

**Specificity of Enzyme Immunoassay for Serologic  
Coccidioidomycosis Diagnosis Compared to  
Immunodiffusion**

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## **Dedication**

For my mom, who suffered from disseminated coccidioidomycosis, which had taken months before an accurate diagnosis was made.

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## Abstract

**BACKGROUND:** Serologic testing for coccidioidomycosis challenges clinicians due to conflicting small studies regarding the sensitivity and specificity of newer enzyme immunoassay (EIA) tests and the lack of a true gold standard diagnostic test for comparison.

**METHODS:** We analyzed all Lab Corp coccidioidomycosis serological test results from February 2008 through February 2009 and calculated the sensitivity, specificity, and positive/negative predictive values of EIA immunoglobulin (Ig)M and IgG. Immunodiffusion IgM and IgG (ID), complement fixation titers (CF), and tissue/culture diagnosis were used as tests for comparison. The comparison test (CT) was considered positive if any comparison test was positive the day of EIA collection or if tissue/culture diagnosis occurred during the time period. Cases required EIA IgM and IgG and  $\geq 2$  comparison tests performed the same day for inclusion. Medical records associated with positive EIA and negative comparison test results were reviewed for coccidioidomycosis symptoms, physician diagnosis, and subsequent positive comparison test results. Sensitivity, specificity, and predictive values were calculated, including those with subsequent positive comparison test results.

**RESULTS:** A total of 1445 laboratory test sets were identified. EIA sensitivity and specificity were 83.8% and 92.6%, respectively. Positive and negative predictive values were 61.5% and 97.6%, respectively. Of 94 “false positive” EIA results, 92 (97.9%) were associated with documented coccidioidomycosis symptoms and 81% with coccidioidomycosis physician diagnosis.

**CONCLUSION:** Based on the largest study of sensitivity and specificity calculated from laboratory surveillance data, EIA sensitivity and specificity for coccidioidomycosis diagnosis are lower than previously reported using only coccidioidomycosis laboratory tests as a comparison. However, association of “false positive” EIA results with coccidioidomycosis symptoms and physician diagnosis suggests that ID and CF laboratory tests alone are not a sufficient confirmation test for diagnosis.

## Table of Contents

Dedication.....	2
Acknowledgements.....	3
Abstract.....	4
Table of Contents.....	5
List of Figures and Tables.....	6
Introduction.....	7
Research Materials and Methods.....	12
Results.....	14
Discussion.....	16
Future Directions.....	19
Conclusions.....	19
References.....	21
Appendix.....	24
Poster.....	30

## **List of Figures and Tables**

Figure 1. Rates of Reported Valley Fever (VF) in Arizona, 1993-2008

Table 1. Learning objectives and implementation plan

Figure 2. Diagram of matching schema for public health reporting validation for laboratories A and B

Figure 3. Summary of “false positive” enzyme immunoassay results

Table 2. Enzyme immunoassay compared to comparison tests

## Introduction

Coccidioidomycosis, or Valley Fever, is an infection caused by the fungus *Coccidioides immitis* or *Coccidioides posadasii*. This fungus grows in the soil of hot, dry climates in the southern and central portions of California, Arizona, New Mexico, Texas, and the southern portions of Nevada and Utah (13). Valley Fever is also endemic in parts of Mexico, Central, and South America (10). Anyone who lives, visits, or travels through the areas where the fungus is endemic may acquire Valley Fever (18). People working in certain occupations such as construction, excavation, agriculture, archaeological digging, and other occupations, which disturb soil in endemic areas, may be at increased risk of exposure (5, 8). Various domestic animals such as dogs, cats, and horses, as well as wild animals are also susceptible.

Valley Fever is acquired by inhaling one or more airborne spores of the fungus *Coccidioides immitis* or *C. posadasii* (5, 13). The spores are carried on dust particles by the wind when the desert soil is disturbed. Approximately 60% of infected individuals are asymptomatic or have mild respiratory symptoms (4, 5, 10, 13, 18). Those who develop symptoms usually develop them within 7-21 days after exposure (4). Most symptomatic people have a pneumonia-like illness, with symptoms of acute influenza-like illness (fever, cough, fatigue), which makes it difficult to distinguish from community-acquired pneumonia (CAP). About 1-4% of symptomatic individuals will develop disseminated disease (6), where the infection spreads to other parts of the body, such as the skin, joints, bones, lymph nodes, and central nervous system. Disseminated coccidioidomycosis always requires treatment and can lead to serious complications, including death. There is no known cure and no licensed vaccine available.

Valley Fever is not spread from human to human, animal to animal, animal to human, or human to animal (13, 16). Because *Coccidioides* spp. are dimorphic fungi, the spores change form from infectious arthrospores in the soil to non-infectious spherules in tissues of the body. *Coccidioides* spp. are very contagious when grown in the laboratory setting and require laboratorians to take special precautions once the fungus is isolated (4). Additionally, since it is a select agent of bioterrorism, once the fungus is isolated in a laboratory, it must be either destroyed or transported to a biosafety level 4 laboratory.

