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The Future of Fungal Serology

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Abstract The incidence of invasive fungal infections continues to grow. Early and rapid diagnosis is essential to prevent morbidity and mortality. The number of assays available for the detection of fungal antigens in human body fluids are increasing in number and becoming part of the basic diagnostic workup for many fungal infections. Detection of specific antibody has been an important component in the diagnosis of fungal infections. Complement fixation and immunodiffusion continue to be the gold standard for antibody detection but are complex to perform, require extensive expertise, and are mostly performed in reference labs. Newer assays are being developed to reduce turn-around time, but have not been fully evaluated. A challenge for improving serologic assays is to move from crude antigens and polyclonal antibodies to purified and/or recombinant antigens and monoclonal antibodies, while retaining good sensitivity and specificity. Recent developments using lateral flow methodology have provided novel point-of-care antigen assays requiring little technical expertise. Such innovative techniques will help to keep the future of fungal serology bright.

Keywords Fungal serology · Fungal diagnosis · Antibody assays · Antigen assays · Opportunistic infections · *Aspergillus* · *Candida* · *Cryptococcus* · *Histoplasma* · *Blastomyces* · *Coccidioides* · *Paracoccidioides*

Introduction

As the number of individuals susceptible to fungal infections continues to grow and the number of fungal infections expands [1], there is an increased need for timely diagnosis and initiation of appropriate antifungal therapy. Standard methods for diagnosis of fungal infections include culture, histopathology, serology and, more recently, DNA-based methodologies. Culture and histopathology provide definitive proof of infection [2]; however, the time required for culture growth and identification and the increased patient-risk in acquiring tissue biopsies does not always provide a timely diagnosis. On the horizon, molecular methods have tremendous potential; however, most methods are still experimental. Serologic methods for the detection and measurement of antibodies and antigens in serum, cerebral spinal fluid, and other body fluids have been used for many years for the diagnosis of fungal infections due to minimal invasiveness in specimen acquisition and generally more rapid turn-around time. Detailed information on current serologic assays and their interpretation is provided elsewhere [3]. This paper reviews the available serologic methods for diagnosis of fungal infection. Detailed descriptions of commercially available serologic assays are shown in Table 1.

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Assays for Opportunistic Infections

One of the most common risk factors for fungal infection is immunosuppression. Opportunistic fungal infections continue to rise in immunocompromised patients, including transplant, neutropenic, cancer and HIV-infected patients. Infections caused by *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans* are the most prevalent opportunistic fungal infections worldwide. Antibody detection, while a useful method for the diagnosis of infection in immunocompetent individuals, is generally not reliable in immunocompromised individuals. Antibody testing for the diagnosis of these infections, while effective in some instances, is often hard to interpret. These organisms are ubiquitous in nature and the presence of antibody titers suggests that prior exposure is common, making it necessary to show a fourfold rise in titer to demonstrate infection. Likewise, antibody formation may be suppressed in highly immunocompromised patients, resulting in false negative antibody reactions. Antigen detection, on the

