despite these multiple potential targets and extensive efforts toward development, only a handful of nonculture-based tests have proven clinically useful, and even fewer have reached commercial availability. As current diagnostic techniques are less than ideal, development of new methods is a priority in medical mycology. This chapter outlines the available immunologic tests according to what component of the invading pathogen or host immune response they target and provides some discussion of their strengths and weaknesses. Given the growing interest in this field, there is also some introduction to newer assays that are currently being investigated. Specific recommendations for utilizing the currently available tests in conjunction with the culture and histopathology are discussed for individual fungal species and specific disease manifestations.

B. Wong (✉) · C. D. Pfeiffer
Division of Infectious Diseases, Department of Medicine, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, L457, Portland, OR 97239, USA
e-mail: wongbri@ohsu.edu

C. D. Pfeiffer
Division of Hospital and Specialty Medicine, Portland VA Medical Center, Portland, OR 97239, USA

Host Antibody Testing

Many tests in current use have been developed to detect host antibodies against specific fungal antigens. These require identification of one or more distinctive antigens to which host antibodies are directed, sufficient immunocompetence on the part of the host to mount a specific antibody response, and the use of a variety of techniques to detect the antibody. Tube precipitin (TP) assays, immunodiffusion (ID) assays, complement fixation (CF) assays, radioimmunoassays (RIA), and enzyme-linked immunosorbent assays (ELISA/EIA) are some such techniques. One major limitation of this general approach is that immunocompromised patients have impaired abilities to mount specific antibody responses. Moreover, these responses may be delayed and antibodies do not necessarily distinguish acute from chronic or a past history of infection. Finally, antibodies to some fungal antigens may be demonstrable in uninfected people, thereby reducing the diagnostic specificity of positive tests.

Fungal Antigen Testing

A second common method to diagnose fungal infection includes tests that use immunologic reagents to identify

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antigenic components of the fungus. These require the presence of unique antigens in a body fluid or tissue specimen that is available for testing and use of a variety of techniques to detect these antigens. Some such techniques include latex agglutination (LA) assays, dot immuno-binding assays, ELISA/EIA, RIA, and lateral flow immunoassays (LFA). Monoclonal or polyclonal antibody is often needed in these assays to help detect the antigen of interest. The major limitations of this general approach are the low level and transient nature of antigenemia in some hosts, cross-reactions between antigens derived from different fungal species, and lack of specificity for a particular antigen when polyclonal antibodies are used.

Fungal Metabolite Testing

Another methodology includes the direct detection of fungal metabolites in patient serum or other samples. These are usually by-products of a specific fungus which are detected either by gas chromatography, mass spectrometry, or an enzymatic reaction. One limitation of these tests is that the metabolic products are not unique to individual fungal species and may be present in small amounts in uninfected individuals. Furthermore, the level of metabolite may not be present in sufficient quantity outside of the local tissue being invaded, making detection difficult.

Fungal Nucleic Acid Testing

There has been a rapid expansion of diagnostic tests targeting fungal nucleic acid which is now discussed in Chap. 3 (discussed in this chapter in a previous edition of this book).

PAN-Fungal Testing: 1,3-Β-D-Glucan

1,3-β-D-glucan (BG) is a major component of the cell wall of many different fungi. The ability of fungal BG to activate an enzyme in the clotting cascade of the horseshoe crab has led to the development of assays capable of detecting very small amounts of BG. Four separate assays are commercially available and each assay uses individualized cutoff values to define positivity. Available assays include Fungitell (formerly Glucatell; Associates of Cape Cod, East Falmouth, MA), Fungitec-G (Seikagaku, Tokyo, Japan), Wako turbidimetric assay (Wako Pure Chemical Industries, Tokyo, Japan), and Maruha colorimetric assay (Maruha-Nichiro Foods, Tokyo, Japan) [3]. The primary clinical scenarios where the accuracy of BG has been evaluated are discussed below and summarized in Table 5.1. In two recently performed meta-analyses, the diagnostic accuracy for invasive fungal infection (IFI) was moderate (sensitivity 77–80 %; specificity 82–85 %) but for *Pneumocystis jirovecii* pneumonia (PCP) was high (sensitivity 95–96%; specificity 84–86%) [4–6]. Due to the low pretest probability for IFI (5–15 % in many clinical settings), the BG test has a high-negative predictive value (i.e., if the test is negative, the likelihood of IFI is quite low). A major limitation of the BG is its inability to differentiate between different species of fungi. This is because BG is a component of the cell walls of many fungi including *Candida, Aspergillus, Pneumocystis, Trichosporon, Fusarium,* and *Saccharomyces* species. Importantly, BG is not found in *Cryptococcus* or the agents of mucormycosis. False-positive results are associated with receipt of hemodialysis, recent surgery, exposure to immunoglobulin or albumin products, receipt of certain medications (e.g., amoxicillin-clavulanic acid), and bacteremia [7–14].

Diagnosis of IFI in High-Risk Hemato-Oncological Patients

The meta-analysis described above for IFI detection included a majority of studies involving the hemato-oncologic patient population. Limiting discussion to this population only, however, BG evaluation in case-control studies has demonstrated a wide range of sensitivity (50–90 %) and specificity (70–100 %) [15–17]. BG assessments in the cohort study setting, which more closely reflects the real world performance of the test, have been performed. These cohort studies are the subject of a systematic review and meta-analysis which evaluated the diagnostic accuracy of BG in high-risk adult hemato-oncological patients. Of the six studies meeting inclusion criteria, the diagnostic performance of two consecutive BG assays was superior to the performance of one test alone for the diagnosis of proven or probable IFI; the sensitivity and specificity of two consecutive positive tests were 49.6 % (95 % CI, 34.0–65.3 %) and 98.9 % (95 % CI, 97.4–99.5 %), respectively [3]. When a threshold of only one positive test was applied, the BG sensitivity and specificity were 61.5 and 90.8 %, respectively. The most common IFIs detected in the six studies were invasive aspergillosis ($n = 90$), invasive candidiasis ($n = 80$), PCP ($n = 14$), and other ($n = 31$). Importantly, similar accuracy was noted across all four available BG assays. The use and interpretation of BG must be mindfully considered in context of local factors specific to a hemato-oncologic program's fungal prophylaxis and detection strategy.

Diagnosis of IFI in High-Risk Intensive Care Unit (ICU) Patients

Several reports have evaluated BG for diagnosis of IFI, primarily invasive candidiasis (IC), in the ICU setting. In a

Table 5.1 Studies of serum 1,3-β-D-glucan (BG) for invasive fungal infection (IFI) and *Pneumocystis jirovecii* pneumonia (PCP)

Population	Assay	Study design	Sensitivity (%)	Specificity (%)	Comments[b]	Ref.
All	Any	Meta-analysis of 16 studies: 2979 patients; 594 with proven/probable IFI. Excluded diagnosis of PCP	77	85	One positive test required; study population = 11 hemato-oncologic; five at risk for IC; one transplant; one miscellaneous; test performance for detection of IC and IA was similar	[4]
	Any	Systematic review and meta-analysis of 31 studies: uncertain number of patients	80	82	One positive test using the test cutoff that offered the best test performance in each individual study; diagnostic accuracy was similar for IC versus. IA; sensitivity and specificity were lower (72 and 78 %, respectively) when analysis restricted to 17 cohort studies	[5]
High-risk hemato-oncologic	Any	Systematic review and meta-analysis of six cohort studies: 1771 patients analyzed; 215 had proven or probable IFI	62	91	One positive test required	[6]
			50	99	Two consecutive positive tests required	
High-risk intensive care unit (ICU)	Fungitell[a]	Prospective cohort: 95 ICU patients with length of stay > 5 days; 16 with IFI (IC = 14)	93	94	One positive test required; analysis performed for proven IC only	[18]
	Fungitell[a]	Prospective cohort: 57 ICU patients, nine developed proven/probable IC	91	57	One positive test required; many false positives occurred on the initial sample (ICU day 3); omitting these samples and requiring two consecutive positive samples increased sensitivity and specificity to 90 and 80 %, respectively	[11]
	Fungitell[a]	Prospective, randomized: 64 ICU patients, six developed proven/probable IC	100	75	Two sequential positive tests required; those with positive tests received empiric antifungal (anidulafungin) therapy	[10]
At risk for PCP	Any	Meta-analysis of 14 studies: 1723 controls (with other medical conditions) and 357 PCP cases	95	86	One positive test using test cutoffs according to (or closest to) the manufacturers' instructions required; HIV status did not impact accuracy	[6]
	Any	Systemic review and meta-analysis of 12 studies: uncertain number of patients	96	84	One positive test using the test cutoff that offered the best test performance in each individual study; HIV status did not impact accuracy	[5]