Sixty percent of US reported coccidioidomycosis cases occur in Arizona. Therefore, of the estimated 150,000 U. S. coccidioidomycosis infections per year, approximately 90,000 are thought to occur in Arizona (predominantly Pima, Maricopa and Pinal Counties), making this region the focal point for investigation of the disease (2). Arizona has had mandated reporting of Valley Fever by both laboratories and physicians since 1997. Figure 1 shows the rates of reported Valley Fever (VF) in Arizona from 1993-2008. Over the last decade, the reported rate of Valley Fever cases in Arizona has more than quadrupled. In 2007, there were 4815 cases (75/100,000 population) reported to the Arizona Department of Health Services (ADHS) (1), whereas only 958 cases (16/100,000 population) were reported to ADHS in 1997. Valley Fever is seasonal with the highest rates of infection in Arizona typically occurring from June through August and from October through November.

For public health surveillance purposes, the Council of State and Territorial Epidemiologists (CSTE) clinical criteria for coccidioidomycosis diagnosis include at least one of the following:

- 1) Influenza-like signs and symptoms, including fever, chest pain, cough, myalgia, arthralgia, headache;
- 2) Pneumonia or other pulmonary lesion, by chest X-ray;
- 3) Rash, including erythema nodosum or erythema multiforme;
- 4) Involvement of bones, joints, or skin;
- 5) Meningitis or other CNS involvement; or
- 6) Involvement of viscera and lymph nodes (20).

The CSTE case definition for a confirmed case of coccidioidomycosis also includes laboratory criteria for diagnosis, which require at least one of the following:

- 1) Cultural, histopathologic, or molecular evidence of presence of *C. immitis* or *C. posadasii* or
- 2) Immunologic evidence of infection (20).

Immunologic evidence can be demonstrated by a) coccidioidal skin test conversion from negative to positive after the onset of clinical signs and symptoms (Note: the skin test reagent is not currently commercially

available in the US), or b) serologic (testing of serum, cerebrospinal fluid, or other body fluid) via 1) detection of coccidioidal immunoglobulin (Ig)M by immunodiffusion, enzyme immunoassay (EIA), latex agglutination, or tube precipitin, or 2) detection of any titer of coccidioidal IgG by immunodiffusion, enzyme immunoassay (EIA), or complement fixation (20). In Arizona, for public health surveillance purposes, a confirmed case is a case that is laboratory confirmed following the CSTE case definition; the clinical criteria are not required. This epidemiological case definition was validated by a study indicating that 95% of all reported laboratory diagnoses of coccidioidomycosis were associated with symptoms meeting the CSTE clinical criteria for coccidioidomycosis diagnosis (21).

For the past half century, detecting anticoccidioidal antibodies has been an important means of establishing a diagnosis of coccidioidomycosis (19). The two major antigens used to detect anticoccidioidal antibodies are the tube precipitin-reacting (TP) antigen --- so called because of the precipitin button that formed in the bottom of the test tube in the originally described "tube precipitin" test --- and the complement-fixing (CF) antigen. During primary infections, IgM antibodies against the TP antigen are usually found in serum earlier than CF antibodies (IgG) and disappear sooner, although exceptions to this rule can occur. In contrast, TP antibodies (IgM) are not usually found in more chronic infections whereas CF antibodies persist. Dual immunodiffusion procedures for both IgM and IgG that can be more readily supplied commercially have been developed as surrogate procedures to detect antibodies of the same specificity as the original TP and CF tests (16). The immunodiffusion tests (ID) are considered to be highly specific and at least as sensitive as the earlier methods (16). Other tests such as the latex agglutination test are available for detecting coccidioidal infections but are at this time of more limited value.

More recently, an enzyme-linked immunosorbent assay (EIA) has been introduced, which may be more specific than latex agglutination tests (9). Although easier to perform and faster than immunodiffusion, the results from these methods have not yet been extensively correlated with results from TP or CF tests. Thus, the sensitivity and specificity of the EIA test has not been clearly defined (7).

Today, serologic testing includes qualitative tests (immunodiffusion, enzyme immunoassay, or latex particle

agglutination), which permit detection in the serum of the major antibody responses: coccidioidal IgM in early coccidioidomycosis and complement fixation (CF) IgG, which appears later and is more persistent (3, 12, 14, 23). Unlike other infectious diseases, a positive IgG, whether by immunodiffusion, CF, or EIA, indicates active disease, since IgG is thought to persist in the blood for less than one year in non-disseminated disease. Further, quantitation of the level (titer) of coccidioidal IgG is useful in prognosis and management of the disease but less for diagnosis. The CF titer is typically higher in more severe or disseminated disease and decreases with treatment response (7).

Because the EIA test is the easiest and least expensive to perform of all serologic tests for coccidioidomycosis, it is one of the most widely used by commercial laboratories and thus is the basis for the majority of Arizona's coccidioidomycosis surveillance data. Like all the other serologic tests for coccidioidomycosis, the EIA test can be falsely negative early in disease because the body requires 1-2 weeks to mount an antibody response. However, some believe that EIA might actually become positive earlier in the course of disease than immunodiffusion, giving it a distinct advantage for diagnosis (3, 12, 14, 23). Likewise, false positives may occur, leading to additional diagnostic testing and unwelcome patient anxiety. Specifically, concerns have been raised among clinicians about false positive EIA IgM results. The literature on this issue is conflicting and difficult to interpret (3, 12, 14, 23). CF testing typically becomes positive after EIA tests but is more specific.