Table 1 List of commercially available fungal serology assays

Disease	Name of assay	Manufacturer	Assay[a]	Analyte	Specimen[b]	USA[c]	FDA[d]
Aspergillosis	*Aspergillus* Antibody Immunodiffusion (not provided as kits but individual reagents) – Multiple sources	IMMY; Norman, OK Meridian Biosciences; Cincinnati, OH	ID	Ab	Serum	Yes	Yes
	Platelia *Aspergillus* Ag	Bio-Rad; Redman, WA	EIA	Ag	Serum, BAL	Yes	Yes
	Platelia *Aspergillus* IgG	Bio-Rad; Redman, WA	EIA	Ab	Serum, Plasma	No	No
Blastomycosis	OMEGA *Blastomyces* Antibody Enzyme Immunoassay	IMMY; Norman, OK	EIA	Ab	Serum	Yes	No
	MVista *Blastomyces dermatitidis* Quantitative Antigen EIA[e]	MiraVista Diagnostics; Indianapolis, IN	EIA	Ag	Urine, Serum, CSF, BAL Plasma	Yes	NA[f]
Candidiasis	Platelia *Candida* Ag Plus	Bio-Rad; Redman, WA	EIA	Ag	Serum, Plasma	No	No
	Platelia *Candida* Ab Plus	Bio-Rad; Redman, WA	EIA	Ab	Serum, Plasma	No	No
	Cand-Tec *Candida* Detection	Ramco Laboratories, Inc; Stafford, TX	LA	Ag	Serum	Yes	Yes
Coccidioido-mycosis	OMEGA *Coccidioides* Antibody Enzyme Immunoassay	IMMY; Norman, OK	EIA	Ab	Serum	Yes	No
	Premier *Coccidioides* EIA	Meridian Biosciences; Cincinnati, OH	EIA	Ab	Serum, CSF	Yes	Yes
	MVista *Coccidioides* Quantitative Antigen EIA[e]	MiraVista Diagnostics; Indianapolis, IN	EIA	Ag	Urine, Serum, CSF, BAL Plasma	Yes	NA
Cryptococcosis	Cryptococcal Antigen Latex Agglutination – Multiple sources	IMMY; Norman, OK Meridian Biosciences, Cincinnati, OH	LA	Ag	Serum, CSF	Yes	Yes
	Premier Cryptococcal Antigen	Meridian Biosciences, Cincinnati, OH	EIA	Ag	Serum, CSF	Yes	Yes
	ALPHA Cryptococcal Antigen Enzyme Immunoassay (CrAg EIA)	IMMY; Norman, OK	EIA	Ag	Serum, CSF	Yes	Yes
	Cryptococcus Antigen Lateral Flow Assay	IMMY; Norman, OK	LFA	Ag	Serum, CSF	Yes	Yes
	Pastorex *Cryptococcus* Plus	Bio-Rad; Redman, WA	LA	Ag	Serum, BAL, CSF, Urine	No	No
Histoplasmosis	ALPHA *Histoplasma* Antigen Enzyme Immunoassay	IMMY; Norman, OK	EIA	Ag	Urine	Yes	Yes
	MVista *Histoplasma capsulatum* Quantitive Antigen EIA[f]	MiraVista Diagnostics; Indianapolis, IN	EIA	Ag	Urine, serum, CSF, BAL, Plasma	Yes	NA
	Histoplasma DxSelect	Focus Diagnostics; Cypress, CA	EIA	Ab	Serum	Yes	No
1,3 β-D-Glucan	Fungitell Assay	Associates of Cape Cod, Inc.; East Falmouth, MA	LAL	Ag	Serum	Yes	Yes

Use of trade names and commercial sources is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention

[a] ID = immunodiffusion, LA = Latex agglutination, EIA = Enzyme immunoassay, LFA = Lateral flow assay, LAL = *Limulus* amebocyte lysate

[b] CSF = Cerebral spinal fluid, BAL = Bronchoalveolar lavage

[c] Commercially available in the United States

[d] FDA cleared

[e] Assay only performed at one laboratory (MiraVista Diagnostics)

[f] Not applicable

other hand, is a method of diagnosis that can be used in both immunocompetent and immunocompromised individuals. As a result, assays for the detection of fungal antigen in immunosuppressed populations have become important tools for the diagnosis of these infections. However, assay sensitivity can be affected by the rate of antigen clearance [4] from the body and by the formation of antibody/antigen complexes unless first disrupted with pronase, EDTA, boiling, etc., prior to the assay.

Cryptococcosis

Cryptococcus neoformans and *Cryptococcus gattii* are polysaccharide encapsulated yeasts that are global in distribution

and are the most common causes of fungal meningitis [5]. Infection is most frequently observed in HIV-infected individuals whose CD4 cell counts fall below 100 cells/μl [6]. The mortality rate in these patients can be substantial if diagnosis is delayed.

