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MINIREVIEW

Review of Clinical and Laboratory Diagnostics for Coccidioidomycosis

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ABSTRACT Coccidioidomycosis is a fungal disease associated with soil exposure that frequently goes undiagnosed due at least in part to its nonspecific presentation and the lack of clinical suspicion by health care providers. Currently available diagnostics for coccidioidomycosis offer qualitative results that can suffer from low specificity, while semiquantitative assays are labor-intensive and complex and can require multiple days to complete. Furthermore, significant confusion exists regarding the optimal diagnostic algorithms and appropriate usage of available diagnostic tests. This review aims to inform clinical laboratorians and treating clinicians about the current diagnostic landscape, appropriate diagnostic strategies, and future diagnostic directions for coccidioidomycosis, which is expected to become more prevalent due to increased migration into areas of endemicity and climate changes.

KEYWORDS *Coccidioides*, coccidioidomycosis, valley fever, serology, diagnostics, complement fixation, immunodiffusion, enzyme immunoassay, antigen, antibody, fungus

Coccidioidomycosis ("valley fever") is caused by the dimorphic fungi *Coccidioides immitis* and *C. posadasii*. These organisms exist in a mycelial form in the soil, and during periods of low precipitation/drought, they produce aerosolizable arthroconidia (spores). Infection generally occurs via the inhalation of airborne arthroconidia, which gives rise to the spherule/endospore phase of infection (1). The majority of those who present with clinically relevant disease exhibit respiratory symptoms within 1 to 3 weeks of exposure, with fever, cough, chills, night sweats, weight loss, and/or joint pain. Coccidioidomycosis causes 17 to 29% of all cases of community-acquired pneumonia (CAP) in regions where the disease is endemic, which were historically concentrated in California and Arizona but appear to be expanding into other central and northwestern states (2). Severe infection can occur naturally in many other mammalian species, including primates, dogs, cats, horses, dolphins, and sea lions (3, 4). The majority of acute pulmonary cases resolve over weeks to months with or without treatment (5). However, roughly 5 to 7% of patients will develop more complicated infections ranging from complicated pulmonary disease (nodules, cavities, or pleural disease) to disseminated disease most frequently affecting the skin, bones/joints, and/or the central nervous system (CNS). The rates of infection and the severity of coccidioidomycosis are higher for males than for females, with observed coccidioidal meningitis (CM) rates of 2.4% in men versus 1.8% in women (6). Immunocompromised (including pregnant women), diabetic, Black, and Filipino patients of both sexes are also at an increased apparent risk of complicated and severe infection courses (7, 8).

Diagnostic criteria for coccidioidomycosis have been developed by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) (9). Criteria for "proven disease" include histopathology or direct microscopy of specimens obtained from the affected site demonstrating

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characteristic forms of *Coccidioides* or the recovery of the fungus from the affected site(s). "Probable disease" requires evidence of exposure (residence or travel to an area of endemicity) and the presence of coccidioidal antibodies in the serum or cerebrospinal fluid (CSF). These criteria were developed for use in clinical trials, the development of diagnostic tests, and epidemiological studies and need not be rigorously adhered to during routine clinical care.

This review summarizes currently available diagnostics for coccidioidomycosis and emphasizes areas of uncertainty and future needs.

CULTURE AND MICROBIOLOGY

The isolation of *Coccidioides* spp. from clinical specimens remains the standard for definitive diagnosis. While culture evaluation can occur in a biosafety cabinet under biosafety level 2 (BSL-2) conditions, the 6th edition of *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) recommends BSL-3 practices when propagating and manipulating sporulating *Coccidioides* cultures (10). Respiratory samples, either sputum cultures or those obtained by bronchoalveolar lavage (BAL), may reveal the organism in as early as 48 to 72 h using routine blood, chocolate, or mycologic media, including Sabouraud dextrose agar incubated at 25 to 30°C. Unlike other dimorphic fungi, the only observable morphotype on routine culture media is mycelium. The addition of cycloheximide to culture medium inhibits some saprobic fungi and is useful as a selective medium as it supports the growth of dimorphic fungi. Routine blood cultures are not helpful for the evaluation of suspected coccidioidomycosis. Detection was possible but rare using fungal isolator blood cultures and usually only for highly immunocompromised individuals (11). However, fungal isolator tubes are being discontinued in the United States, which should encourage reliance on tissue-based culture and serologic diagnostic modalities. In addition to lower respiratory tract samples, material for microscopy and culture can be collected from other presumed sites of infection, including cutaneous and soft tissue lesions, bone lesions, CSF, or joint aspirates, among others. While observable using low-power microscopy without a stain, calcofluor white can improve spherule detection (Fig. 1A), although the sensitivity of microscopy (even with calcofluor white staining) may not exceed 22% compared with culture (12).

The sensitivity of cultures for the detection of *Coccidioides* spp. depends on the host's immune status, the fungal burden, and the site of infection. Culture success is most common from respiratory specimens, but the overall culture sensitivity for coccidioidomycosis cases is unclear and is estimated to not exceed 50% (13, 14). Isolate recovery rates have been observed to be 13% from pleural fluids, 30% from CSF specimens (from CM cases), and 44% from respiratory specimens from established coccidioidomycosis cases (13–15). While only limited data are available, pleural biopsy specimens may be among the more rewarding specimen types for culture success when pleural disease is present (16).

Following inoculation onto solid culture media, mycelial fungal growth may be observable in as early as 2 to 3 days. Combined with its ability to grow on routine bacteriology media, this can present significant and serious infection risks for unsuspecting clinical laboratorians, underscoring the imperative for proper training such that cultures containing mold are neither manipulated nor opened outside a biosafety cabinet. Arthroconidia present in culture are abundant at levels several orders of magnitude higher than those observed in the environment, and laboratory-acquired infection is unfortunately frequent and well documented (17). Optimally, cultures should be examined daily for the first 10 days and then weekly thereafter (17). It may be necessary to hold cultures for up to 6 weeks, particularly in patients currently receiving antifungal therapy, as this may significantly delay fungal growth. Macroscopically, colonies are typically white to gray, although they may produce variably colored pigment (yellow/brown to orange) over time. Discrete concentric rings of developmentally distinct mycelium may be observed (Fig. 1C). Microscopically, hyphae are thin, hyaline, and septate. As arthroconidia mature, the alternating disjunctor cells undergo lytic degradation, releasing "boxcar"- or "barrel"-shaped arthroconidia (~2 to 5 μm in length) easily visible with lactophenol cotton blue staining (Fig. 1B). Arthroconidia may also be seen with other genera, including *Malbranchea* and *Geotrichum*, so confirmation of the culture identity is considered necessary in most laboratories.