It is recommended that the ID test be run in parallel with the CF test (11). It is also recommended when performing EIA tests for diagnosis, that both IgM and IgG be performed simultaneously, as this greatly increases the sensitivity and specificity of the test (12). Additionally, some literature suggests the use of EIA IgM and IgG as a screening test only (12), which when positive should be confirmed with ID. Subsequently, if the EIA test is positive and the ID is negative, laboratories that use EIA as a screening test report the test results as negative. This raises concerns about missed diagnoses if the EIA is either more sensitive or becomes positive earlier in the course of disease than the test used for confirmation. Other laboratories report any positive EIA result as positive. This inconsistency among laboratory reporting practices significantly affects the quality of Arizona's coccidioidomycosis surveillance data. Therefore, it is important for

public health purposes to take into account these variable laboratory reporting practices.

The consistent reporting of positive laboratory results for reportable infectious diseases is critical to public health surveillance. Surveillance is used to identify changing trends in diseases, detect outbreaks, and control disease spread. However, if new cases are not reliably reported, the public health system cannot compile an accurate picture of disease in the community. For this reason, conducting validations of surveillance data periodically is an important way to determine how well the public health system is capturing the burden of disease in the community. Therefore, prior to utilization of Arizona coccidioidomycosis surveillance data for this investigation, a validation study was performed.

The main research objective of this investigation is to define the specificity of enzyme immunoassay (EIA) for coccidioidomycosis diagnosis compared to immunodiffusion, complement fixation, or tissue/culture diagnosis, although it is acknowledged that there is not a true gold standard available. Secondary objectives are to define the sensitivity and positive/negative predictive values of enzyme immunoassay (EIA) for coccidioidomycosis diagnosis compared to immunodiffusion, complement fixation, or tissue/culture diagnosis, with the same caveat for the comparison tests. As mentioned previously, in order to accomplish the primary and secondary objectives, the public health reporting of coccidioidomycosis was evaluated during a selected period for two commercial Arizona laboratories and laboratory reporting of coccidioidomycosis results was validated from one major Arizona laboratory.

A program plan was established with a goal to gain practical skills and experiences in analyzing diagnostic testing methods for coccidioidomycosis. The planned outcomes were 1) to determine the specificity and positive predictive value of EIA for coccidioidomycosis diagnosis, 2) validate Arizona's laboratory reporting of coccidioidomycosis from one major laboratory, and 3) provide information to clinicians, laboratories, and public health practitioners to maximize accuracy of coccidioidomycosis diagnosis and public health surveillance. An evaluation plan (Table 1) was designed to measure our success in achieving the project's goals in the following ways: report to a supervising mentor at ADHS, submit quarterly progress notes to the

supervising mentor, and meet monthly with staff to discuss the progress of the project and address any issues.

## **Research Materials and Methods**

### *Validation of public health reporting in Arizona*

In 2007, two laboratories accounted for a combined total of 46% of the initial reports of coccidioidomycosis, Laboratory A (30%) and Laboratory B (16%). In 2008, both laboratories provided independent reports of positive coccidioidomycosis tests; these data were used to validate public health reporting of coccidioidomycosis from the time period of March 1, 2008, through May 31, 2008.

The study examined data from the ADHS surveillance system from March 1, 2008 through May 31, 2008 to ensure that all positive test results from two major laboratories (Laboratories A and B) were reported to ADHS. Positive results from Laboratory A included results for immunodiffusion and complement fixation tests. At that time, enzyme immunoassays (EIAs) were used as a screening test and positive results were not reported to physicians or public health unless the EIA was confirmed by immunodiffusion or complement fixation. Positive results from Laboratory B included immunodiffusion, complement fixation, and EIA, even if no confirmatory test had been run. Surveillance data at ADHS, including all positive coccidioidomycosis reports, have been entered in the Medical Electronic Disease Surveillance Intelligence System (MEDSIS) since January 2006. When multiple positive tests of coccidioidomycosis are received for a single person, only the first report is counted. When data entry staff enter a new laboratory report, they first check that the case has not been previously entered in MEDSIS, and also check against a database with cases reported from 1998 through 2005 (the “historic data”). Because the surveillance databases capture data on the person, not on the individual tests, multiple positive tests on one individual (based on patient name and date of birth) from the laboratory data were eliminated so that matching was done on only one positive test per person.

Data from both laboratories were first matched with MEDSIS, using an exact match on first and last name and birth date. Laboratory

reports that did not match MEDSIS cases were then matched against the database of historic cases (1998-2005) using the same criteria of an exact match on first and last name and date of birth. Laboratory-reported results that did not match either public health databases were then hand-matched to MEDSIS and the historic database to identify matches with possible misspellings or missing information. County of resident was sometimes used as a criterion for the hand-match to confirm or reject suspected matches on name and birth date, such as if the date of birth was missing or a close match. A positive match was considered to be a patient reported by the laboratory that was found in either database during the automated or hand-matching steps. Tests that did not match were reviewed for test type and date to look for trends among cases that may not have been reported.