The detection of cryptococcal polysaccharide capsular antigen (CrAg) in serum and CSF by latex agglutination (97–100 % sensitive and 93–100 % specific [7, 8]) and enzyme immune assay (EIA) (Premier™ Cryptococcal Antigen) (85–100 % sensitive and 96–99 % specific [7–10]) have been very successful in providing accurate and timely diagnosis. The LA assay utilizes polyclonal antibody-coated latex beads, while the EIA employs polyclonal antibody-coated microwells for antigen capture and an enzyme-conjugated monoclonal antibody for antigen detection. While similar in sensitivity and specificity, EIA has the advantage in its ability to easily process large number of specimens, if necessary.

Recently, a new EIA for the detection of CrAg has been made commercially available (ALPHA Cryptococcal Antigen Enzyme Immunoassay). Manufacturer analysis states, upon comparison with the Premier EIA, there was a positive and negative agreement of 98.5 % and 97.7 % for serum and 93.5 % and 99.2 % for CSF, respectively. Analytical specificity, evaluated with 118 sera and 15 fungal culture filtrates, was 96.1 %; 67 % of *Paracoccidioides* culture filtrates were positive. Further independent evaluation of this assay is required.

The recently developed CrAg Lateral Flow Assay (CrAg-LFA) is a monoclonal, anticryptococcal antigen immunochromatographic assay. Through capillary action, diluted serum/CSF travels along a filter test strip to a section containing immunogold-conjugated antibody. If antigen is present, antibody/antigen complexes form, continue down the test strip, and are trapped by a line of immobilized anti-CrAg monoclonal antibodies. A purple immunogold line appears denoting a positive reaction. Results from this low-complexity assay are available in 10 minutes. It is 96–100 % sensitive and 96–100 % specific compared to EIA [11•, 12•], 96–100 % sensitive and 94–100 % specific compared to LA, and 98–100 % sensitive and 94–100 % specific compared to culture [13]. The limit of sensitivity is 1 ng/ml for serotypes A and B and 4–16 ng/ml for serotypes C and D [14]. The assay is inexpensive and does not require significant laboratory infrastructure or refrigeration, making it an ideal point-of-care test for use in underdeveloped countries where the incidence of disease is extremely high and where critical laboratory infrastructure is lacking [5]. The potential to detect antigen prior to onset of symptoms may reduce morbidity and mortality through the initiation of earlier treatment [6, 15].

Aspergillosis

Aspergillus species are globally distributed and ubiquitous in nature. Pulmonary infections in immunocompetent individuals can present as allergic bronchopulmonary aspergillosis (ABPA), aspergilloma or a fungus ball which forms in old tuberculosis lung cavities, or chronic necrotizing aspergillosis. Detection of *Aspergillus* antibody in these patients is variable, generally with good specificity but low sensitivity. Sensitivity can be improved by concentration of the serum prior to assay [16]. In the US, commercial immunodiffusion (ID) assays detect multiple antigens where increasing number of bands observed represents worsening of infection [16]. Commercial EIAs for detection of antibody are used in Europe but are not available in the US.

Invasive aspergillosis (IA) is a disease of great morbidity and mortality in immunocompromised patients. The Platelia *Aspergillus* Ag EIA, which uses monoclonal antibody EB-A2 [17] for detection of galactomannan antigen (GM) in serum, has proven extremely helpful in the diagnosis of IA in these patients. Initial evaluation of the assay demonstrated a specificity of 97.5 % and sensitivity of 76.3 %. Reducing the assay cutoff (from OD index of 1.5 to 0.5) increased the sensitivity to 97.4 % with a slight reduction in specificity to 90.5 % [18, 19]. The assay has been shown to demonstrate false positive results due to infection with other fungal and bacterial organisms [20–23] and antibiotic therapy [24–26] making it necessary to closely evaluate positive results. Assay specificity increases in patients with two consecutive positive results and was a predictor for developing IA [27, 28]. This assay has recently been approved for use in bronchoalveolar lavage (BAL) fluids. BAL fluids from lung transplant patients demonstrate a sensitivity of 81.8 % in proven or probable cases of IA [29]. Specificity in lung transplant patients not colonized with *Aspergillus* was 95.8 % and 96.6 % when using cutoffs of 0.5 and 1.0, respectively. In a recent update, the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) consensus group has now included a positive GM result as one of the factors required in the definition of probable and possible fungal infections [30].