IC invasive candidiasis, *IA* invasive aspergillosis, *IFI* invasive fungal infection, *PCP Pneumocystis jirovecii* pneumonia
[a]Associates of Cape Cod, East Falmouth, MA
[b]Positive test cutoff according to the manufacturer's instructions used unless otherwise noted

prospective study of 95 nonneutropenic ICU patients with signs of sepsis and a length of stay > 5 days, 16 (17 %) were diagnosed with IFI (14 IC, one aspergillosis, one fusariosis). In this setting, a single BG test demonstrated 94 % sensitivity and 93 % specificity; moreover, for all 13 candidemia cases, the BG assay was positive 24–72 h before a positive blood culture result [18]. Mohr et al. evaluated BG in 57 consecutively enrolled surgical ICU patients, of whom nine had proven or probable IC [11]. Overall, when only one positive sample was required, the sensitivity and specificity of the BG for IC were 91 and 57 %, respectively. Many of the false-positive tests occurred early during the ICU stay (day 3), a finding that requires further examination. BG performance was much improved (sensitivity 90 %; specificity 80 %) if the samples obtained within 72 h of ICU admission were omitted and two consecutive positive samples were required. Finally, a prospective randomized pilot study was performed and involved 64 ICU patients, six of whom developed proven or probable IC [10]. Optimal assay performance in that study was found to use two sequential positive BG tests; the

sensitivity for IC was 100% and specificity 75%. Subjects randomized to the intervention group with two positive BG tests received preemptive anidulafungin therapy, which was safe and well tolerated (the study was not powered to detect clinical outcomes). The utility of BG as an adjunctive test in the ICU setting remains to be fully evaluated.

Diagnosis of Pneumocystis jirovecii Pneumonia

BG assay has also been evaluated for the detection of PCP. Two large meta-analyses were performed to evaluate the diagnostic accuracy of BG for PCP. The first report evaluated 12 studies and found a pooled sensitivity and specificity of 96 and 84%, respectively [5]. This study found nominal differences when test performance was stratified according to HIV status, assay type (Fungitell vs. Fungitec G test), study design (cohort vs. case-control), and methodological quality. A subsequent meta-analysis of 14 studies (nine of which were included in the first meta-analysis) evaluating 357 PCP cases and 1723 controls found BG to have a sensitivity of 95% and specificity of 86% [6]. All studies in this setting relied upon only one positive BG test. Given the high sensitivity, BG testing may be sufficiently sensitive to effectively exclude a diagnosis of PCP without bronchoscopy.

Conclusions and Recommendations

See Box 5.1 for recommendations on how test use is recommended. More data regarding the optimal use of BG in various clinical scenarios is undoubtedly forthcoming.

Box 5.1 Recommendations for using the serum 1,3-β-D-glucan (BG) assay

1. A positive test generally indicates IFI but is not pathogen-specific; always interpret the results in the context of other clinical and laboratory findings.
2. Serum BG can be employed as part of IFI surveillance in a similar fashion to serum GM (e.g., perform 1–2/week on at-risk hemato-oncologic patients). The test has moderate accuracy in this setting.
3. Serum BG appears to be a sensitive test when incorporated into a screening protocol for ICU patients at high risk for invasive candidiasis (IC). However, one positive test alone, particularly early in the ICU course, may be falsely positive and should ideally be confirmed with a second test.
4. Serum BG has excellent performance characteristics for the diagnosis of PCP and may be particularly helpful in ruling out disease for patients suspected of PCP when BAL fluid cannot be readily obtained.

Candidiasis

Candida species are now the fourth most common microorganism isolated from the bloodstream of hospitalized patients in the USA and sixth most common nosocomial pathogen overall [19]. Rapid detection of IC is critical and warrants the development of nonculture diagnostic approaches. Recent attention has focused primarily on the pan-fungal BG assay (discussed above), while other *Candida*-specific investigations (discussed below) have garnered less success.

Antibody Detection

Antibody detection assays were the area of earliest interest but yielded tests with poor sensitivity and specificity. These tests have since fallen out of favor and are not recommended for routine use.

Antigen Detection

Several tests that target a variety of cell wall and cytoplasmic components have been developed to detect macromolecular *Candida* antigens (Table 5.2). Some of these are no longer available, such as an assay to detect enolase antigen. Of the available tests, the earliest was the Cand-Tec LA assay (Ramco Laboratories, Stafford, TX), which was designed to detect circulating *Candida* antigen in patients with serious, disseminated infection. Unfortunately, there are conflicting reports on its overall sensitivity and specificity, especially in patients with renal failure or rheumatoid factor positivity, making it difficult to confirm the diagnosis of candidiasis by the Cand-Tec assay alone [20, 21].

The mannan component of the *Candida* cell wall is a major antigen and the target of many serum detection assays. These assays vary in the laboratory method and type of antibody used for antigen detection. Two tests that use the same monoclonal antibody are the Pastorex *Candida* LA test (Bio-Rad, Marnes-la-Coquette, France) and the Platelia *Candida* Antigen EIA test (Bio-Rad, Marnes-la-Coquette, France). Although the EIA test is more sensitive than the LA test, they are both limited by the rapid clearance of man-

Table 5.2 Studies of serum immunological assays for *Candida* infection (excluding BG)

Target	Assay	Population tested	Sensitivity	Specificity	Comments	Ref.
Antigens as detected by						
Unknown	Cand-Tec LA[a]	Retrospective case control: 39 candidemia cases; 40 controls (20 healthy volunteers, ten patients with *Candida* colonization only, ten patients with other deep mycoses)	77 %	88 %	Titer of 1:4 considered positive	[20]
		Prospective cohort of patients at risk for IC: 202 patients; 23 developed IC	70 %	69 %	Titer of 1:4 considered positive	[21]
Mannan	Pastorex LA[b]	As above	26 %	100 %	–	[20]
		Retrospective case control: 43 cases of proven IC; 150 controls included ICU patients, patients with other deep mycoses, and healthy volunteers	28 %	100 %	–	[22]
	Platelia *Candida* Antigen EIA[b]	Per-patient analysis of 14 studies (13 retrospective) including 453 patients (proven/probable IC) and 767 controls (healthy volunteers and high-risk patients without IC). The population studied was split (seven studies mainly hemato-oncologic; seven studies mainly ICU/surgery)	58 %	93 %	Cutoffs used were adopted from the primary study and varied Combined testing performance was superior	[23]
	Platelia *Candida* Antibody EIA		59 %	83 %		
	Platelia Candida Antigen and Antibody EIA[b]		83 %	86 %		
	Platelia *Candida* Ag Plus EIA	Retrospective case control: 56 candidemia cases; 200 controls (100 bacteremic, 100 nonbacteremic)	59	98	Cutoffs used per manufacturer's recommendations; *C. parapsilosis* and *C. guilliermondii* were not detected by Platelia *Candida* Ag Plus; BG in comparison: sensitivity 88 %, specificity 86 %	[24]
	Platelia *Candida* Ab Plus EIA		63	65		
	Platelia *Candida* Ag/Ab Plus EIA		89	63		
Metabolites as detected by						
D-arabinitol	Enzymatic–chromogenic	Prospective analysis of high-risk oncology patients and control patients that included those with fever, neutropenia, and mucosal colonization with *Candida* but no culture evidence of IC, and those without these and also without culture evidence of IC	31/42 (74 %)	178/206 (86 %)	Candidemia patients	[25]
			25/30 (83 %)	–	Persistent candidemia patients	
			4/10 (40 %)	–	IC patients w/o candidemia	
			7/16 (44 %)	–	Deep mucosal candidiasis patients	
	Enzymatic-fluorometric	Retrospective evaluation of patients with candidemia and healthy control patients	63/83 (76 %)	89/100 (89 %)	Candidemia patients	[26]
			25/30 (83 %)	–	Persistent candidemia patients	