#### *Specificity of EIA compared to immunodiffusion or complement fixation*

All coccidioidomycosis testing results including serological tests and cultures from February 2008 through February 2009 were obtained from Laboratory Corporation (Lab Corp). The data were cleaned and duplicates were removed. Duplicates were identified by last name, first name, middle initial when available, and date of birth. The tests were then categorized based on the test performed. Results were categorized as positive or negative. If a test result was listed as indeterminate or had an equivocal value as a result, it was considered negative. If a test result was listed as test not performed, it was excluded. A CF was considered positive at titer levels equal to or greater than 1:2. EIA was considered positive when the absorbance value was equal to or greater than 0.200 and if at least one EIA test was positive (IgM or IgG).

Individual test results were grouped together into sets by last name, first name, date of birth, and test date, so that each set represented all the tests run for a single patient from a single day. If a person had more than one set of tests done, only the first set of tests was included in the original calculation of the sensitivity, specificity, and positive/negative predictive values. The inclusion criterion for the analysis was: presence of two coccidioidomycosis serologic EIA tests (both IgM and IgG) and two or more ID/CF tests run on the same day (from the same blood sample).

Sensitivity, specificity, and positive/negative predictive values (PPV or NPV) of EIA (IgM and IgG) were calculated using the following

comparison tests (CT): if any immunodiffusion IgM or IgG (ID) or complement fixation titers (CF) were positive the day of EIA collection, then the CT was considered positive. A false positive test set is one in which at least one EIA test was positive and all CT tests were negative. After the sensitivity, specificity, and positive/negative predictive values were calculated, medical records associated with test sets consisting of at least one positive EIA result and all negative ID/CF tests, referred to as “false positives” (FPs), were requested. Records were reviewed for coccidioidomycosis symptoms as defined by the CSTE coccidioidomycosis clinical case definition, physician diagnosis, and subsequent positive CT laboratory results. The subsequent positive laboratory results examined included tissue/culture diagnosis from any time during the test period if listed in the medical records, a thorough review of the all of the Lab Corp data used in the original analysis, and review of additional Lab Corp test results through December of 2010. After review of the medical records and additional laboratory data, test sets were re-classified as being positive for the CT if they had tissue or culture diagnosis from any time in the study period or positive ID or CF tests identified through any of these methods. Sensitivity, specificity, PPV, and NPV were then recalculated. The final analysis also included 1) the number of FP test sets that met the CSTE clinical case definition, 2) the number of FP test sets that were associated with a physician diagnosis of coccidioidomycosis, and 3) whether the test sets were positive for EIA IgM, IgG, or both.

## Results

### *Validation of public health reporting in Arizona*

The results of the validation study are found in Figure 2.

Laboratory A reported positive results for 664 patients during this time. Of those, 509 matched with MEDSIS data (2006-2008) and 73 matched with the older surveillance data (1998-2005). After examining the remaining 82 Laboratory A results by hand, 69 matched. The primary reasons for why results did not originally match the surveillance data included name misspellings, inclusion or exclusion of a middle initial in the name fields, and discrepant birthdates that appear to be typographical errors. The final validation for Laboratory A

showed a positive match for 651 of 664 (98%) patients with positive tests reported.

Laboratory B reported positive results for 517 patients during this time. Of these, 360 matched with MEDSIS data (2006-2008) and 36 matched with the older surveillance data (1998-2005). After examining the remaining Laboratory B results by hand, 41 of 121 matched. The primary reasons for why lab results did not originally match the surveillance data were similar to those for Laboratory A and included name misspellings, inclusion or exclusion of a middle initial in the name fields, and discrepant birthdates that appear to be typographical errors. The final validation for Laboratory B showed a positive match for 427 of 517 (85%) patients with positive tests reported.

Of the thirteen test results that did not match from Laboratory A, most were EIA tests, either IgM or IgG, and were mostly in the months of March and May. The unreported positive tests from Laboratory B, which had a greater number unmatched than Laboratory A, were spread evenly across the three months. However, the type of test among the unreported results was more likely to be EIA (89%) and less likely to be immunodiffusion (5%) compared to the matched tests (67% and 22%, respectively).

#### *Specificity of EIA compared to immunodiffusion or complement fixation*

There were 3942 CF tests, 3867 ID IgM tests, 6486 ID IgG tests, 18,750 EIA IgM tests, 18,698 EIA IgG tests, and 18,617 combined EIA IgM and IgG tests available for review. A total of 1445 lab test sets met the inclusion criteria. A total of 125 “false positives” (FPs) were identified and their associated clinical records were requested and reviewed. We received all 125 records that we requested. Of those, 31 (25%) were re-classified as true coccidioidomycosis cases with tissue/culture diagnosis and/or a positive serologic comparison test (CT) reported in the clinical record or identified in review of the additional laboratory data (Figure 3). The 94 remaining FPs were used to calculate sensitivity, specificity, PPV, and NPV (Table 2). EIA sensitivity equaled 83.8%; EIA specificity equaled 92.6%; positive predictive value equaled 61.5%; and negative predictive value equaled 97.6%.