Recently, another *Aspergillus* monoclonal antibody (JF5), specific for a protein epitope on a glycoprotein antigen, was incorporated into an LFA [31]. Monoclonal antibody JF5 reacted with 19 species of *Aspergillus* and cross-reacted with only *Paecilomyces variotii* and 12 species of *Penicillium*. It did not react with four other species of *Penicillum*, including *P. marneffei*, and 39 other fungal species. In a guinea pig model of invasive aspergillosis, the LFA was more sensitive and specific than the GM and 1,3-β-D-glucan assays [32]. A retrospective study compared LFA analysis to GM and polymerase chain reaction (PCR) analysis in hematology patients with IA [33•]. LFA was more sensitive and specific than GM (81.8 % vs. 77.3 % and 98.0 vs. 91.5 %, respectively. PCR was more sensitive (95.5 %) but less specific (96.6 %) than LFA. However, combining PCR and LFA provided the best sensitivity (100 %) and specificity

(100 %). Currently, there is no commercial source for this assay.

Candidiasis

Candida species are found worldwide and are frequently members of normal human gastrointestinal flora. Invasive infection occurs in immunosuppressed patients. Diagnosis by antibody detection is insensitive since antibody synthesis is poor in these patients and most healthy humans already produce abundant antibody to *Candida*. ID reagents are commercially available and shown to be 85 % sensitive and 94 % specific. EIA assays for the detection of antibodies to *Candida* mannan, a major component of the *Candida* cell wall, generally lack sensitivity and specificity [34, 35] but have displayed some usefulness when used in combination with mannan antigen assays [36].

Use of recombinant *Candida* antigens in an EIA format may be helpful in the detection of anti-*Candida* antibodies [37, 38]. Clancy et al. [37] detected antibodies in serum of systemic candidiasis patients using a panel of four recombinant antigens, which displayed a sensitivity of 96.6 % and specificity of 95.6 %. It is suggested that the use of a panel of recombinant *Candida*-specific enzymes and hyphal-specific antigens improves utility in the diagnosis of candidiasis, as antibody titers to individual antigens vary during infection [38]. *Candida* antigen assays, while specific, lack adequate sensitivity as a result of rapid antigen clearance [4]. The only *Candida* antigen assay available in the US is the Cand-Tec *Candida* detection system. Using latex beads coated with rabbit anti-*Candida* antibodies, the assay has a sensitivity of 13–38 % and specificity of 82–93.9 % [35, 39]. Outside the US, the Platelia *Candida* EIA for the detection of *Candida* mannan antigen is commercially available. In a 2012 report from the European Conference of Infections in Leukemia (ECIL) Laboratory Working Group [40•], the Platelia *Candida* Antigen assay provided a sensitivity of 58 % and a specificity of 93 %. When combined with the *Candida* Antibody assay sensitivity rose to 83 %; however, specificity declined to 86 %. Positive results were detected 6 to 7 days earlier than a positive blood culture in 73 % of patients and 16 days prior to radiographic lesion in 86 % of patients with hepatosplenic invasive candidiasis [40•].

1,3 beta–D-Glucan (Fungitell)

1,3-beta-D-glucan (BDG) is a fungal cell wall component that is found in all fungal organisms except for mucormycetes, *Cryptococcus* and the yeast-phase of *Blastomyces*. During infection, BDG is sloughed off growing hyphae into the blood stream. The method of detection does not use a standard antibody for detection, but a modification of the limulus amebocyte lysate (LAL) assay which uses horseshoe crab amebocyte lysate for the detection of BDG. When the amebocyte lysate is exposed to BDG, an enzymatic cascade is initiated, ending in the production of a chromophore that is detected spectrophotometrically. The Fungitell assay is used for BDG detection in serum and has been evaluated in cases of aspergillosis [41•], candidiasis [42, 43] and most recently, infections with *Pneumocystis jiroveci* [44]. Fungitell is FDA approved and has a general sensitivity and specificity of 83.3 % and 92.6 % [45]. This assay has also been used to detect BDG in CSF samples [46], however, the use of CSF in this assay is not FDA approved and a cutoff value has not been determined.