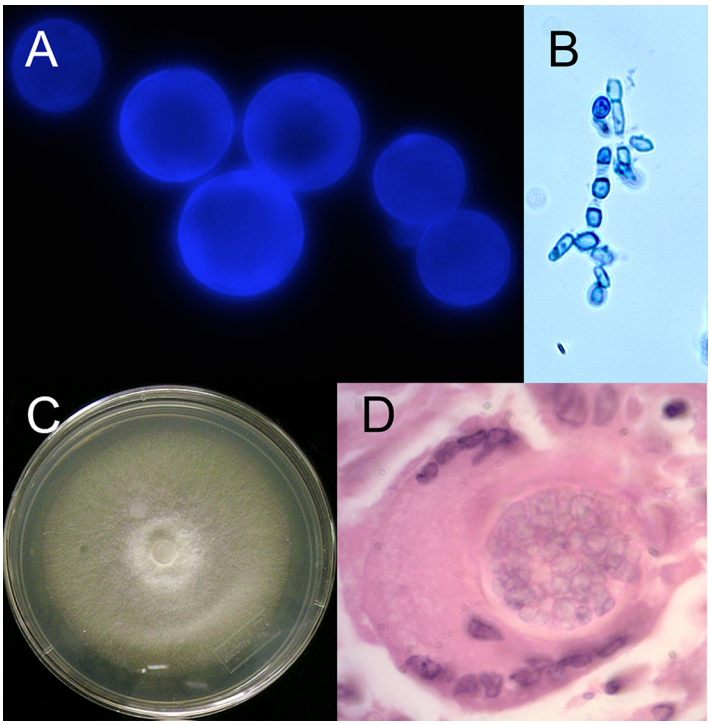

FIG 1 Morphotypes and culture morphology of *Coccidioides*. (A) Calcofluor white-stained fluorescent spherules, approximately 10 to 15 μm in diameter (magnification, $\times$600). (B) Mycelial arthroconidia, approximately 2 to 5 μm (magnification, $\times$1,000), stained with lactophenol cotton blue. (C) Coccidioides mold growing on solid culture medium following extended incubation. (D) A spherule, approximately 120 μm in diameter (magnification, $\times$1,000), engulfed in a multinucleated giant cell. Splenic tissue was stained with hematoxylin and eosin.

Until 2021, the *Coccidioides* AccuProbe assay (Hologic, Sunnyvale, CA, USA) was the most common and convenient mechanism for confirming the identity of *Coccidioides* culture isolates (18). However, this test was discontinued in 2021, necessitating new workflows for culture identification. A common response has been to utilize matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) (following culture inactivation). The recently released, FDA-approved V3 FDA database for the Vitek MS system (bioMérieux, Marcy-l'Étoile, France) includes *Coccidioides immitis*/*C. posadasii*, along with many other dimorphic fungal species, allowing convenient in-house confirmation for some laboratories. At the time of manuscript preparation, no FDA-approved database was available for the MALDI Biotyper (Bruker, Billerica, MA, USA) that could identify *Coccidioides* spp. To address this deficit, some laboratories with Bruker MALDI instruments have turned to custom research use only (RUO) spectral library creation using in-house isolates; at least one database is publicly available via a material transfer agreement (19). Alternatively, DNA sequencing or PCR can be employed to confirm the isolate identity but would currently necessitate the development and implementation of a laboratory-developed test (LDT) as no commercial options for these methods exist to test culture isolates (13, 20–22).

PATHOLOGY

Within the host (37°C), arthroconidia transform into spherules (up to 120 μm in diameter), which are thick-walled spherical structures (Fig. 1D) containing hundreds of endospores, each approximately 2 to 4 μm, that are released upon spherule rupture. Each endospore can further propagate to a spherule, continuing the life cycle of *Coccidioides* spp. Tissue sections should be stained with periodic acid-Schiff (PAS), Grocott-Gomori methenamine silver (GMS), or hematoxylin and eosin (H&E) stain to assist in the detection of the characteristic large, thick-walled spherules of *Coccidioides* spp. Microscopic examination of wet preparations of sputum, bronchoalveolar lavage fluid, pus, or other samples treated with potassium hydroxide (KOH)

may be helpful, but these methods are less sensitive. Histologic "mimickers" exist, including *Prototheca wickerhamii*, which can resemble small spherules, and *Rhinosporidium seeberi*, which can appear similar to larger ones. Adiaspores from *Adiaspiromyces* species must also be distinguished from the spherules of *Coccidioides* spp. Adiaspores do not contain endospores, and adiaspores are typically much larger than empty *Coccidioides* spherules. The molecular methods discussed below may be helpful when uncertainty exists about the identity of structures observed in tissue.

SUSCEPTIBILITY TESTING

While rarely warranted or recommended, antimicrobial susceptibility testing can be performed via broth macrodilution using arthroconidia according to the CLSI M38-A3 reference standard (23), and MICs are read as the lowest concentration that results in ≥80% inhibition of growth compared with the antifungal-free control well (24). No susceptibility breakpoints for *Coccidioides* spp. have been established by the CLSI, FDA, or EUCAST to date, and no automated susceptibility platforms are currently available for testing filamentous fungi. Accordingly, susceptibility testing usually requires the shipment of culture isolates to reference laboratories, which further requires that laboratory staff are properly trained and competent in category A shipping requirements as *Coccidioides* spp. are considered a UN2814 infectious substance affecting humans.

MIC data from 581 isolates sent to the Fungal Testing Laboratory at the University of Texas, San Antonio, revealed that the geometric mean MIC of fluconazole (7.71 μg/mL) was significantly higher than those of other triazole antifungals like itraconazole (0.25 μg/mL), posaconazole (0.14 μg/mL), or voriconazole (0.11 μg/mL) (25). This finding is concerning as fluconazole remains among the most commonly prescribed antifungals for coccidioidomycosis. While these *in vitro* findings have not yet been correlated with patient outcomes and may be subject to some degree of selection bias, they raise concerns about the optimal therapy of serious or refractory infections (26).