Clinical chart review of the 94 FP results revealed that 92 (97.9%) were associated with documented coccidioidomycosis symptoms, and 76 (80.9%) were associated with documented physician-diagnosed disease. In the 18 cases without documented physician-diagnosed disease, the physician had not yet given the patient a diagnosis or it was not documented in the medical record.

Further laboratory review of the 2009 data from Lab Corp revealed that 30/94 (32%) had subsequent coccidioidomycosis serology testing done at Lab Corp in 2009. Of those 30, 25 had at least one comparison test run, which were all negative, 3/30 (10%) had both positive EIA tests, 10/30 (33%) had one positive EIA test, and 17/30 (57%) had all negative tests. Further laboratory review of the 2010 data from Lab Corp revealed that only 9/94 (10%) FP had coccidioidomycosis serology testing done, including comparison tests run, at Lab Corp in 2010. Of those 9, none had any positive CT, 2/9 (22%) had one positive EIA test, and 7/9 (78%) had all negative tests.

## **Discussion**

This is the largest investigation of EIA specificity for coccidioidomycosis diagnosis and the only validation of laboratory reporting of coccidioidomycosis in Arizona. Based on laboratory tests alone, the EIA specificity is calculated to be 93% with a PPV of 62%, and 85% of positive coccidioidomycosis laboratory tests performed at a major commercial laboratory were reported to public health as mandated by statute.

The calculated EIA specificity and PPV from this investigation are lower than those previously reported in the literature, which range from 96%-100% and 96%-98% for specificity and PPV, respectively (3, 12, 14, 23). The lower EIA specificity and PPV from our investigation is likely due to the epidemiologic design of the study and the fact that there is no known gold standard for the diagnosis of coccidioidomycosis, especially in early disease. In our investigation, there were 18,617 specimens where both EIAs were run. A total of 7.7% are positive for IgM or IgG. In the final data set, however, there are 1445 specimens, and 16.9% are positive. Our methods apparently select for positive EIA tests, which might mean we are missing a lot from the “negative EIA” column, which would drive down specificity, since the positivity among

the CTs does not seem as much affected. Additionally, because all coccidioidomycosis serology tests from February 2008 through February 2009 were reviewed for inclusion in the analysis, rather than testing serum from selected patients with known disease and known absence of disease, it is much easier for misclassification bias to occur with an epidemiologic design than a laboratory design. Previous studies have been based on a laboratory design using serum from patients with known coccidioidomycosis diagnosis (confirmed by a serologic test, tissue or culture) as a gold standard. In our investigation, patients presenting to their clinicians early in the disease process are much more likely to have falsely negative serology results because it can take up to several weeks for the body to mount an immune response. These patients would have to return for a repeat test in order to be accurately identified as a case. Further, if the EIA does become positive earlier than ID, which is suggested by some studies (3, 22), any case, which is tested during this “window period,” would have a positive EIA and negative comparison test and be misclassified as a FP in our investigation. In a laboratory design, patients who are further along in the disease process can be selected to ensure they would test positive by ID. Additionally, because this was done as part of public health surveillance, patients with FP results could not be contacted and asked to obtain repeat testing to ensure they had not seroconverted.

Due to the fact that no sensitive and specific gold standard laboratory test for coccidioidomycosis exists (other than culture, which is rarely obtained), we reviewed the medical records associated with all FP results to determine if subsequent coccidioidomycosis diagnostic testing had been performed and to determine if the patient had clinical illness consistent with coccidioidomycosis and/or a physician diagnosis of disease. This review revealed that 25% of “false positive” EIA results represented laboratory confirmed disease. Additionally, almost all patients with FP results had a clinical illness consistent with the disease and >80% had a physician’s diagnosis of coccidioidomycosis. In the cases without documented physician-diagnosed disease, the physician had not yet given the patient a diagnosis or it was not documented in the medical record. Unfortunately, the latter information cannot be included in the specificity or PPV calculations because we do not know whether the physician diagnosed coccidioidomycosis on the basis of the “FP” EIA test result or based on some other clinical suspicion or a combination of both.

It is concerning that major commercial laboratories continue to use EIA as a screening test and report only those with positive ID results as positive, both to the ordering physician and to public health. It is also concerning that this practice is recommended by the EIA kit manufacturer (15). The result of this practice is that at least 1 in 4 individuals with a positive EIA and negative ID are actually infected with coccidioidomycosis and are likely misdiagnosed. Further, of the 94 remaining “false positive” EIA results in our investigation, 21 (22%) were positive for both IgM and IgG, which increases the likelihood that they represent true disease, since it is unlikely that both tests would be falsely positive (12). Of the 31 FPs that were subsequently confirmed as positive cases based on a comparison test, 26 (94%) had a positive EIA IgG and 8 (26%) were positive for both EIA IgG and IgM. EIA IgG has been shown to be more specific than EIA IgM when done in isolation (98.3% vs. 84.6%) (23). Based on the increased specificity of EIA IgG, the 54 (57%) “false positives” that were positive for EIA IgG are more likely to represent actual disease (probably early in the course of disease). Finally, Blair and Currier investigated 28 patients with isolated EIA IgM positive serology results and negative ID results among patients suspected of having coccidioidomycosis and found that all 28 eventually developed laboratory confirmed disease either by subsequent ID testing, or based on microbiologic or histopathologic evidence of disease (3). All of this information supports that the specificity calculated from our epidemiological dataset is likely an underestimate of the true EIA specificity for coccidioidomycosis diagnosis.