While the detection of BDG does not specifically identify the fungal agent involved, it provides a rapid detection of fungal infection, which is particularly useful for those infections that have no faster means of diagnosis. However, one must take care interpreting the results as false positive results can occur in patients that had recently undergone hemodialysis, received immunoglobulin products, or have had recent contact with surgical gauze [41•], as well as during the performance of the assay and in a variety of other circumstances. In a recent update, the EORTC/MSG included a positive BDG assay in defining probable and possible fungal infection [30].

Antigen Assays for Thermally Dimorphic Fungi

Histoplasmosis

An EIA antigen capture assay for the detection of *Histoplasma* antigen in urine, serum and BAL fluid is commercially performed at a single reference laboratory in the US (MiraVista Laboratories). A recent study [47] compared the efficacy of conventional antibody detection and antigen detection in urine for the diagnosis of histoplasmosis. While the antigen assay was more sensitive for the diagnosis of disseminated infection in immunocompromised patients (69–84 % [antibody] vs. 92–94 % [antigen]), antibody diagnosis was more sensitive in immunocompetent patients with disseminated or localized pulmonary infections (86–100 % [antibody] vs. 39–80 % [antigen]). This study demonstrates that both antigen and antibody assays are useful in their specific patient populations. The antigen assay is more sensitive if performed during the first 3 weeks of infection, prior to the detection of antibody [48, 49].

Other *Histoplasma* antigen assays have been developed for the detection of antigenuria [50, 51]. Cloud et al. [50] demonstrated a positive agreement of 92 % and a negative agreement of 98 % when split specimens were compared with the reference laboratory assay. A commercially available antigen assay (*Histoplasma* Antigen Enzyme Immunoassay), based on the Cloud et al. protocol, has a sensitivity and specificity of 80.9 % and 98.7 %, respectively, as stated by the manufacturer. There are currently no other independent reports in the

literature that describe the performance of the *Histoplasma* Antigen EIA.

Recently, Kushnir et al. [52•] examined urine specimens from patients with *Histoplasma* antigenuria, patients with increased proteinuria from different causes, and healthy individuals. Comparing protein found in the urine of these patients, this preliminary study revealed the presence of five proteins found only in the urine of *Histoplasma* patients. Whether any of these are immunogenic and specific to histoplasmosis remains to be seen.

Blastomycosis

An EIA for detecting *Blastomyces* antigenuria [53] has a reported sensitivity of 92.9 % and specificity of 79.3 % with cross-reactions occurring in patients with histoplasmosis (96.3 %), paracoccidioidomycosis (100 %), and *Penicilliosis marneffei* (70 %). The *Blastomyces* antigen assay appears to correlate well with confirmed cases of blastomycosis [54, 55]; however, the general usefulness of this assay for the diagnosis of unconfirmed blastomycosis remains uncertain because of its low specificity and cross-reactions with the other major fungal pathogens. The assay has recently been converted to a quantitative assay, developed using urine spiked with purified *Blastomyces* galactomannan [56]. Sensitivity was 89.9 % and specificity 99 % in nonfungal infected and healthy controls. However, cross-reaction was still observed with samples from 95.6 % of patients with histoplasmosis. *Blastomyces* antigenemia was observed in 35.7 % of cases increasing to 57.1 % after pretreating patient serum with EDTA at 104 °C to disrupt antigen/antibody complexes increased.

Coccidioidomycosis

An antigen assay for the detection of *Coccidioides* antigens in urine (*Coccidioides* Antibody Enzyme Immunoassay), has been shown to be 70.8 % sensitive [57]. Polyclonal capture antibodies for this assay were produced by injecting rabbits with a mixture of purified *Coccidioides* galactomannan and formalin-killed mold. The specificity of the assay was 99.4 % when compared to healthy individuals and patients with nonfungal infections. There was a 10.7 % cross-reaction with other endemic molds.