SEROLOGY

In immunocompetent patients, coccidioidomycosis typically manifests with detectable serum IgM within 1 to 3 weeks of symptom onset, followed shortly thereafter by IgG production. Due to the nonspecific presentation of acute coccidioidomycosis, associated diagnostic delays, and the insensitivity/impracticality of other front-line diagnostics, most patients are diagnosed using serological methods. While antibodies can be detectable in many body fluids, antibody titers are nearly always highest in serum (Ian H. McHardy and George R. Thompson III, unpublished observations). Apart from CSF, antibody testing of other body fluids is not generally recommended and is unlikely to be informative. Among highly immunocompromised individuals, the sensitivity of antibody detection can be as low as 67% (27), so multiple diagnostic modalities, including antigen detection and culture, may be necessary. Available serologic diagnostics currently include enzyme immunoassays (EIAs), immunodiffusion (ID), complement fixation (CF), and a lateral flow assay (LFA). These assays exhibit differing sensitivities and specificities and have different roles in a diagnostic algorithm for coccidioidomycosis (Fig. 2). ID and CF remain the benchmarks for coccidioidal serologic testing but are complex and less standardized and require considerable technical experience maintained in very few clinical laboratories. Laboratories can mitigate unnecessary diagnostic delays associated with reference laboratory utilization by exploring the FDA-approved serologic options described below for in-house implementation.

Lateral flow assay. More recently, an LFA has been developed (Sōna; Immy, Norman, OK, USA). This rapid test was designed to detect *Coccidioides*-specific IgG and IgM, and results can be available within 1 h. This test has been evaluated in a prospective evaluation of 392 patients (293 hospitalized patients and 99 outpatients) with suspected coccidioidomycosis (28). In this trial, the LFA was found to have 31% sensitivity and 92% specificity compared to EIA results. The optical density values of positive EIA IgM results were significantly higher for LFA-positive results than for LFA-negative results. In the subset of patients where CF results were available, titers were frequently

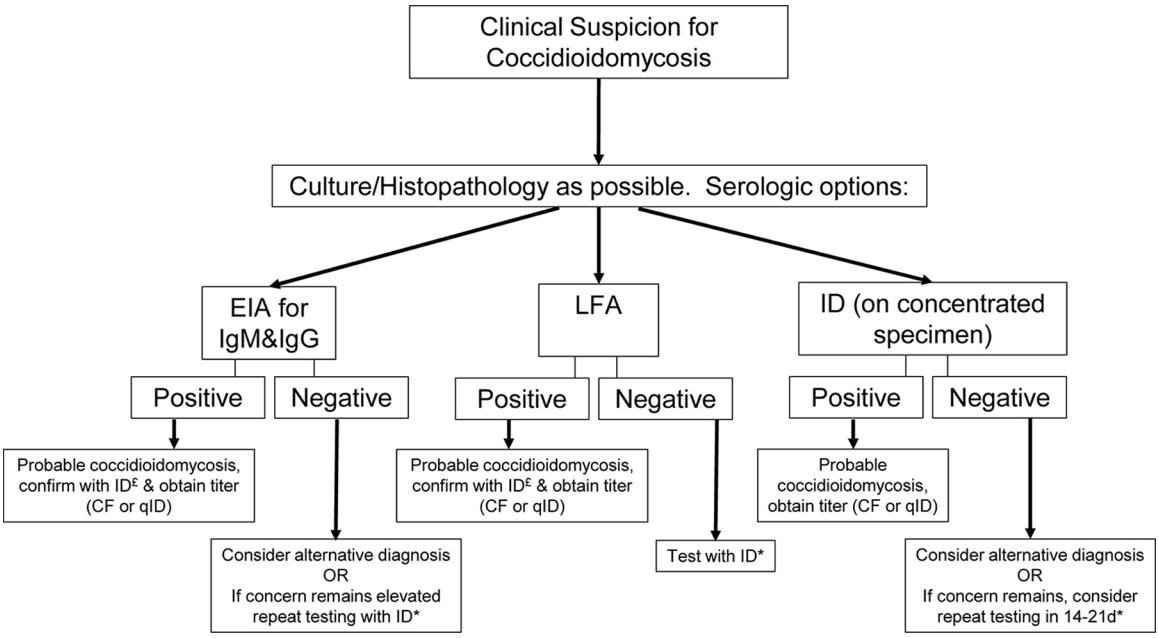

FIG 2 Recommended algorithm for the diagnosis of coccidioidomycosis. *, coccidioidal antigen testing may be beneficial for immunocompromised patients with negative serologic results. £, confirmation by ID may not be necessary for positive IgG results by EIA and LFA results in highly endemic areas.

low but detectable in those with negative LFA results. Accordingly, while the positive predictive value of the test may be high, particularly for disseminated cases where titers are frequently ≥1:16, confirmatory ID testing is recommended for negative LFA results. While possibly suboptimal for ruling out coccidioidomycosis, the LFA may have value for antimicrobial stewardship; antibacterial agents might be safely discontinued in patients who test positive via an LFA in clinically appropriate situations.

EIAs. EIAs are qualitative or semiquantitative assays that are attractive due to their relative simplicity, speed, and fair, but overall inferior, analytic characteristics compared with CF and ID. Several commercial kits are available to measure anticoccidioidal IgM and IgG antibodies (Clarus [Immy, Norman, OK, USA] and Premier [Meridian Bioscience, Cincinnati, OH, USA]). Separately, a laboratory-developed EIA is also performed by MiraVista Diagnostics (MVista). Importantly, EIAs suffer from some deficiencies in sensitivity and high false positivity rates compared to other methodologies and should not be used for definitive assessment, particularly of CSF specimens, for this reason (29).

A comparative study of different EIA kits to a composite clinical standard showed that the sensitivity and specificity of the Immy EIA IgM test were 35% and 86%, respectively; for the IgG test, the sensitivity was 53%, and the specificity was 97%. The sensitivity and specificity of the Meridian EIA IgM test were observed to be 57% and 70%, respectively; for the IgG test, the sensitivity was 69%, and the specificity was 95% (30).