Other than the limitation regarding a lack of CT described previously, our investigation has several additional limitations. First, upon reviewing the 2009 and 2010 data for repeat comparison tests, only 30 (in 2009) and 9 (in 2010) of 94 individuals with FP results had repeat testing done at Lab Corp during that time period. The absence of repeat serology data prevented a final determination of whether the FP tests represented true disease. This could be due to the fact that the patients stopped getting coccidioidomycosis serology testing done, or they were tested at a different laboratory. Secondly, serologic test results were only reviewed from one major laboratory in Arizona, and due to differences between laboratories in testing methodologies, correlation with other laboratories’ results is needed. Thus, these results may not be generalizable to all laboratories in Arizona.

For all of the reasons cited above, the calculated specificity and PPV of EIA for the diagnosis of coccidioidomycosis in this investigation likely represent underestimates of the true values. The findings of our investigation support those of Blair and Currier and suggest that serologic testing for coccidioidomycosis should always be performed in combination. Further, use of EIA as a screening test that is only reported as positive when confirmed with ID leads to missed diagnoses, which is a disservice to patients, physicians, and public health. For patients with clinical illness consistent with coccidioidomycosis and isolated EIA positivity, clinicians should consider close observation and repeat serologic testing or performance of other diagnostic methods such as culture or biopsy. Furthermore, clinicians should inform patients of positive EIA results irrespective of CF/ID results and explain that further laboratory testing and monitoring of the illness may be needed to establish a diagnosis.

## **Future Directions**

Once completed, this project will not have to be repeated by the same researchers. Correlation with other laboratories' results is needed along with an evaluation of the impact of the results within the community. Recommendations to laboratories about how to report coccidioidomycosis serological testing results along with instructions to physicians about how to interpret the reported results are also necessary.

## **Conclusions**

Based on the largest study of sensitivity and specificity calculated from laboratory surveillance data, EIA sensitivity and specificity for coccidioidomycosis diagnosis are lower than previously reported using only coccidioidomycosis laboratory tests as comparison tests. However, 25% of positive EIA results with negative comparison tests were found to have subsequent confirmatory test results, suggesting that single immunodiffusion or complement fixation tests are not a sufficient comparison tests/gold standards for coccidioidomycosis diagnosis. Association of "false positive" EIA results

with coccidioidomycosis symptoms suggests that some of these may represent missed diagnoses. Repeat serologic or other coccidioidomycosis diagnostic testing for patients with isolated positive EIA test results may improve EIA diagnostic utility.