BG 1,3-β-D-glucan, *ICU* intensive care unit, *IC* invasive candidiasis, *EIA* enzyme immunoassay
[a]Ramco Laboratories, Stafford, TX
[b]Bio-Rad, Hercules, CA

nan antigenemia. In an effort to overcome this, an anti-mannan antibody EIA was developed and marketed individually (as the Platelia *Candida* Antibody test) and combined with the Platelia *Candida* Antigen (as the Platelia *Candida* Antibody and Antigen test) [22]. In a review of 14 studies that evaluated the three Platelia *Candida* assays, the pooled per-patient sensitivity of the Platelia *Candida* Antigen, Platelia *Candida* Antibody, and both tests combined was 58, 59, and 83 %, respectively, while the corresponding specificities were 93, 83, and 86 %, respectively [23]. Both Platelia *Candida* tests have been recently refined and are now marketed as *Candida* Ag Plus and *Candida* Ab Plus. In one initial evaluation of their performance using a case–control design, their sensitivity and specificity were, respectively,

59 and 98 % for *Candida* Ag Plus, 63 and 65 % for *Candida* Ab Plus, and 89 and 63.0 % for the tests combined (*Candida* Ag/Ab Plus) [24]. Further evaluation of the newer test is warranted before widespread clinical application.

Detection of Fungal Metabolites

D-arabinitol (DA) is a five carbon polyol metabolite that is produced by several pathogenic *Candida* species (except for *C. krusei* and *C. glabrata*). It has been shown to be present in higher serum concentrations in humans and animals with IC than in uninfected or colonized controls, making it potentially useful as a diagnostic marker for IC. There are two general methods to measure DA: gas chromatography or an enzymatic method. The former is labor intensive and not readily available in most hospital laboratories, while the latter is more suited to a commercial test kit, as is currently marketed in Japan as Arabinitec-Auto (Marukin Diagnostics, Osaka, Japan). This assay is also available for DA testing on urine samples. Several studies have shown that DA can be detected earlier than the presence of *Candida* in blood cultures and that serial measurements correlate well with clinical response to therapy [25, 26].

Conclusions and Recommendations

Immunodiagnostic tests are promising additional diagnostic strategies in the detection of IC, but they have yet to supplant traditional methods. In general, there appears to be utility for serum BG, serum mannan antigen/antibody, and serum DA as adjunctive tests in combination with cultures, histopathology, and radiology. As yet, no single test has been demonstrated to have optimal sensitivity and specificity. Their greatest value appears to be in serial testing of high-risk populations where the trend (rather than a single value) will allow an earlier, accurate diagnosis and monitor the effectiveness of empirically instituted antifungal therapy. An emerging strategy is to use these tests in combination with one another and/or molecular assays to optimize a diagnostic approach to these complex patients. Further, rigorous, prospective clinical trials are needed to determine which tests or combinations thereof will offer the greatest clinical utility.

Aspergillosis

There are a variety of clinical manifestations of aspergillosis, including aspergillomas, allergic bronchopulmonary aspergillosis (ABPA), chronic invasive aspergillosis, and IA. Different types of immunologic tests have shown different utility for this spectrum of disease.

Antibody Detection

The diagnosis of aspergilloma is made by combined radiologic and serologic testing, where IgG antibodies are usually positive. Similarly, for ABPA, a combination of routine blood tests, radiographic findings, skin testing for *Aspergillus* sensitivity, and both IgG and IgE antibody positivity are used for diagnosis. Conversely, antibody detection is less useful and not recommended for invasive disease since the immunocompromised patients most at-risk are less likely to mount a sufficient response.

Antigen Detection

Galactomannan (GM) is a polysaccharide component of the *Aspergillus* cell wall. It has been demonstrated in the serum of some patients with IA and thus has been the target of several serum detection assays. An earlier test called the Pastorex *Aspergillus* (Bio-Rad, Hercules, CA) utilized an LA method with a monoclonal antibody. This test yielded disappointing results with low sensitivity unless multiple samples were used and false-positive reactions from cross-reactivity of the antibody with several other fungal species (see Table 5.3) [27, 28]. A newer, commercially available test is the Platelia *Aspergillus* Ag (Bio-Rad, Hercules, CA). This sandwich ELISA uses the same monoclonal antibody but has the ability to detect GM at much lower limits, thereby improving the test's sensitivity and allowing earlier detection of IA. In a retrospective review of stored serum specimens on bone marrow transplant and leukemia patients, the study leading to its FDA approval, this assay had a sensitivity of 81 % and specificity of 89 % [29]. However, subsequent investigations have shown a lower sensitivity; for example, a meta-analysis of 27 studies of the Platelia *Aspergillus* assay in serum, performed in the setting of repeated surveillance in high-risk patients, demonstrated an overall sensitivity of 61 % and specificity of 93 % in proven or probable IA cases [30]. The sensitivity of the test, when limited to proven cases only, was 71 %. In subgroup analyses, the sensitivity was 79 % in those studies which reported using an optical density (OD) cutoff at the US FDA-cleared threshold of 0.5 (as opposed to a higher OD cutoff of 1 or 1.5; $n=5$). GM test performance is best in the hemato-oncologic population and is less sensitive in solid organ transplant patients [30]. An emerging role for serum GM testing is to monitor treatment response; several recent reports have demonstrated that the trend of GM during therapy predicts outcome [31–33]. False-negative results can occur due to limited angioinvasion, low fungal load, high antibody titers, or the use of prophylactic or preemptive antifungals. Alternatively, false positives occur due to cross-reactivity of the assay with other fungal species, co-administration of pipercillin-tazobactam, or several fungal-derived antibiotics.

Table 5.3 Studies of *Aspergillus* galactomannan antigen detection

Assay/body fluid	Study design and population	Sensitivity (proven/probable IA) (%)	Specificity (not IA) (%)	Comments	Ref.
Pastorex LA[a]/serum	Retrospective cohort: 61 at-risk neutropenic patients; ten developed proven or possible IA	70	86	A single positive test (only) required ELISA also performed: 90 % sensitive, 83 % specific	[27]
	Retrospective cohort of 215 at-risk bone marrow transplant recipients: 25 proven IA, 15 probable IA, and eight indeterminate infection cases	27	100	A single positive test (only) required ELISA also performed: 93 % sensitive, 82 % specific	[28]
Platelia[a] ELISA/Serum	Meta-analysis of 27 studies –21 prospective; six retrospective –23 hemato-oncologic; 3 SOT; one not specified –GM sampled in at-risk patients 1–2/week in all studies	61	93	Positive tests required: 1 (11 studies); 2 (16 studies) Proven cases only: sensitivity 71 %, specificity 89 % SOT subgroup: sensitivity 41 % Sensitivity by OD cutoff for positivity: 79 % for 0.5; 65 % for 1.0; 48 % for 1.5	[30]
Platelia ELISA/BAL	Meta-analysis of 30 studies –14 prospective; 16 retrospective –12 hemato-oncologic; 4 SOT; 14 other –GM typically performed as part of a diagnostic BAL	87	89	One positive BAL GM required OD≥0.5 available in 24 studies and used for primary analysis OD≥1 (21 studies) performance: sensitivity 86 %; specificity 95 % Serum GM: 65 % sensitive; 95 % specific	[37]
Platelia ELISA/BAL	Systematic review of 19 studies for PCR –7 compared PCR to GM (OD≥0.5) –75 % of population was hemato-oncologic	82	96	Reference standard imperfect: positive GM or PCR	[38]