Separately, an independent assessment of the Meridian and Immy EIA kits for the diagnosis of coccidioidomycosis and the interlaboratory agreement of test results was jointly conducted by the Arizona Department of Public Health and the Centers for Disease Control and Prevention (CDC). This analysis showed sensitivity and specificity results similar to those outlined above (IgM sensitivity of 11%, IgM specificity of 100%, IgG sensitivity of 64%, and IgG specificity of 99% for Immy; IgM sensitivity of 36%, IgM specificity of 92%, IgG sensitivity of 65%, and IgG specificity of 99% for Meridian). This study also found more interlaboratory variability in the performance of the Meridian test kit.

An industry-sponsored evaluation has shown a higher sensitivity using the MVista EIA than using the Meridian or Immy assays, although the sensitivity of the MVista EIA for IgM was still only 61.2% (31). The specificities for both IgM and IgG detection in this study remained high for all three assays (≥90%).

Importantly, due to the imperfect sensitivity of EIAs, negative EIA results for patients with strong clinical suspicion for coccidioidomycosis should be retested and/or confirmed via ID. Similarly, all positive IgM-only results by EIAs and positive IgG results by EIAs in areas with a low prevalence should also be confirmed with ID. However, in areas where the disease is highly endemic, a positive IgG result by an EIA may be considered a probable infection, but downstream clinical staging by CF testing still allows prognostic information and is useful to monitor during therapy (30).

Immunodiffusion. Immunodiffusion, or "Ouchterlony double immunodiffusion," relies on the ability of antibodies and antigens in different wells of ~0.8% gellan plates to diffuse and form a visible band(s) of precipitation at their intersection point. If performed in experienced laboratories, ID can be highly sensitive and specific for the detection of *Coccidioides*-specific antibodies, with the specificity exceeding 95% (32, 33). Estimates of the sensitivity of ID vary and depend on the duration of symptoms and the patient's immune status, but it is among the most sensitive serologic methods and is currently the serologic diagnostic method of choice for confirmatory testing (34). Of the available FDA-approved ID assays, only Meridian's package insert includes language supportive of the use of preconcentrated specimens; off-label or independent LDT verification may be necessary for use with other reagents. ID performance with unconcentrated specimens is not recommended and may yield suboptimal sensitivity.

When present, the position of the precipitation band between wells containing the culture filtrate antigen and an antibody-containing specimen can provide a preliminary assessment of IgG antibody abundance (i.e., CF titer); precipitation bands observed close to the culture filtrate antigen well are usually associated with high titers, while bands close to the serum well are associated with low to undetectable CF titers (our unpublished observations).

Historical nomenclature is occasionally referenced with coccidioidal ID results, which may lead to some confusion without context. First, coccidioidal IgM may be referred to as "precipitin" (also "tube precipitin" or "TP"), which references an antiquated tube precipitin assay that assayed for solid, flake-like "precipitin" formation when serum containing anti-*Coccidioides* IgM was mixed with heat-stable antigen (including glycosylated β-glucosidase-2) found in *Coccidioides* species culture filtrates (35, 36). Some laboratories continue to report ID reactions for IgM as "IDTP" (ID tube precipitin). Similarly, the antibody responsible for CF reactivity was found to be IgG, so ID results for IgG are occasionally referred to as "IDCF" (immunodiffusion complement fixation).

Complement fixation. Complement fixation testing provides a semiquantitative assessment of coccidioidal IgG antibody and can be performed on sera, CSF, and other body fluids (e.g., synovial and pleural fluids, etc.) (14). The test relies on two intrinsic properties of complement: (i) free complement (unbound to IgG antibody) will lyse sensitized (hemolysin-treated) red blood cells (RBCs), and (ii) complement will bind to IgG antibodies that are bound to their target antigens. Complement that is bound to IgG antibodies will not lyse sensitized RBCs. Thus, when coccidioidal antigen-antibody complexes are present in sufficient quantities to bind all exogenous complement in a mixture, sensitized RBC lysis is prevented, which can be qualitatively or semiquantitatively assessed by turbidity or spectrophotometry, respectively. In this way, serial 1:2 dilutions of patient sera or CSF are tested, and the titer is reported as the concentration of the specimen in the last tube containing intact RBCs.

CF is less sensitive than ID (performed on preconcentrated specimens), and over 25% of uncomplicated pulmonary cases develop no detectable CF titer due to the small quantity of IgG present in these cases (34, 37). Accordingly, CF should be used not for diagnosis but rather for disease staging, prognosis, and antifungal response monitoring (37, 38). In general, complicated (i.e., disseminated and chronic pulmonary) cases tend to exhibit CF titers of $\geq$1:32, while uncomplicated (i.e., acute/nondisseminated) cases tend to exhibit lower CF titers of $<$1:32 (37). However, there are many exceptions, so this should be considered not a rigid "rule" but rather general guidance; clinical findings are often more informative than CF titers. For example, 16% of uncomplicated cases develop titers of $\geq$1:32, and 56% of meningitis cases exhibit maximum serum titers of $\leq$1:32 (37). In most cases, maximal titers develop within the first 31 to 50 days following serologic diagnosis.

Quantitative immunodiffusion (qID) can also be used instead of CF to semiquantitatively assess IgG titers in cases where CF is uninterpretable due to the myriad of frequently encountered technical failures associated with CF (e.g., anticomplementary activity) (34). Like CF, serial 1:2 dilutions of patient sera or CSF are plated adjacent to a well containing the antigen (*Coccidioides* culture filtrate containing chitinase), and the titer is reported after 48 h as the last dilution with an observable band of precipitation.

The kinetics of antibody decline during successful therapy vary depending on the type of disease; those with uncomplicated pulmonary infection exhibit a more rapid antibody titer decline than those with complicated (e.g., chronic pulmonary or disseminated) disease (37, 38). Some patients exhibit persistent elevation, or stasis, of their CF titers despite clinical improvement. Some of these patients are likely serofast, while others may experience repeated environmental exposure or have subclinical persistence of active infection (38).