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## Appendix

Figure 1. Rates of Reported Valley Fever (VF) in Arizona, 1993-2008

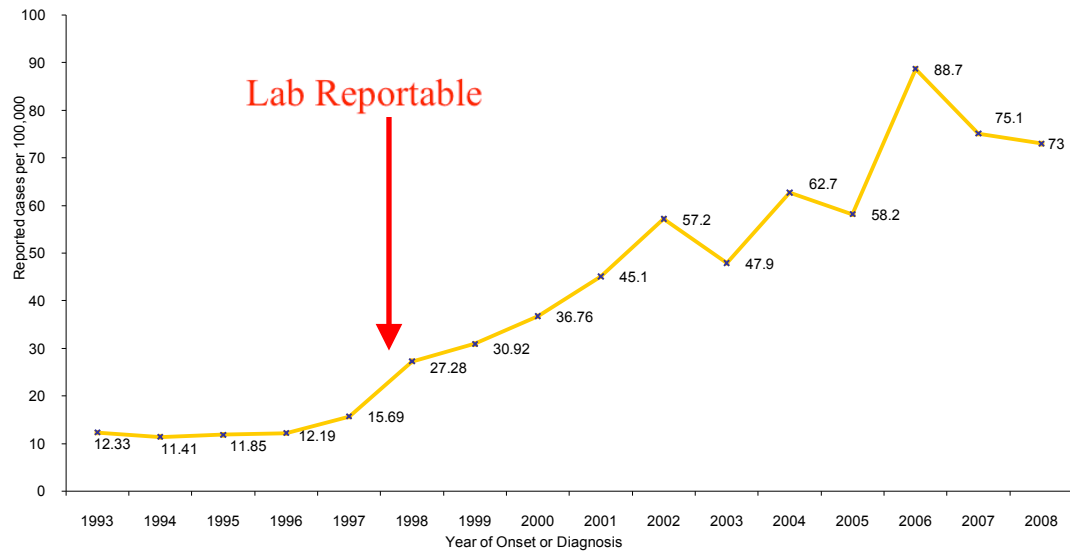


Table 1. Learning objectives and implementation plan

Learning objectives	Implementation plan
Describe the impact of coccidioidomycosis in AZ and why establishing a diagnosis is difficult.	<ol style="list-style-type: none"> <li>1. Conduct a literature search.</li> <li>2. Learn about the various diagnostic methods.</li> </ol>
Establish the sensitivity and specificity of EIA for coccidioidomycosis diagnosis compared to ID and CF as the comparison tests.	<ol style="list-style-type: none"> <li>1. Request data from selected laboratories.</li> <li>2. Clean, validate, and analyze the data.</li> <li>3. Compare the results with published studies.</li> </ol>
Determine the positive predictive value of EIA by comparing it to a combination of ID and CF, as well as clinical symptoms as the comparison tests.	<ol style="list-style-type: none"> <li>1. Create a clinical review form.</li> <li>2. Extract data from selected records.</li> <li>3. Recalculate the PPV using the results from the clinical review.</li> </ol>
Specify the best way for laboratories to report diagnostic testing results for coccidioidomycosis to healthcare providers.	<ol style="list-style-type: none"> <li>1. Report the results of the study to the participating laboratory directors with recommendations.</li> </ol>
Gain knowledge of the process and strengths and limitations in collecting and analyzing reported laboratory data to the state health department.	<ol style="list-style-type: none"> <li>1. Request data from selected laboratories.</li> <li>2. Clean, validate, and analyze the data.</li> <li>3. Compare the results with published studies.</li> </ol>
Gain knowledge on the process and strengths and limitations in collecting and analyzing clinical data reported to the state health department.	<ol style="list-style-type: none"> <li>1. Request data from selected providers.</li> <li>2. Create a form to extract the data from the records.</li> <li>3. Analyze the data.</li> <li>4. Compare the results with published studies.</li> </ol>

Learning objectives	Implementation plan
Construct a database and create a statistical program to calculate true and false positives and true and false negatives to determine sensitivity, specificity, PPV, and NPV.	<ol style="list-style-type: none"> <li>1. Analyze laboratory data to determine which variables need to be included in the database.</li> <li>2. Meet with a biostatistician to create a statistical program.</li> <li>3. Define sensitivity/specificity/PPV/NPV.</li> </ol>

Figure 2. Diagram of matching schema for public health reporting validation for laboratories A and B

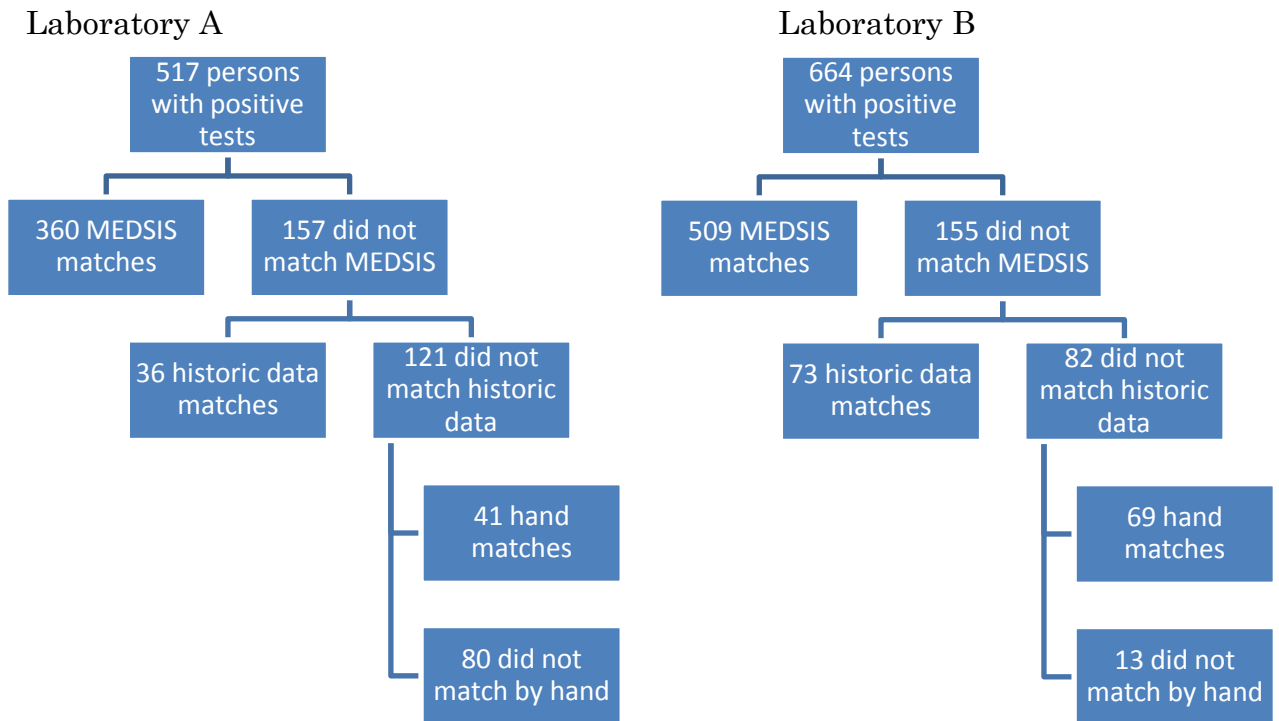


Figure 3. Summary of “false positive” enzyme immunoassay results



FP = “false positive”  
 IgG = immunoglobulin G  
 IgM = immunoglobulin M  
 pos = positive  
 neg = negative