Prov proven, *Prob* probable, *Poss* possible, *ELISA* enzyme-linked immunosorbent assays, *BAL* bronchoalveolar lavage, *PCR* polymerase chain reaction, *GM* genetically modified, *IA* invasive aspergillosis, *LA* latex agglutination
[a]Bio-Rad, Hercules, CA

The Platelia *Aspergillus* assay was FDA-cleared for use on bronchoalveolar lavage (BAL) fluid in 2011, and its use has increased due to the improved sensitivity (generally >75 %) as compared to serum across various patient populations [34–36]. A meta-analysis of 30 studies demonstrated a sensitivity of 87 % and specificity of 89 % for the BAL-GM [37]. In a systematic review of seven studies comparing BAL GM (OD cutoff 0.5) to *Aspergillus* PCR, BAL GM performed similarly; BAL GM sensitivity and specificity were reported as 82 and 97 %, respectively [38]. As with serum GM, test performance depends on the selected OD cutoff required for test positivity (FDA-cleared at 0.5). While specificity is improved, sensitivity is sacrificed if higher OD cutoffs (e.g., 1.0 or 1.5) are used to indicate a positive result. Though it can be detected in other body fluids, the GM assay is validated only for serum and BAL samples at this time.

Detection of Fungal Metabolites

Mannitol, a six-carbon acyclic polyol is produced in large amounts by many different fungi, including several *Aspergillus* species in culture. Unfortunately, available data do not support the usefulness of mannitol as a diagnostic marker.

Conclusions and Recommendations

Despite the broad spectrum of disease caused by *Aspergillus* organisms, it is the invasive disease that is most important and most challenging diagnostically. The Platelia *Aspergillus* assay supports a diagnosis of IA in the appropriate clinical setting. This serum test has shown increased specificity with serial sampling and is ideally used to screen patients on a weekly or twice weekly basis during periods of severe im-

munosuppression and may be useful to monitor patients on therapy. All clinicians should keep in mind the potential for false positive and negative results and incorporate the GM results into the general clinical assessment of the patient, rather than as the sole basis on which to change management. For instance, a change in the assay from negative to positive in an immunosuppressed patient under surveillance should prompt a more thorough investigation for IA, while a change from positive to negative should lend support to other evidence that proper therapy has been instituted. Additionally, it is important to remember that the positive predictive value of this test is highest in populations with a high pretest probability; using it for routine diagnosis in lower risk populations will likely increase the chance that a positive result is a false positive. Also, BAL fluid GM testing demonstrates very good sensitivity and specificity although similar cautions regarding test interpretation should be considered. Despite its limitations, this assay is a suitable, noninvasive adjunct for diagnosing and managing IA (see Box 5.2 for recommendations).

Box 5.2 Recommendations for using the Platelia *Aspergillus* galactomannan (GM) ELISA assay

1. Serum GM is ideally used as a part of IA surveillance and performed 1–2/week on at-risk hemato-oncologic patients. The test has moderate accuracy in this setting.
2. BAL GM is a useful adjunctive diagnostic test for at-risk patients presenting with compatible clinical illness. The test has moderate-to-high accuracy in this setting.
3. Consider using serum GM testing to monitor treatment response.
4. Always interpret the results in the context of other clinical and laboratory findings.

Cryptococcosis

The worldwide burden of cryptococcosis, primarily due to *Cryptococcus neoformans,* increased in parallel with the HIV epidemic. More recently, although historically a pathogen of tropical and subtropical regions, *C. gattii* has caused outbreaks of invasive cryptococcosis in the Pacific Northwest, USA and British Columbia, Canada [39]. While culture and histopathology remain the gold standards for diagnosing cryptococcal disease, detection of cryptococcal polysaccharide antigen is a critical test which affords a more rapid diagnosis and allows for earlier disease treatment.

Antibody Detection

Tests for cryptococcal antibodies are not useful and are not widely available for clinical use because they have high false positive and false negative rates.

Antigen Detection

Some of the most important and rapid serodiagnostic tests available for any fungi are those used to detect the cryptococcal capsular polysaccharide antigen, glucuronoxylomannan (GXM) (Table 5.4). These tests utilize a variety of different laboratory techniques (e.g., LA and EIA) for antigen detection. Recently, a point-of-care LFA (IMMY, Norman, OK) was FDA-cleared in 2011. In several studies, the available assays have been directly compared; assay performance is generally excellent in serum (with some important inter-assay differences) and outstanding in CSF (with minimal inter-assay differences) [40–44]. Potential advantages of the LFA are that it is easily stored (room temperature), rapid, and simple to perform/interpret. However, the LFA requires further evaluation and particularly validation of quantitative cryptococcal antigen titers before its widespread adoption.

The performance characteristics of the cryptococcal antigen assays to diagnose cryptococcosis depend on both the disease status (localized vs. disseminated) and host status (HIV, transplant, otherwise immunosuppressed, or immune competent). Due to the multiple possible combinations, the ability to tease out these performance differences is difficult. In general, a higher fungal burden (as is typical for CNS disease) is associated with higher antigen titers. For example, the sensitivity of serum cryptococcal antigen is lower for isolated pulmonary disease than that for meningitis. Indeed, in immunosuppressed patient, a positive serum cryptococcal antigen from a patient who may only appear to have localized pulmonary disease should prompt the clinician to strongly consider checking blood cultures and CSF fluid to rule out dissemination [45]. There has been much investigation regarding the differing performing characteristics of the ability of serum and CSF antigen tests to detect cryptococcal meningitis. In a review of nine studies, the sensitivity of serum and CSF antigen titers to detect CNS disease in the following patient groups was reported: 92 % (307/333) and 99 % (181/183) in HIV-positive patients; 88 % (38/43) and 100 % (65/65) in transplant recipients; 87 % (91/105) and 97 % (141/154) in otherwise immunocompromised patients; and not reported and 92 % (72/78) in immune competent patients, respectively [46]. Overall, the sensitivity across all reviewed patient populations for serum (94 %) and CSF (94 %) antigen titers was much better than that of India ink (60 %) and similar (or slightly better) to that of CSF culture (92 %).