Serial CF testing of the CSF in cases of meningitis has shown a correlation with concurrent CSF parameters (cell count, protein, and glucose), although both serology and routine CSF values respond more slowly than clinical signs and symptoms of disease (39). Importantly, given the lifelong repercussions for patients diagnosed with CM, laboratorians should be aware that any positive CF results performed on a CSF specimen can result in a diagnosis of CM. Accordingly, appropriate labeling of specimen sources on both tubes and requisitions is critically important. Complicating the challenge of CM diagnosis is the finding that high serum titers may "spill over" into CSF, resulting in false-positive ID results for CSF samples. This is why complement fixation, being less sensitive than ID, is the recommended diagnostic modality for CM diagnosis (40).

ANTIGEN DETECTION

The detection of circulating coccidioidal antigens in patient samples for diagnosis has focused on the recognition of spherule-derived antigens (41), galactomannan (42), and, more recently, chitinase-1 (CTS1) (43). Initial studies evaluating spherule-derived antigens in patient sera detected circulating antigen in 35 of 233 patients, and antigen was detected infrequently following the development of IgM and/or IgG antibodies (41). Following the recognition of the cross-reactivity of coccidioidomycosis with the *Histoplasma* urine antigen EIA, a specific *Coccidioides* (galactomannan) antigen assay was developed and evaluated in a cohort of 24 patients (16 patients with acute coccidioidomycosis and 12 of these with HIV). Antigenuria was detected in 71% of patients in this study (42), although the performance characteristics in nonimmunosuppressed patients have been less favorable, and the sensitivity in the veterinary population has been found to be less than 20% (44). Several case reports and case series have also shown positivity in the CSF of patients with coccidioidal meningitis (45), although attempts to use this test serially to monitor the response to therapy have thus far yielded conflicting results. More recently, the detection of CTS1 in patient blood samples has been explored, and antigen was detected in 87% of patients with proven or probable coccidioidomycosis, although this assay was later determined to also detect anticoccidioidal antibodies, limiting its utility in monitoring patients longitudinally (43).

Serum (1→3)-β-D-glucan (BDG) has undergone limited evaluation for the detection of coccidioidomycosis and appears to have a limited role, although sensitivity may not exceed 44% (46, 47). BDG has been found to be positive in the CSF of patients with coccidioidal meningitis, with a sensitivity of 96% and a specificity of 82% in one study (48). Serial antigen or BDG testing of the serum, urine, or CSF is not routinely performed due to the lack of data supporting this approach.

MOLECULAR METHODS

Numerous PCR-based methods have been developed and applied for the detection of *Coccidioides* species from clinical specimens, culture, and the environment (13, 14, 21, 22, 49). However, only one has received FDA approval (GeneSTAT, St. George, UT, USA) and is approved only for bronchial alveolar lavage and bronchial wash specimens.

The sensitivity of PCR on clinical specimens appears to be similar to that of culture in the limited studies that have been published to date.

Emerging next-generation sequencing (NGS)-based assays have also been used to detect *Coccidioides* species in clinical specimens and formalin-fixed, paraffin-embedded tissue (50, 51). While the sensitivities may vary, this hypothesis-free methodology could be particularly helpful for coccidioidomycosis where significant diagnostic delays occur due to low clinical suspicion or awareness. However, the lack of FDA-approved solutions, rigorous technical requirements, and high start-up costs currently limit the widespread implementation of these assays in clinical laboratories. Outside clinical laboratories, NGS has also been used in outbreak investigations and can reliably link isolates epidemiologically (52, 53). Similarly, differentiation between *C. immitis* and *C. posadasii* is neither clinically necessary nor possible using most available diagnostic modalities, but it is achievable using molecular approaches like NGS.

IMMUNOSURVEILLANCE

In the early 20th century, the detection of delayed-type hypersensitivity following the subcutaneous injection of a coccidioidal culture filtrate was reportedly a successful form of immunosurveillance (34). A reformulated skin test (Spherusol; Nielsen BioSciences) is available and approved for patients recovering from acute pneumonia, but contemporary studies have raised questions about the overall sensitivity, which may not exceed 72% compared to current diagnostics (54–56). Furthermore, many patients with disseminated coccidioidomycosis test negative by skin tests (54). Accordingly, future applications using this method may be limited.

FUTURE DIAGNOSTIC TEST DEVELOPMENT

The available diagnostic repertoires described above largely derive from developments in the early to mid-20th century, are highly labor-intensive, and necessitate significant expertise. Meanwhile, the prevalence of coccidioidomycosis continues to increase with the increasing migration of naive hosts into areas of endemicity and the pathogen potentially expanding its ecological range due to climate change (57). Accordingly, there is interest in the development of new diagnostic and prognostic tools with increased throughput that simultaneously have reduced complexity and a shorter turnaround time.

Given the high morbidity and mortality rates associated with the reactivation of dormant *Coccidioides* in some patient populations, there is a need for more sensitive immunosurveillance modalities to more accurately detect previous infection and, thus, inform the appropriate management of high-risk (e.g., oncology, transplant, and some allergy and immunology) patients (24). Accordingly, efforts have been made to develop *in vitro* diagnostic tests that assess the cellular immune response of coccidioidomycosis patients. Promising initial studies indicate that cytokine profiles correlate with skin test results and might one day be used for both the diagnosis and prognosis of infection (58, 59). While such approaches may prove fruitful, clinical validation and calibration against disease severity/outcome, as were done for CF, would be valuable (33).

SUMMARY AND CONCLUSIONS

Due to the restricted geographic range and variable impacts on patients, investment into new diagnostic approaches for coccidioidomycosis has been relatively low compared with similarly virulent pathogens with broader endemicity. It is hoped that recent funding efforts at the federal and state levels will reverse this trend as much work remains to develop and tailor the next generation of diagnostic and prognostic tools to fit into the current rapidly evolving diagnostic landscape. In the absence of more rapid and high-throughput alternatives, clinical laboratories are encouraged to educate their clinician clients about local trends and consider implementing commercially available EIAs or ID assays as appropriate to better balance diagnostic access, sensitivity, and turnaround time compared with culture- and reference laboratory-based diagnostics.