Table 2. Enzyme immunoassay compared to comparison tests

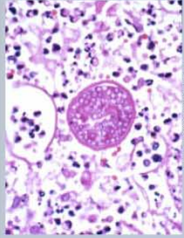
	CT positive (CF/ID)	CT negative (CF/ID)	Total
EIA positive	150	94	244
EIA negative	29	1172	1201
Total	179	1266	1445

EIA = enzyme immunoassay

CT = comparison test

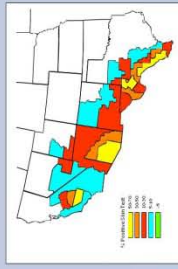
CF = complement fixation

ID = immunodiffusion



#### Coccidioidomycosis (Valley Fever)

- Respiratory infection caused by soil fungus
- Transmission
  - Cough, Fever, Fatigue
- Disseminated illness: spreads to other parts of the body – fatal without treatment
- No licensed vaccine available
- Laboratory and provider reportable in AZ



\*F. C. Edwards and C. E. Palmer. The pattern of results in the serological reference test specific and nonspecific reactions to coccidioidomycosis and to histoplasmosis. *Dis. Chest* 33:55-59, 1957

#### Cocci Epidemiology

##### Valley Fever in the U.S.

- Endemic areas: Southwestern US, Mexico, parts of Central and South America
- 80% of US disease in AZ
- Mode of transmission
  - Inhalation of spores from soil and dust
- Incubation period: 1 to 4 weeks (primary infection)

#### Rates of Reported Valley Fever (VF) in Arizona, 1993-2008



# Specificity of Enzyme Immunoassay for Serologic Coccidioidomycosis Diagnosis Compared to Immunodiffusion

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#### Coccidioidomycosis Diagnosis

- Tissue diagnosis and culture most specific
- Serology to detect antiococcidial antibodies is most sensitive and most used
- Immunodiffusion (ID)
  - Traditional and most studied
  - Very specific, but not sensitive in early disease
- Enzyme immunoassay (EIA)
  - Newer, less expensive and easier to perform
  - Thought to be more sensitive in early disease
  - Concern about specificity, especially IgM

#### Need & Relevance

- Need
  - Of the estimated 150,000 U.S. coccid infections per year, approximately 60% occur in Arizona, making this state the focal point for investigation of the disease
  - The EIA is the easiest and least expensive to perform, but its sensitivity and specificity can be problematic
  - Early in disease the test can be falsely negative
  - Low sensitivity and specificity require additional diagnostic testing and involve patient anxiety
  - The results from using EIA has not yet been extensively correlated with immunodiffusion
- Relevance
  - Valley Fever grows in desert Southwest soil in low abundance
  - Anyone who lives, visits, or travels in an endemic area may acquire Valley Fever
  - Wilds, disturbances, construction and activities that stir up soil, dust, debris, and spores can be inhaled and inhaled spores can be inhaled and inhaled
  - People working in construction, landscaping, excavation, agriculture, & archaeology are at increased risk
  - Tourists who are immun-naïve are at risk of increased risk, especially if engaging in activities that stir up dust

#### Summary of "False Positive" EIA Results



#### Clinical Review of 84 False Positive Results

- 92/84 (97.9%) were associated with documented coccidioidomycosis symptoms
- 76/84 (90.5%) were associated with documented physician-diagnosed disease

#### Summary

- Largest investigation of EIA specificity for coccidioidomycosis diagnosis
- EIA specificity = 93% (PPV 62%) based on laboratory tests alone
- 25% of "false positive" EIA results represent lab confirmed disease
- 23% of false positive EIA results are both IgG and IgM positive, increasing the likelihood that they represent true disease

#### Results

- 1445 lab test sets met inclusion criteria
  - EIA IgM & IgG with 2 GS tests run on the same date
  - 125 false positives (FP) identified
  - 125 false positives (FP) identified
  - 31 FP → true positive with tissue culture diagnosis and/or positive GS test reported in clinical record
  - 94 false positives
  - Reported sensitivity, specificity, PPV, NPV at this level

	GS+GS+ (Disease)	GS- (No Disease)
EIA+	150	94
EIA-	29	1172

EIA sensitivity = 83.8%  
EIA specificity = 92.8%  
Positive predictive value = 61.5%  
Negative predictive value = 97.6%

#### Conclusions

- ≥ 25% of positive EIA results with negative "gold standard" tests represent true disease
- Single immunodiffusion/complement fixation tests are not a sufficient "gold standard" for coccidioidomycosis diagnosis
- Association of "false positive" EIA results with coccidioidomycosis symptoms and diagnosis suggests clinical correlation may improve EIA diagnostic utility

#### Limitations

- Repeat serologic test results occurring after February 2009 were not available possibly leading to missed diagnoses
- Serologic test results were only reviewed from only one laboratory - correlation with other laboratories' results is needed
- Laboratory methods may vary in different laboratories

#### Future Directions

- Correlation with other laboratories' results is needed
- Evaluation of the impact of the results within the community
- Recommendations to laboratories about how to report coccid serological testing results

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