Table 5.4 Studies of *Cryptococcus* antigen detection

Study design/reference	Assay	Fluid	Sensitivity (%)	Specificity (%)	Comments
Case-control: 90 serum (19 positive) and 182 CSF (30 positive) samples from 47 HIV-positive patients; unknown control population Tanner et al [42]	Crypto LA[a]	CSF	100	98	IMMY LA: used pronase on CSF and serum; CALAS: used pronase on serum
		Serum	83	98	
	Myco-Immune LA[b]	CSF	100	97	
		Serum	83	100	
	IMMY LA[c]	CSF	93	93	
		Serum	97	93	
	CALAS[d]	CSF	100	96	
		Serum	97	95	
	Premier EIA[d]	CSF	100	98	
		Serum	93	96	
Prospective cohort for serum (634 samples, nine cases) and retrospective cohort for CSF (51 samples, 18 cases); unknown patient population—studied at a reference laboratory CALAS in serum and Premier EIA in CSF were used as the "gold standard" references Binnekar et al. [44]	Premier EIA	CSF	Gold standard		Note: culture data were not incorporated in this study
		Serum	56	100	
	CALAS LA	CSF	100	100	
		Serum	Gold Standard		
	IMMY LFA	CSF	100	100	
		Serum	100	99.8	
	IMMY Alpha EIA[c]	CSF	100	100	
		Serum	100	99.7	
Prospective cohort: 106 serum, 42 CSF, and 20 urine samples obtained from 92 patients. 25 cases (four HIV+, nine other immuno-compromised, 12 immune competent) McMullan et al. [43]	IMMY LFA	CSF	100	100	–
		Serum	100	100	
		Urine	94	100 ($n=2$)	
	CALAS	CSF	100	100	
		Serum	91	93	
Prospective cohort: 100 HIV-positive patients with CNS infections; 58 cases of *Cryptococcus* meningitis Asawavichienjinda et al. [47]	Pastorex LA[e]	Serum	91	83	Note: This study used serum to screen for CNS disease

CSF cerebrospinal fluid, *LA* latex agglutination, *EIA* enzyme immunoassay, *CNS* central nervous system, *CALAS* cryptococcal antigen latex agglutination system,
[a]International Biological Labs, Cranbury, NJ
[b]American MicroScan, Mahwah, NJ
[c]IMMY, Norman, OK
[d]Meridian Diagnostics, Cincinnati, OH
[e]Sanofi Diagnostic Pasteur, France

The argument follows that for HIV-positive patients unable to undergo lumbar puncture (LP) or with vague central nervous system (CNS) symptoms not warranting an LP, a serum antigen test may be a reasonable surrogate for meningitis screening [46, 47]. On the other hand, in immune competent patients with meningitis symptoms, the utility of a serum antigen is less clear (i.e., CSF antigen is required) [48, 49].

The prognostic value of antigen titers is controversial given the above mentioned issues of disease and host factors. In the majority of studies, higher initial titers are associated with increased mortality and relapsed disease; however, other recent studies have not confirmed this association [39, 47, 50–54]. Also controversial is the use of sequential antigen titers obtained during therapy. This practice is best studied in the HIV patient population, in which setting the use of titers to monitor treatment response is probably unreliable and therefore not recommended [45, 55]. In certain situations, sites other than serum and CSF may be useful in detection of cryptococcal disease including the pleural fluid and urine [41, 56].

All cryptococcal antigen assays appear to have excellent accuracy for *C. neoformans*. However, because of different binding affinity to different GXM serotypes, the tests may have differing abilities to detect *C. gattii* (which has Serotype C GXM). In vitro data and a recent clinical report indicate that the Meridian EIA and CALAS kits may be less sensitive than IMMY LFA and EIA for *C. gattii* detection [40].

The only limitations of these assays are the occasional false-negative results in patients with extremely low or high *Cryptococcus* organism burden and infrequent false-positive results, generally resulting in low titers, in patients with other infections including disseminated trichosporonosis, *Capnocytophaga canimorsus* sepsis, and *Stomatococcus* infection [57–59].

Detection of Fungal Metabolites

Cryptococcus species, like *Aspergillus* species, also produce large amounts of mannitol, but it has not proven useful as a diagnostic marker for this disease either.

Conclusions and Recommendations

Cryptococcal infection is the rare condition where a serodiagnostic test has extremely high accuracy. A positive cryptococcal antigen result is highly suggestive of infection and can be the sole basis for initiating targeted therapy. However, definitive proof of disease still requires culture or histopathology and efforts to prove the diagnosis by these means are always warranted. The clinical utility of the antigen test depends on the extent of disease and host immune status (see Box 5.3 for recommendations). Care must be taken not to compare titers derived from different kits given the lack of standardization among manufacturers.

Box 5.3 Recommendations for using cryptococcal antigen tests in different host populations

HIV-positive patients

1. For meningeal symptoms, check both a serum and CSF antigen. If unable to do LP or the neurologic symptoms are vague, one can use the serum antigen (which has a high sensitivity for meningitis) as a surrogate screening test.
2. For nonmeningeal symptoms, check a serum antigen. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid).
3. A positive serum antigen is associated with disseminated disease and warrants blood cultures and CSF evaluation (cultures and antigen testing).
4. Initial antigen titers correlate with disease burden and likely provide prognostic information; serial antigen titers probably do not provide prognostic information during therapy (and therefore are not recommended).

Solid organ transplant patients

1. For meningeal symptoms, check both a serum and CSF antigen. The sensitivity of a serum test to screen for CNS disease also appears to be high in this population, however an LP is mandatory to evaluate for *Cryptococcus* and other disease.
2. For nonmeningeal symptoms, check a serum antigen. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid).
3. A positive serum antigen is associated with disseminated disease and warrants blood cultures and CSF evaluation (cultures and antigen testing).
4. Initial antigen titers correlate with disease burden and likely provide prognostic information; it is unknown whether serial titers provide prognostic information during therapy (and therefore are not specifically recommended).

Immune competent patients

1. For meningeal symptoms, check both CSF and serum antigen; serum antigen alone may be insufficient to rule out meningitis.
2. For nonmeningeal symptoms, check a serum antigen. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid).
3. Initial antigen titers correlate with disease burden and likely provide prognostic information; some experienced clinicians monitor serial titers during therapy to document therapeutic response and predict relapse, but others do not.

Histoplasmosis

The standard method for the diagnosis of histoplasmosis remains isolation and specific identification of the causative organism. Unfortunately, this process can take 2–4 weeks and the necessary specimens can be difficult to obtain. Immunologic tests offer a more rapid alternative and in some manifestations of the disease are the preferred means of establishing a diagnosis.

Antibody Detection

The two *Histoplasma capsulatum* species-specific antigens against which host antibodies are made are the H and M antigens, which are both components of histoplasmin. Antibodies against H antigen, a β-glucosidase, form during acute histoplasmosis, while antibodies against M antigen, a catalase, may be formed in active or chronic histoplasmosis and are usually the first to arise upon seroconversion (Table 5.5). These antibodies can be detected by either ID or CF assays; the CF assay uses two antigens including histoplasmin and a suspension of killed yeast phase cells, the latter of which renders CF more sensitive but less specific than ID. Antibodies against M antigen are detected 6–8 weeks after exposure in 50–80 % of patients, but can persist for years in patients who have recovered from infection; therefore their presence does not distinguish remote infection from current disease. On the other hand, antibodies against H antigen are detected in only 10–20 % of exposed patients, but their presence signifies an active infection. In general, asymptomatic patients are less likely to have detectable antibody levels,

Table 5.5 Studies of *Histoplasma* antibody and antigen detection

Assay	PDH in patients with AIDS	PDH in patients without AIDS	Limited disease (type)[b]	Controls[b]	Specimen type[b]	Ref.[b]
Antibodies as detected by						
ID	32/52 (62)	14/21 (67)	65/81 (80)	–	Serum	[89]
	–	17/21 (81)	210/255 (82)	4/767 (1)	Serum	[90]
	–	–	5/29 (17); acute	–	Serum	[91]
ID plus CF	9/13 (69)	37/53 (71); other IC 8/9 (89); non-IC	4/6 (67); acute 39/41 (95); subacute 5/6 (83); chronic	–	Serum	[88]
CF	29/46 (63)	14/22 (64)	75/83 (90)	–	Serum	[89]
	–	–	18/28 (64)	–	Serum	[91]
	0/3 (0)	7/10 (70)	–	–	CSF	[92]
	0/3 (0)	8/9 (89)			Serum	
	–	12/21 (57)	212/255 (83)	15/357 (4)	Serum[c]	[93]
	–	17/21 (81)	197/255 (77)	8/357 (2)	Serum[d]	
Antigens as detected by						
RIA[a]	3/4 (75)	2/10 (20)	–	1/28 (4)	CSF	[92]
	3/4 (75)	4/10 (40)		–	Urine	
	2/4 (50)	2/10 (20)			Serum	
	75/79 (95)	22/27 (82)	24/82 (29)	–	Urine	[89]
	54/63 (86)	7/11 (64)	6/26 (23)		Serum	
	38/40 (95)	12/16 (75)	11/30 (37)	1/96 (1)	Urine	[60]
EIA[a]	38/40 (95)	12/16 (75)	11/30 (37)	1/96 (1)	Urine	[60]
	–	–	84/130 (65); acute	n/a	Urine	[91]
			24/35 (68); acute	n/a	Serum	
	53/56 (95)	81/87 (93); other IC 11/15 (73); non-IC	5/6 (83); acute 14/46 (30); subacute 7/8 (88); chronic	n/a	Urine	[88]