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REFERENCES

1. Bays DJ, Thompson GR, III. 2021. Coccidioidomycosis. Infect Dis Clin North Am 35:453–469. https://doi.org/10.1016/j.idc.2021.03.010.
2. Mazi PB, Sahrmann JM, Olsen MA, Coler-Reilly A, Rauseo AM, Pullen M, Zuniga-Moya JC, Powderly WG, Spec A. 2022. The geographic distribution of dimorphic mycoses in the United States for the modern era. Clin Infect Dis.
3. Sunenshine RH, Anderson S, Erhart L, Vossbrink A, Kelly PC, Engelthaler D, Komatsu K. 2007. Public health surveillance for coccidioidomycosis in Arizona. Ann N Y Acad Sci 1111:96–102. https://doi.org/10.1196/annals.1406.045.
4. Crum NF. 2022. Coccidioidomycosis: a contemporary review. Infect Dis Ther 11:713–742. https://doi.org/10.1007/s40121-022-00606-y.
5. Blair JE, Chang Y-HH, Cheng M-R, Vaszar LT, Vikram HR, Orenstein R, Kusne S, Ho S, Seville MT, Parish JM. 2014. Characteristics of patients with mild to moderate primary pulmonary coccidioidomycosis. Emerg Infect Dis 20:983–990. https://doi.org/10.3201/eid2006.131842.
6. McHardy I, Reagan KL, Sebastian JF, Barker B, Bays DJ, Dandekar S, Cohen SH, Jennings KE, Sykes J, Thompson GR, III. 2022. Sex differences in susceptibility to coccidioidomycosis. Open Forum Infect Dis 9:ofab543. https://doi.org/10.1093/ofid/ofab543.
7. Crum NF, Lederman ER, Stafford CM, Parrish JS, Wallace MR. 2004. Coccidioidomycosis: a descriptive survey of a reemerging disease. Clinical characteristics and current controversies. Medicine (Baltimore) 83:149–175. https://doi.org/10.1097/01.md.0000126762.91040.fd.
8. Rosenstein NE, Emery KW, Werner SB, Kao A, Johnson R, Rogers D, Vugia D, Reingold A, Talbot R, Plikaytis BD, Perkins BA, Hajjeh RA. 2001. Risk factors for severe pulmonary and disseminated coccidioidomycosis: Kern County, California, 1995-1996. Clin Infect Dis 32:708–715. https://doi.org/10.1086/319203.
9. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, Clancy CJ, Wingard JR, Lockhart SR, Groll AH, Sorrell TC, Bassetti M, Akan H, Alexander BD, Andes D, Azoulay E, Bialek R, Bradsher RW, Bretagne S, Calandra T, Caliendo AM, Castagnola E, Cruciani M, Cuenca-Estrella M, Decker CF, Desai SR, Fisher B, Harrison T, Heussel CP, Jensen HE, Kibbler CC, Kontoyiannis DP, Kullberg B-J, Lagrou K, Lamoth F, Lehrnbecher T, Loeffler J, Lortholary O, Maertens J, Marchetti O, Marr KA, Masur H, Meis JF, Morrissey CO, Nucci M, Ostrosky-Zeichner L, Pagano L, Patterson TF, Perfect JR, Racil Z, et al. 2020. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis 71:1367–1376. https://doi.org/10.1093/cid/ciz1008.
10. Centers for Disease Control and Prevention, National Institutes of Health. 2020. Biosafety in microbiological and biomedical laboratories, 6th ed. Centers for Disease Control and Prevention, Atlanta, GA.
11. Mess T, Daar ES. 1997. Utility of fungal blood cultures for patients with AIDS. Clin Infect Dis 25:1350–1353. https://doi.org/10.1086/516140.
12. Saubolle MA. 2007. Laboratory aspects in the diagnosis of coccidioidomycosis. Ann N Y Acad Sci 1111:301–314. https://doi.org/10.1196/annals.1406.049.
13. Vucicevic D, Blair JE, Binnicker MJ, McCullough AE, Kusne S, Vikram HR, Parish JM, Wengenack NL. 2010. The utility of Coccidioides polymerase chain reaction testing in the clinical setting. Mycopathologia 170:345–351. https://doi.org/10.1007/s11046-010-9327-0.
14. Thompson GR, III, Sharma S, Bays DJ, Pruitt R, Engelthaler DM, Bowers J, Driebe EM, Davis M, Libke R, Cohen SH, Pappagianis D. 2013. Coccidioidomycosis: adenosine deaminase levels, serologic parameters, culture results, and polymerase chain reaction testing in pleural fluid. Chest 143:776–781. https://doi.org/10.1378/chest.12-1312.
15. Mathisen G, Shelub A, Truong J, Wigen C. 2010. Coccidioidal meningitis: clinical presentation and management in the fluconazole era. Medicine (Baltimore) 89:251–284. https://doi.org/10.1097/MD.0b013e3181f378a8.
16. Lonky SA, Catanzaro A, Moser KM, Einstein H. 1976. Acute coccidioidal pleural effusion. Am Rev Respir Dis 114:681–688. https://pubmed.ncbi.nlm.nih.gov/970744/.
17. Sutton DA. 2007. Diagnosis of coccidioidomycosis by culture: safety considerations, traditional methods, and susceptibility testing. Ann N Y Acad Sci 1111:315–325. https://doi.org/10.1196/annals.1406.005.
18. Sandhu GS, Kline BC, Stockman L, Roberts GD. 1995. Molecular probes for diagnosis of fungal infections. J Clin Microbiol 33:2913–2919. https://doi.org/10.1128/jcm.33.11.2913-2919.1995.
19. Lau AF, Drake SK, Calhoun LB, Henderson CM, Zelazny AM. 2013. Development of a clinically comprehensive database and a simple procedure for identification of molds from solid media by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 51:828–834. https://doi.org/10.1128/JCM.02852-12.