Antibody Assays for Thermally Dimorphic Fungi

Most serologic methods employed today for the detection of fungal antibody still use some of the original techniques initially developed in the 1950s and 1960s. Serodiagnosis of the endemic dimorphic pathogens *Histoplasma*, *Blastomyces*, *Coccidioides*, and *Paracoccidioides* continues to rely upon complement fixation (CF) [58] and immunodiffusion (ID) assays. Generally, while sensitivity of these assays can be quite variable, specificity is good. Optimal sensitivity and specificity is obtained by performing both CF and ID on all specimens. Due to cross-reactivity between antigen preparations, it is recommended to test each serum with antigens from all dimorphic pathogens, and included *Paracoccidioides* antigen when testing sera from Central and South America. More detail about the performance and interpretation of these assays can be found elsewhere [3]. While still considered the serologic gold standard for diagnosis for these infections, these methods are labor and time intensive, requiring a great deal of technical expertise in both assay performance and interpretation. Therefore, development of simpler, more rapid assays is necessary to improved turn-around-time and patient care.

Histoplasmosis

Histoplasmin, a *Histoplasma* mycelial culture filtrate antigen, is a major component of the CF and ID antibody assays for *H. capsulatum*. Incorporating histoplasmin into an EIA format for the detection of antibodies to *Histoplasma* was 93 % specific but had poor sensitivity at 57 % [59]. Pretreatment of histoplasmin with sodium meta-periodate, removing antigen blocking and cross-reacting carbohydrate, improved the sensitivity to 92 % and specificity to 96 % [59]. Sensitivity of the assay varied among patients with various forms of histoplasmosis: 100 % for patients with acute histoplasmosis and mediastinal histoplasmosis, 90 % during chronic disease, 89 % with disseminated disease, and 86 % in disseminated disease in HIV-infected patients [60]. These assays are a vast improvement to the old CF and ID assays, especially in ease of use and technical time, but are still considered experimental.

A commercially produced EIA for histoplasmosis antibodies (Histoplasma DxSelect) uses “inactivated” histoplasmin antigen to coat microtiter wells. The manufacturer states the assay has 82.4 % sensitivity and 88 % specificity when compared to sera with CF titers of ≥1:8. When compared to CF titers of ≥1:32 the assay was 93.3 % specific but only 58 % sensitive. There are currently no independent reports in the literature evaluating the performance of this commercial assay.

Blastomycosis

Current antibody assays for *Blastomyces* using purified A-antigen [61] derived from yeast culture filtrate concentrate [16], while specific, have poor sensitivity. Using purified A-antigen in an enzyme immunoassay improves sensitivity (86-100 %) but shows less specificity (87-92 %) [62–64]. Most of the current development of *Blastomyces* serodiagnostic assays has been performed with serum from dogs which are also frequently infected in endemic areas. EIAs using yeast cell

lysates from different *Blastomyces* strains have shown variable immunoreactivity [65, 66]. Using strains with the greatest reactivity, sensitivity was 100 % (36/36). Whether these assays can be used in the diagnosis of human disease is unknown.

A commercial EIA (*Blastomyces* Antibody Enzyme Immunoassay) uses a proprietary mixture of fungal yeast-phase antigens. The manufacturer describes sensitivity as 83.3 % when compared to positive CF at ≥1:8 and 100 % at ≥1:16. When compared to ID results the sensitivity and specificity were 100 %. There are currently no independent reports evaluating this assay.