PDH progressive disseminated histoplasmosis, *RIA* radioimmunoassay, *EIA* enzyme immunoassay, *ID* immunodiffusion, *CF* complement fixation, *CSF* cerebrospinal fluid
[a]Mira Vista Diagnostics, Indianapolis, IN
[b]Number of positive tests/total number of subjects (%)
[c]Antibody to yeast phase antigen
[d]Antibody to mycelial phase antigen

and if present, they are usually in lower titers. This is evidenced by the low levels of antibody detected in ~10% of healthy patients residing in an endemic area. Antibody titers generally decline over several months following exposure, but may remain positive for years in some chronic forms of the disease. False-negative tests occur during the early stages of infection and are more common in immunocompromised patients. False-positive results occur in ~15% of patients mainly due to cross-reaction with the agents of coccidioidomycosis or blastomycosis.

Antigen Detection

One of the major developments in diagnostic strategies for histoplasmosis was the introduction of antigen detection assays that could recognize a histoplasmosis polysaccharide antigen (Table 5.5). Depending on the disease manifestation, this antigen can be present in urine, serum, plasma, CSF, or bronchoalveolar lavage (BAL) fluid. The original assay was a RIA that was costly and posed a risk to laboratory personnel because of its radioactivity. A newer assay, now in its third generation, is the MVista *Histoplasma* antigen EIA (MiraVista Diagnostics, Indianapolis, IN). This assay is a quantitative sandwich EIA that uses polyclonal rabbit *anti-Histoplasma* antibodies to bind antigen and has demonstrated favorable results compared to its earlier generations and to the original RIA test [60, 61]. Two other polyclonal antibody EIAs which have performed well in initial reports include a test developed at the Centers for Disease Control (intended for resource poor countries) and an FDA-cleared in vitro diagnostic assay, the *Histoplasma* Antigen EIA (IMMY, Norman, OK) [62, 63]. Antigen detection assays are especially useful for establishing a diagnosis in immunosuppressed patients and patients with progressive disseminated disease (i.e., those with higher fungal burden), while they are less sensitive in detection of isolated pulmonary histoplasmosis. Furthermore, these tests are useful for monitoring antigen levels during treatment, where levels decrease with appropriate therapy and increase with disease

Table 5.6 Recommendations for immunologic test selection stratified by clinical syndrome for histoplasmosis, blastomycosis, and coccidioidomycosis

Disease/clinical syndrome	Antigen sensitivity	Antibody sensitivity	Recommendations
Histoplasmosis			
Progressive disseminated disease	95 % in urine: AIDS ~75–95 % in urine: other hosts	Poor	Antigen testing of serum and urine is recommended
Acute pulmonary disease	35–70 % in urine	> 80 % (at 4–6 weeks)[a]	Antibody (ID and CF) testing is recommended. Also consider antigen testing of serum and urine, particularly early in disease
Subacute pulmonary disease	~20 % in urine	90 %	Antibody (ID plus CF) testing is recommended
Chronic pulmonary histoplasmosis	~20 % in urine (note 88 % in one study)	80–100 %	Sputum or BAL fluid for culture and antigen testing are preferred. Send antibody (ID and CF) for remaining cases
Fibrosing mediastinitis Broncholithiasis Asymptomatic lung granuloma Chronic mediastinal lymphadenopathy	Antigen test usually negative	50–65 %	Antibody (ID and CF) testing is recommended
Meningitis	~20–70 % in CSF ~40–70 % in urine ~20–50 % in serum	0–70 % in CSF 0–80 % in serum	Antigen testing in CSF, serum, and urine plus antibody testing in serum and CSF (use CF assay) is recommended
Blastomycosis			
All forms of disease	85–93 % in urine 57 % in serum	Poor	Obtain culture and histopathology; perform urine antigen testing in cases where immunologic diagnosis is needed
Coccidioidomycosis			
Acute pulmonary disease: immune competent	Unknown	80–95 % (ID + CF)	Send serum antibodies (ID or EIA); if positive, obtain CF
Acute disease: immunosuppressed	50–70 % in urine ~70 % in serum	50–70 % (ID + CF)	Send serum antibodies (ID + CF +/− EIA) and consider sending serum and urine antigen (not well studied)
Meningitis	Unknown	~40–80 % in CSF ~75–195 % in serum	Send serum antibodies (ID + CF +/− EIA). Culture CSF (alert the laboratory) and send for EIA and/or CF

EIA enzyme immunoassay, *CF* complement fixation, *CSF* cerebrospinal fluid *ID* immunodiffusion, *BAL* bronchoalveolar lavage
[a]Data and recommendations from references [60, 67, 70, 72, 74, 79, 80, 87–98]

relapse. Though urinary and serum antigen detection are the most sensitive for these patients as a whole, CSF and BAL fluid testing may prove more valuable in patients with disease at those specific sites. Cross-reactivity of the assay occurs commonly with penicilliosis, paracoccidioidomycosis, and blastomycosis; less frequently in coccidioidomycosis; rarely in aspergillosis; and possibly in sporotrichosis [64]. False-negative results can also occur depending on the population tested and the severity of illness.

Detection of Fungal Metabolites

No tests of this nature are currently available.

Skin Testing

Skin testing with histoplasmin antigen is a useful epidemiologic tool to document past exposure and to investigate histoplasmosis outbreaks. It is of little use in the diagnosis of individual cases. Prior skin test positivity can be lost with disseminated disease or immunosuppression.

Conclusions and Recommendations

Immunodiagnostic tests for histoplasmosis are a proven adjunct to the usual diagnostic methods of culture and histopathology. Due to the wide spectrum of disease with histoplasmosis, there are different recommendations to help guide the appropriate use of immunologic tests (see Table 5.6 for recommendations). Patients with acute localized disease and a low burden of organisms or patients with chronic sequelae of a prior histoplasmosis infection should predominantly be diagnosed via antibody testing. Conversely, patients with a high burden of organisms (e.g., progressive disseminated disease) are diagnosed primarily via antigen testing. Also, combining antigen with antibody testing likely adds sensitivity in certain clinical situations.

Finally, measuring antigen levels is recommended during and after completion of therapy for progressive disseminated disease to monitor response to therapy and for relapse, respectively [65].

Blastomycosis

A high level of suspicion for *Blastomyces dermatitidis* infection is important to its successful diagnosis since no clinical syndrome is characteristic for infection with this organism. While definitive diagnosis requires the growth of the organism from clinical specimens, a presumptive diagnosis can be made by histological characteristics and further supportive evidence can be gained from immunologic tests.

Antibody Detection

Early serologic tests for blastomycosis were directed toward detecting host antibodies against *B. dermatitidis* A antigen. These tests utilized different laboratory techniques including ID, CF, and ELISA. The ID test has the most specificity (while sacrificing sensitivity); the ELISA test has the most sensitivity (and sacrifices specificity); and the CF test has poor sensitivity and specificity [66, 67]. However, all the tests have limited sensitivity for diagnosing acute disease because the mean peak seroprevalence of antibody occurs 50–70 days after the onset of symptoms [68]. An additional limitation is the presence of detectable antibodies for 1 year or more even after successful treatment. Antibody detection assays directed against the WI-1 antigen of the outer cell wall of *B. dermatitidis* have also been explored with promising results, but are not currently available commercially [69].