20. Binnicker MJ, Buckwalter SP, Eisberner JJ, Stewart RA, McCullough AE, Wohlfiel SL, Wengenack NL. 2007. Detection of Coccidioides species in clinical specimens by real-time PCR. J Clin Microbiol 45:173–178. https://doi.org/10.1128/JCM.01776-06.
21. Bowers JR, Parise KL, Kelley EJ, Lemmer D, Schupp JM, Driebe EM, Engelthaler DM, Keim P, Barker BM. 2019. Direct detection of Coccidioides from Arizona soils using CocciENV, a highly sensitive and specific real-time PCR assay. Med Mycol 57:246–255. https://doi.org/10.1093/mmy/myy007.
22. Chaturvedi S, Victor TR, Marathe A, Sidamonidze K, Crucillo KL, Chaturvedi V. 2021. Real-time PCR assay for detection and differentiation of Coccidioides immitis and Coccidioides posadasii from culture and clinical specimens. PLoS Negl Trop Dis 15:e0009765. https://doi.org/10.1371/journal.pntd.0009765.
23. CLSI. 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard, 3rd ed. M27-A3. CLSI, Wayne, PA.
24. Galgiani JN, Ampel NM, Blair JE, Catanzaro A, Geertsma F, Hoover SE, Johnson RH, Kusne S, Lisse J, MacDonald JD, Meyerson SL, Raksin PB, Siever J, Stevens DA, Sunenshine R, Theodore N. 2016. 2016 Infectious Diseases Society of America (IDSA) clinical practice guideline for the treatment of coccidioidomycosis. Clin Infect Dis 63:e112–e146. https://doi.org/10.1093/cid/ciw360.
25. Thompson GR, III, Barker BM, Wiederhold NP. 2017. Large-scale evaluation of in vitro amphotericin B, triazole, and echinocandin activity against Coccidioides species from U.S. institutions. Antimicrob Agents Chemother 61:e02634-16. https://doi.org/10.1128/AAC.02634-16.
26. Thompson GR, III, Lewis JS, II, Nix DE, Patterson TF. 2019. Current concepts and future directions in the pharmacology and treatment of coccidioidomycosis. Med Mycol 57:S76–S84. https://doi.org/10.1093/mmy/myy029.
27. Blair JE, Coakley B, Santelli AC, Hentz JG, Wengenack NL. 2006. Serologic testing for symptomatic coccidioidomycosis in immunocompetent and immunosuppressed hosts. Mycopathologia 162:317–324. https://doi.org/10.1007/s11046-006-0062-5.
28. Donovan FM, Ramadan FA, Khan SA, Bhaskara A, Lainhart WD, Narang AT, Mosier JM, Ellingson KD, Bedrick EJ, Saubolle MA, Galgiani JN. 2021. Comparison of a novel rapid lateral flow assay to enzyme immunoassay results for early diagnosis of coccidioidomycosis. Clin Infect Dis 73:e2746–e2753. https://doi.org/10.1093/cid/ciaa1205.
29. Pappagianis D, Krasnow RI, Beall S. 1976. False-positive reactions of cerebrospinal fluid and diluted sera with the coccidioidal latex-agglutination test. Am J Clin Pathol 66:916–921. https://doi.org/10.1093/ajcp/66.5.916.
30. Grys TE, Brighton A, Chang Y-H, Liesman R, Bolster LaSalle C, Blair JE. 2019. Comparison of two FDA-cleared EIA assays for the detection of Coccidioides antibodies against a composite clinical standard. Med Mycol 57:595–600. https://doi.org/10.1093/mmy/myy094.
31. Malo J, Holbrook E, Zangeneh T, Strawter C, Oren E, Robey I, Erickson H, Carranza-Chahal R, Durkin M, Thompson C, Hoover SE, Ampel NM, Wheat LJ, Knox KS. 2020. Comparison of three anti-Coccidioides antibody enzyme immunoassay kits for the diagnosis of coccidioidomycosis. Med Mycol 58:774–778. https://doi.org/10.1093/mmy/myz125.
32. Malo J, Holbrook E, Zangeneh T, Strawter C, Oren E, Robey I, Erickson H, Chahal R, Durkin M, Thompson C, Hoover SE, Ampel NM, Wheat LJ, Knox KS. 2017. Enhanced antibody detection and diagnosis of coccidioidomycosis with the MiraVista IgG and IgM detection enzyme immunoassay. J Clin Microbiol 55:893–901. https://doi.org/10.1128/JCM.01880-16.
33. Smith CE, Saito MT, Beard RR, Kepp RM, Clark RW, Eddie BU. 1950. Serological tests in the diagnosis and prognosis of coccidioidomycosis. Am J Hyg 52:1–21. https://doi.org/10.1093/oxfordjournals.aje.a119404.
34. Pappagianis D, Zimmer BL. 1990. Serology of coccidioidomycosis. Clin Microbiol Rev 3:247–268. https://doi.org/10.1128/CMR.3.3.247.
35. Smith CE, Saito MT. 1957. Serologic reactions in coccidioidomycosis. J Chronic Dis 5:571–579. https://doi.org/10.1016/0021-9681(57)90099-1.
36. Kruse D, Cole GT. 1992. A seroreactive 120-kilodalton β-1,3-glucanase of Coccidioides immitis which may participate in spherule morphogenesis. Infect Immun 60:4350–4363. https://doi.org/10.1128/iai.60.10.4350-4363.1992.
37. McHardy IH, Dinh BN, Waldman S, Stewart E, Bays D, Pappagianis D, Thompson GR, III. 2018. Coccidioidomycosis complement fixation titer trends in the age of antifungals. J Clin Microbiol 56:e01318-18. https://doi.org/10.1128/JCM.01318-18.
38. Galgiani JN, Blair JE, Ampel NM, Thompson GR. 2020. Treatment for early, uncomplicated coccidioidomycosis: what is success? Clin Infect Dis 70:2008–2012. https://doi.org/10.1093/cid/ciz933.
39. Galgiani JN, Catanzaro A, Cloud GA, Higgs J, Friedman BA, Larsen RA, Graybill JR. 1993. Fluconazole therapy for coccidioidal meningitis. The