Coccidioidomycosis

The incidence of infection has been increasing significantly since 2008 [67]. Providing timely and accurate diagnosis is important, particularly in high-risk patients. Conventional serodiagnosis using coccidioidin, a *Coccidioides* culture-filtrate antigen, provides adequate specificity (80–95 %) but variable sensitivity (75–98 %)[68]. A commercial EIA (Premier *Coccidioides* EIA) for the detection of both IgG and IgM antibodies against *Coccidioides* reports a sensitivity/specificity of 92 %/97 % for IgG and 74 %/96 % for IgM, and 97 %/ 94 % when the two assays are combined [69, 70]. However, there has been some concern regarding the specificity of the IgM assay. Initial reports by the manufacturer observe a 4–6 % false positivity rate. Subsequently, independent reports have shown false positive results in 0–18 % of patients without confirmed coccidioidomycosis infection, one report observing an 82 % false positivity rate [71–73]. Crum et al. [72] concluded that specificity was greatest when testing only serum from patients with a high suspicion of coccidioidomycosis. The highest rate of false positivity was observed when the assay was used to screen individuals as part of disease surveillance [72, 74]. Greater false-positive IgM results were observed in serum from HIV-infected patients (6.3 %) compared to 0.9 % in HIV negative patients [72]. Antifungal treatment also appears to attenuate the production of IgG if started within 2 weeks of the onset of symptoms [75].

Symptoms of coccidioidomycosis are often nonspecific and overlap with other fungal infections and bacterial infections. Sera from histoplasmosis patients are known to show cross-reactivity in *Coccidioides* EIA and CF assays [68]. Other agents such as *Mycobacteria tuberculosis* (Mtb), which in some geographic locations is equally as prevalent as *Coccidioides,* may also be responsible for false positive reactions. It has been demonstrated that *Coccidioides* and Mtb share a common antigen, 3-O-methylmannose [68], which may be a source of cross reactivity.

Recently, another commercial EIA for the antibody diagnosis of coccidioidomycosis has become available (OMEGA *Coccidioides* Antibody Enzyme Immunoassay) which uses a mixture of recombinant and native antigens. Compared to CF, the manufacturer states that the assay is 92.5 % sensitive and 98.7 % specific in a "normal" population. Compared to sera from patients with other fungal infections the specificity was 78.6 %, with 16.7 % indeterminate and 4.8 % (2/42) false positive. Independent studies to determine the efficacy of this assay have not been reported in the literature.

Paracoccidioidomycosis

Paracoccidioides is a dimorphic fungus that is endemic in areas of Central and South America. Serodiagnosis for *Paracoccidioides* has been based upon antigen preparations using either mycelial or yeast cell lysates. While a series of immunoreactive antigens are present in these lysates (27 kDa, 43 kDa, 70 kDa and 87 kDa), the predominant antigen is a 43 kDa glycoprotein, gp43. The only commercial assay available uses an immunodiffusion method with a cell lysate known to contain gp43 (*Paracoccidioides* ID Antigen; IMMY).

Discussion/Conclusion

When applied properly, serodiagnosis can be very useful for diagnosis of fungal infections. Serology results should be used to confirm clinical suspicion of infection and should be part of an overall diagnostic strategy combining medical (clinical signs and symptoms), microbiological (culture, if available), histopathological, and serological data. A travel history can be important, especially if the patient had recently visited an area where an endemic fungus is prevalent. In cases with nonspecific signs and symptoms, a panel of tests based on the most-likely causes should be used to provide an accurate and timely diagnosis; serology assays should not be used as a "shot gun" approach of ordering a large panel of assays and then attempting to interpret the positive result(s).

It is also very important to determine when and how many sera to obtain from a patient. Serodiagnosis using results from a single serum can be a limiting factor in interpreting results. An antibody assay with an equivocal or low positive result may represent the early rising titer of an acute infection, the declining titer of a past infection, or the suppressed antibody response of an immunocompromised patient. The serologic gold standard for proof of infection is seroconversion from a negative to a positive result or fourfold or greater increase in antibody titer. Therefore, a second convalescent serum obtained 3–4 weeks later is recommended for testing in parallel with the acute serum. This, however, requires the patient to return to the physician's office for a second blood draw, often after the patient has recuperated.

Interpretation of serological test results must be performed carefully. All assays are subject to false-positive and/or false-negative results, often due to the quality of assay reagents. Most of the antibody assays discussed here uses crude antigen preparations either for production or detection of antibodies. These assays usually provide good sensitivity due to their ability to react with more antigens, but specificity can be reduced if cross-reacting antigens are present. Some antigen preparations may contain glycosylated proteins that often cross-react with other fungal agents; this situation is common in crude antigen preparations made with culture filtrates of dimorphic organisms such as *Histoplasma* and *Coccidioides*. These reagents are produced in a finite quantity which can be difficult to produce repeatedly with equal reactivity in every batch. Some preparations may contain antigens that react only with antibodies produced later in infection. Likewise, patient serum obtained too early in infection, before detectable antibody is produced, may appear falsely negative.