Antigen Detection

A quantitative antigen detection assay for use on urine, serum, plasma, CSF, and BAL fluid specimens of patients with suspected blastomycosis is available (MVista *Blastomyces* Antigen EIA; MiraVista Diagnostics, Indianapolis, IN). This assay targets a glycoprotein antigen that unfortunately is not genus-specific. The test has high sensitivity in the urine, ranging from 85 to 93 % across three studies [70–72]. However, the sensitivity is much lower in serum (in contrast to histoplasmosis). While the specificity is high for patients without fungal disease, the cross-reactivity is similar to the MVista *Histoplasma* Antigen EIA (common cross-reaction with penicilliosis, paracoccidioidomycosis, and blastomycosis; less frequent with coccidioidomycosis; rarely in aspergillosis; and possibly in sporotrichosis) [64]. The correlation between longitudinal measurements of antigen levels with response to therapy and/or relapse is not well established and hence, at this time, monitoring levels during treatment is not recommended.

Detection of Fungal Metabolites

No tests of this nature are currently available.

Conclusions and Recommendations

Several clinical features of blastomycosis make serodiagnosis relatively less important. Unlike histoplasmosis or coccidioidomycosis, where many of the recognized cases are acute pulmonary infections with negative sputum smears and cultures, identified blastomycosis cases are usually chronic pulmonary infections or disseminated infections of the skin and bones. In both of these conditions, histopathology and cultures are usually positive and easy to acquire. Blastomycosis urinary antigen testing should be used for the subset of cases when histopathology is unavailable or negative; however, caution is advised given the high test cross-reactivity with other fungal diseases. On the other hand, the poor accuracy of *Blastomyces* antibodies renders these tests generally unhelpful for diagnosing blastomycosis. Therefore, a negative antibody test should never be used to rule out disease, nor should a positive test be an indication to start treatment (see Table 5.6 for recommendations).

Coccidioidomycosis

Culture and histopathology are the gold standards for diagnosing coccidioidomycosis, but have several limitations. First, although *Coccidioides* species are readily cultured, growth is relatively slow and the cultures need to be performed under Biosafety Level 2 conditions as they pose a certain degree of risk to laboratory personnel. Second, since *Coccidioides* species are listed by the CDC as potential bioterrorism threats, laboratories working with these fungi must follow extensive security practices (and work under Biosafety Level 3 practices). Third, direct examination is insensitive because of the small number of *Coccidioides* organisms present in most clinical specimens. And finally, the mycelial form of growth rarely allows microscopic identification of *Coccidioides* species, requiring further testing to detect coccidioidal antigen in the fungal extract or a specific ribosomal RNA sequence using a DNA probe. This often has to be carried out by a reference laboratory. Given these limitations, immunologic tests are an important adjunct in helping to establish a diagnosis of coccidioidomycosis.

Antibody Detection

The cornerstone of serologic diagnosis is based on the detection of anti-coccidioidal antibodies via several different laboratory methods (Table 5.7). Assays initially developed included the TP and CF tests. Subsequently, two ID tests, the IDTP and IDCF, were developed which detect the same antibodies as the aforementioned TP and CF assays, respectively. The TP test (which is no longer used) and IDTP assay detect primarily IgM antibodies directed against the TP antigen, a heat-stable carbohydrate antigen of the fungal cell wall. These antibodies form early during infection with ~90 % of patients developing them in the first 3 weeks of symptomatic disease. For patients with a self-limited illness, these antibodies decline to less than five percent within 7 months. In contrast, the CF assay detects primarily IgG antibodies directed against the chitinase antigen, an enzyme of the fungal cell wall, which is often detectable while the disease is active. In the 1990s the Premier EIA (Meridian Diagnostics Inc., Cincinnati, OH) became available which measures IgM and IgG antibodies directed against the TP and CF antigens. Subsequently, other EIAs have been developed. An LA assay was also developed; unfortunately, while the LA is simple and provides rapid results, its use is limited by a higher number of false-positive reactions compared to the other assays.

Three tests are widely employed today: EIA, ID (both IDTP and IDCF), and CF. The EIAs are relatively simple to perform, can be performed on serum and CSF, and are generally quite accurate. However, an isolated positive IgM (when IgG is negative) may be falsely positive. This relatively common finding (~10 % of positive tests) has been the subject of several recent publications with quite discrepant conclusions (false-positive rate range 0–82 %) [73]. In particular, one should question the validity of an isolated positive IgM test in an asymptomatic patient or one without classic symptoms [74]. In general, EIAs should be confirmed with ID and/or CF.

Although several ID tests are commercially available (e.g., Meridian Diagnostics, Cincinnati, OH and IMMY Norman, OK), their performance is typically limited to reference laboratories. ID assays are sensitive (although perhaps not as sensitive as EIA or the CF test) and highly specific; they are often used to confirm the results of other serologic tests. They are limited to testing serum.

The CF assay, also limited to reference laboratories, is quantitative and can be performed on body fluids other than serum. Importantly, the antibody concentrations measured by CF are expressed as titers and generally reflect the extent of infection. As variation between testing results from facility to facility exists, it is suggested that serial measurements be conducted using the same laboratory to allow comparison. Use of serial testing can help the clinician gauge disease progression, remission, or cure. Early treatment of suspected coccidioidomycosis with fluconazole may abrogate the IgG response (i.e., the CF assay does not become positive) [75].

Notably, these assays are less reliable in the immunosuppressed population. In a recent publication evaluating 27 solid organ transplant recipients with newly acquired coccidioidomycosis, the sensitivity of all assays (EIA, ID, and CF) used in conjunction was only 77 % (20/26), whereas the positivity of any single assay was limited [76].

The EIA and CF may be used to detect antibody in the CSF; the tests are generally less sensitive than serum testing but, when positive, are highly specific for meningitis [77].

Antigen Detection

Research laboratories have demonstrated the ability to detect coccidioidomycosis antigen in both acute and chronic disease [78]. This sparked the development of the MVista *Coccidioides* antigen EIA (MiraVista Diagnostics, Indianapolis, IN). In the initial report of patients who were mostly immunosuppressed and who had moderate to severe disease, the test in urine demonstrated a 71 % (17/24) sensitivity and 99 % (159/160) specificity compared to healthy volunteers; however cross-reactivity was seen in 11 % (3/28) with other endemic mycoses (two histoplasmosis, one paracoccidioidomycosis) [79]. A second study evaluated the same test in serum (pretreated with EDTA at 100 °C) and urine in 28 patients with milder disease; in this limited dataset, the sensitivity in serum and urine were 73 and 50 %, respectively [80].

Detection of Fungal Metabolites

No tests of this nature are currently available.

Skin Testing

Skin testing with coccidioidin antigen or spherulin antigen is a useful epidemiologic tool to document past exposure. It may also be useful in patients in whom pulmonary coccidioidomycosis has already been proven by other means. A negative skin test in such a patient may be a bad prognostic sign, suggesting current or impending dissemination. In 2011, the FDA cleared a re-formulated test, the *Coccidioides immitis* Spherule Derived Skin Test Antigen (Spherusol; Allermed Laboratories, San Diego, CA). In a published report, this test demonstrated good safety, no histoplasmosis cross-reactivity, sensitivity 98 % in patients with a history of pulmonary coccidioidomycosis, and 98 % specificity in adult volunteers living outside an endemic area [81]. The niche for this test in clinical practice remains unclear.