NIAID-Mycoses Study Group. Ann Intern Med 119:28–35. https://doi.org/10.7326/0003-4819-119-1-199307010-00005.
40. Pappagianis D, Saito M, Van Hoosear KH. 1972. Antibody in cerebrospinal fluid in non-meningitic coccidioidomycosis. Sabouraudia 10:173–179. https://doi.org/10.1080/00362177285190341.
41. Galgiani JN, Grace GM, Lundergan LL. 1991. New serologic tests for early detection of coccidioidomycosis. J Infect Dis 163:671–674. https://doi.org/10.1093/infdis/163.3.671.
42. Durkin M, Connolly P, Kuberski T, Myers R, Kubak BM, Bruckner D, Pegues D, Wheat LJ. 2008. Diagnosis of coccidioidomycosis with use of the Coccidioides antigen enzyme immunoassay. Clin Infect Dis 47:e69–e73. https://doi.org/10.1086/592073.
43. Grill FJ, Grys TE, Grill MF, Roeder A, Blair JE, Lake DF. 2021. Development of a quantitative antigen assay to detect coccidioidal chitinase-1 (CTS1) in human serum. Open Forum Infect Dis 8:ofab344. https://doi.org/10.1093/ofid/ofab344.
44. Kirsch EJ, Greene RT, Prahl A, Rubin SI, Sykes JE, Durkin MM, Wheat LJ. 2012. Evaluation of Coccidioides antigen detection in dogs with coccidioidomycosis. Clin Vaccine Immunol 19:343–345. https://doi.org/10.1128/CVI.05631-11.
45. Bamberger DM, Pepito BS, Proia LA, Ostrosky-Zeichner L, Ashraf M, Marty F, Scully E, Wheat LJ. 2015. Cerebrospinal fluid Coccidioides antigen testing in the diagnosis and management of central nervous system coccidioidomycosis. Mycoses 58:598–602. https://doi.org/10.1111/myc.12366.
46. Thompson GR, III, Bays DJ, Johnson SM, Cohen SH, Pappagianis D, Finkelman MA. 2012. Serum (1→3)-β-D-glucan measurement in coccidioidomycosis. J Clin Microbiol 50:3060–3062. https://doi.org/10.1128/JCM.00631-12.
47. Al-Obaidi MM, Ayazi P, Shi A, Campanella M, Connick E, Zangeneh TT. 2022. The utility of (1→3)-β-D-glucan testing in the diagnosis of coccidioidomycosis in hospitalized immunocompromised patients. J Fungi (Basel) 8:768. https://doi.org/10.3390/jof8080768.
48. Stevens DA, Zhang Y, Finkelman MA, Pappagianis D, Clemons KV, Martinez M. 2016. Cerebrospinal fluid (1,3)-β-D-glucan testing is useful in diagnosis of coccidioidal meningitis. J Clin Microbiol 54:2707–2710. https://doi.org/10.1128/JCM.01224-16.
49. Sheff KW, York ER, Driebe EM, Barker BM, Rounsley SD, Waddell VG, Beckstrom-Sternberg SM, Beckstrom-Sternberg JS, Keim PS, Engelthaler DM. 2010. Development of a rapid, cost-effective TaqMan real-time PCR assay for identification and differentiation of Coccidioides immitis and Coccidioides posadasii. Med Mycol 48:466–469. https://doi.org/10.3109/13693780903218990.
50. Larkin PMK, Lawson KL, Contreras DA, Le CQ, Trejo M, Realegeno S, Hilt EE, Chandrasekaran S, Garner OB, Fishbein GA, Yang S. 2020. Amplicon-based next-generation sequencing for detection of fungi in formalin-fixed, paraffin-embedded tissues: correlation with histopathology and clinical applications. J Mol Diagn 22:1287–1293. https://doi.org/10.1016/j.jmoldx.2020.06.017.
51. Mao Y, Li X, Lou H, Shang X, Mai Y, Yang L, Peng F, Fu X. 2021. Detection of Coccidioides posadasii in a patient with meningitis using metagenomic next-generation sequencing: a case report. BMC Infect Dis 21:968. https://doi.org/10.1186/s12879-021-06661-z.
52. Engelthaler DM, Chiller T, Schupp JA, Colvin J, Beckstrom-Sternberg SM, Driebe EM, Moses T, Tembe W, Sinari S, Beckstrom-Sternberg JS, Christoforides A, Pearson JV, Carpten J, Keim P, Peterson A, Terashita D, Balajee SA. 2011. Next-generation sequencing of Coccidioides immitis isolated during cluster investigation. Emerg Infect Dis 17:227–232. https://doi.org/10.3201/eid1702.100620.
53. Barker BM, Rajan S, De Melo Teixeira M, Sewnarine M, Roe C, Engelthaler DM, Galgiani JN. 2019. Coccidioidal meningitis in New York traced to Texas by fungal genomic analysis. Clin Infect Dis 69:1060–1062. https://doi.org/10.1093/cid/ciz052.
54. Ampel NM, Robey I, Nguyen CT. 2019. An analysis of skin test responses to spherulin-based coccidioidin (Spherusol) among a group of subjects with various forms of active coccidioidomycosis. Mycopathologia 184:533–538. https://doi.org/10.1007/s11046-019-00356-5.
55. Mafi N, Murphy CB, Girardo ME, Blair JE. 2020. Coccidioides (spherulin) skin testing in patients with pulmonary coccidioidomycosis in an endemic region. Med Mycol 58:626–631. https://doi.org/10.1093/mmy/myz099.
56. Wheeler C, Lucas KD, Derado G, McCotter O, Tharratt RS, Chiller T, Mohle-Boetani JC. 2018. Risk stratification with coccidioidal skin test to prevent valley fever among inmates, California, 2015. J Correct Health Care 24:342–351. https://doi.org/10.1177/1078345818792679.
57. Gorris ME, Treseder KK, Zender CS, Randerson JT. 2019. Expansion of coccidioidomycosis endemic regions in the United States in response to climate change. Geohealth 3:308–327. https://doi.org/10.1029/2019GH000209.
58. Ampel NM, Nelson DK, Chavez S, Naus KA, Herman AB, Li L, Simmons KA, Pappagianis D. 2005. Preliminary evaluation of whole-blood gamma interferon release for clinical assessment of cellular immunity in patients with active coccidioidomycosis. Clin Diagn Lab Immunol 12:700–704. https://doi.org/10.1128/CDLI.12.6.700-704.2005.
59. Ampel NM, Hector RF, Lindan CP, Rutherford GW. 2006. An archived lot of coccidioidin induces specific coccidioidal delayed-type hypersensitivity and correlates with in vitro assays of coccidioidal cellular immune response. Mycopathologia 161:67–72. https://doi.org/10.1007/s11046-005-0218-8.