Incorporation of specific recombinant antigens and/or monoclonal antibodies into serodiagnostic assays has the advantage in that these products can be generated in unlimited supply with little variability in quality. Assays using recombinant antigens or monoclonal antibodies are generally more specific, but can be less sensitive than their crude antigen/polyclonal antibody counterparts. However, assays that incorporate multiple recombinant antigens or monoclonal antibodies may provide both sensitivity equivalent to a polyclonal antibody preparation and the specificity of a monoclonal antibody assay.

An inherent feature of serologic assays is that they detect antigens or antibodies related to a single organism and therefore multiple assays must be performed for a diagnosis. DNA-based detection methods have an advantage in that testing can be performed for multiple pathogens in one assay using DNA amplified with universal fungal primers followed by sequence identification or other multiplex detection methods. Furthermore, exact reproduction of nucleic acid primers and probes is performed with relative ease. Molecular methods are available for fungal culture identification [76, 77]; in some cases, applications for detection of fungal DNA directly from patient specimen have been successful. There are currently no standardized DNA extraction methods, but a number of studies of this topic have been published [78–81] . Many fungal cell walls are difficult to disrupt, affecting sensitivity, particularly when fungal organisms are present in small numbers, but in some cases free fungal DNA has been detected in human tissues and fluids [78].

Sufficient challenges exist in the diagnosis of fungal infections not mentioned in this review, particularly other agents of infection in the compromised host. Voriconazole prophylaxis to protect against *Aspergillus* infections may create an opportunity for other molds, such as the voriconazole-resistant mucormycetes, to cause rapid and often fatal disease. *Scedosporium* and *Fusarium* are other opportunists with increasing prevalence. There are currently no specific serologic assays for the detection of these pathogens. Mucormycetes do not produce glucans that can be detected with the BDG assay. Furthermore, the BDG does not differentiate among fungal species. New, rapid assays need to be developed to diagnose disease caused by these emerging pathogens.

Noncommercial, "in-house" developed, "research use only" serologic assays or laboratory-developed tests (LDTs) use laboratory specific methods and reagents to fulfill a need when there is no commercial assay available. In the United States, future development of new assays may be affected by new Food and Drug Administration (FDA) regulations. The FDA considers an LDT as an in vitro diagnostic product (IVD) or a medical device requiring FDA-approval prior to use (21 CFR 809.3). New LDTs or any modifications to already FDA-cleared assays must demonstrate to the FDA, prior to use, accuracy, precision, analytical sensitivity, analytical specificity to include interfering substances, reportable range of test results for the test system, reference intervals (normal values), and any other performance characteristic required for test performance [42 CFR 493.1253 (b)(2)]. These regulations are to be applied to assays put into use after April 24, 2003. Enforcement of these rules may decrease the number of laboratories performing LDTs, relying on commercial products. However, current commercial development of fungal serologic assays has been slow, possibly due to the cost of development and maintenance of reproducible and reliable reagents and the low volume of potential users.

Finally, the future of fungal serology is still to be seen. While future molecular technologies may supplant serologic methodologies, molecular methods for performance directly from patient specimens still need standardization including improvement in time, technology and cost. The current complexity of fungal serology testing relegates most antibody testing and some antigen testing to only larger clinical and reference laboratories. For fungal serology to continue to progress, development of sufficiently sensitive and specific, rapid and simple methods that can be performed at local laboratories is necessary for improved turn-around-times and earlier initiation of specific therapy. The recent development of antigen assays incorporating lateral flow technology have shown great promise for producing new assays that are easy to perform even in the smallest laboratories, brightening the future of fungal serology.

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Compliance with Ethics Guidelines

Conflict of Interest Mark Lindsley declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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