Table 5.7 Studies of *Coccidioides* antibody and antigen detection

Assay	Population tested	Sensitivity	Specificity	Comments	Ref.
Antibodies as detected by					
TP	Retrospective analysis of antibody detection *in serum* of a large group of patients with various forms of coccidioidomycosis	2524/3219 (78 %)	–	Pulmonary disease	[95]
		89/226 (39 %)	–	Disseminated disease	
		33/73 (45 %)	–	Meningeal disease	
CF	As above	1790/3219 (56 %)	–	Pulmonary disease	[95]
		222/226 (98 %)	–	Disseminated disease	
		69/73 (95 %)	–	Meningeal disease	
	Retrospective analysis of patients with coccidioidal *meningitis*	29/30 (97 %)	–	Serum samples	[96]
		25/30 (83 %)	–	CSF samples	
	Retrospective analysis of HIV patients with disseminated coccidioidomycosis	6/8 (75 %)	–	Serum samples	[97]
		4/6 (67 %)	–	CSF samples	
	Retrospective analysis of antibody detection *in CSF* of a group of patients with various forms of coccidioidomycosis and control patients without coccidioidomycosis	0/9 (0 %)	13/13 (100 %)	Pulmonary disease (CSF)	[98]
		0/2 (0 %)	–	Disseminated disease (CSF)	
		14/33 (42 %)	–	Meningeal disease (CSF)	
	Retrospective review of all patients diagnosed with coccidioidomycosis at a tertiary medical center	35/52 (67); IC 188/252 (75); Non-IC	–	–	[87]
IDCF + IDTP	As above	21/40 (53); IC 180/248 (73); Non-IC	–	–	[87]
IDCF[b]	As above	0/9 (0 %)	13/13 (100 %)	Pulmonary disease (CSF)	[98]
		1/3 (33 %)	–	Disseminated disease (CSF)	
		10/19 (53 %)	–	Meningeal disease (CSF)	
	Retrospective analysis: patients with proven coccidioidomycosis; control patients with noncoccidioidal pulmonary illness, other fungal illness, HIV disease and no illness	47/47 (100 %)	362/362 (100 %)	–	[99]
EIA[c]	As above	43/47 (92 %)	352/362 (97 %)	IgG alone	[99]
		36/47 (77 %)	354/362 (98 %)	IgM alone	
		47/47 (100 %)	347/362 (96 %)	IgG and IgM together	
	As above	38/57 (67); IC 212/244 (87); Non-IC	n/a	IgG and IgM together	[87]
LA	As above	8/9 (89 %)	0/13 (0 %)	Pulmonary disease (CSF)	[98]
		3/3 (100 %)	–	Disseminated disease (CSF)	
		31/33 (94 %)	–	Meningeal disease (CSF)	
Antigens as detected by					
EIA[a]	Retrospective analysis of mostly immunosuppressed/HIV-positive patients with coccidioidomycosis	17/24 (70) in urine	159/160 (99 %)	More severe disease	[79]
	Retrospective analysis of mostly immunosuppressed patients with coccidioidomycosis; serology positive in 19/19 cases	12/24 (50) in urine 19/26 (73) in serum	–	Moderately severe disease	[80]

TP tube precipitin, *CF* complement fixation, *IDCF* immunodiffusion using complement fixation antigen, *EIA* enzyme immunoassay, *LA* latex agglutination
[a]Mira Vista Diagnostics, Indianapolis, IN
[b]IMMY, Norman, OK
[c]Meridian Diagnostics, Cincinnati, OH

Conclusions and Recommendations

The manifestations of most early coccidioidal infections overlap with those of other respiratory infections; therefore, specific laboratory testing is required to establish a diagnosis of coccidioidomycosis (see Table 5.6 for recommendations). Serum antibodies are important in the diagnosis of this disease and develop in most immune competent patients; however, antibodies are less reliable in the immunosuppressed host, particularly transplant, for whom the addition of serum and/or urine antigen tests may help augment disease detection. For most patients who resolve their infection, the antibody concentrations decrease to undetectable levels during the course of illness, so measurable antibodies are more likely to represent a recent or active illness. In general, screening for coccidioidomycosis is performed using either an EIA or ID. If EIA is used, it should be confirmed with ID and/or CF. The CF assay is primarily used to follow titers which gauge clinical progression and response to therapy. If initial serology is negative, repeat testing, potentially using all available assays (antibody and possibly antigen) in conjunction, will improve the diagnostic yield. Finally, skin testing may emerge as an improved method to screen individuals for past exposure.

Paracoccidioidomycosis

A definitive diagnosis of paracoccidioidomycosis requires either direct visualization of the organism in body fluids or tissues or its isolation and growth in culture. Immunologic assays are useful and rapid adjuncts for diagnosing this infection and following clinical outcomes, but unfortunately they are not widely available in the USA.

Antibody Detection

Initial efforts at antibody detection caused significant cross-reactivity with other fungal pathogens, leading to the development of antibody tests (ID, LA, counterimmunoelectrophoresis, ELISA) using more specific *P. brasiliensis* antigens. The most commonly used method is ID, which is very specific and has varying sensitivity depending on the antigen preparation used [82]. For example, the sensitivity of ID using the Ag7 antigen preparation was 84 % in one multinational study. More recently, a 43-kDa glycoprotein (gp43) from culture filtrates, now believed to be the dominant immunoantigen of *P. brasiliensis,* has been the target of improved serodiagnosis. A large report on 422 patients under investigation for paracoccidioidomycosis in Brazil evaluated the use of double immunodiffusion (DID) and Western blot (WB) using a crude exoantigen (primarily consisting of gp43). In this study, the sensitivity and specificity of DID were 80 and 95 %, respectively, and the corresponding values for WB were 92 and 64 % [83]. CF can be used to quantify antibody titers. The major limitation of these different assays is that antibodies can be detected for years after apparent successful therapy, so their presence does not help determine disease activity. Additionally, there is still some cross-reactivity with other regional diseases including histoplasmosis and leishmaniasis, and antibody responses are less robust in immunosuppressed patients.

Antigen Detection

The same cell wall and cytoplasmic components were also used as targets in early antigen detection assays. Unfortunately, these assays were also limited because of significant cross-reactivity in sera from patients with other mycoses (namely aspergillosis and histoplasmosis). To improve on this, a new target was sought and gp43 has been used (see “antibody detection” section above). An immunoblotting assay, performed on urine specimens, has demonstrated good sensitivity and excellent specificity for the detection of this antigen [84]. An ELISA technique with a monoclonal antibody has successfully detected this antigen in serum, CSF, and BAL fluid of patients with confirmed acute and chronic disease states [85]. Furthermore, these antigen levels can be followed as a marker of treatment response [86].

Detection of Fungal Metabolites

No tests of this nature are currently available.

Conclusions and Recommendations

Immunologic tests are useful for rapid diagnosis in suspected cases of paracoccidioidomycosis as ~ 80–90 % of patients with clinical disease have specific antibodies at the time of diagnosis. Furthermore, in disseminated disease, antibody production is elevated and titers are high, providing useful prognostic information. Antibody testing is limited, though, as their presence does not differentiate disease activity and their absence does not rule out disease, especially in patients with early disease or those who are severely immunocompromised. These are the populations where antigenemia may be detectable, prior to the development of immune complexes. It is currently advisable to use more than one test for the diagnosis of paracoccidioidomycosis. Serum antibody and serum, urine or site-specific antigen tests should both be ordered and any positive results should be monitored while on treatment. There is a concern that the assays may be detecting infection with other mycoses, so the results should be evaluated in the context of the entire clinical picture.

Other Mycoses

Immunodiagnostic tests have also been investigated for several other fungal infections, namely, mycetoma, mucormycosis, penicilliosis, sporotrichosis, and dermatophytoses. While they target a variety of antigens, antibodies, and nucleic acids, they are unfortunately still limited by a lack of prospective trials and commercial availability and cannot yet be recommended for routine clinical use.

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