Received: 30 November 2020 | Accepted: 19 February 2021

DOI: 10.1111/jvim.16087

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Clinical performance of a point-of-care *Coccidioides* antibody test in dogs

Krystle L. Reagan[1] | Ian McHardy[2] | George R. Thompson III[3] | Jane E. Sykes[1]

[1]Department of Medicine and Epidemiology, University of California-Davis, Davis, California

[2]Department of Medical Microbiology and Immunology, University of California-Davis, Davis, California

[3]Department of Internal Medicine, Division of Infectious Diseases, University of California Davis Medical Center, Sacramento, California

Correspondence
Krystle L. Reagan, Department of Medicine & Epidemiology, University of California-Davis, Davis, CA.
Email: kreagan@ucdavis.edu

Abstract

Background: Point-of-care (POC) *Coccidioides* antibody assays may provide veterinarians with rapid and accurate diagnostic information.

Objectives: To determine the agreement of a POC lateral flow assay (LFA), sona Coccidioides (IMMY, Norman, Oklahoma) with the current diagnostic standard, the immunodiffusion assay (agar gel immunodiffusion [AGID]; Coccidioidomycosis Serology Laboratory, University of California, Davis, California).

Animals: Forty-eight sera specimens from 48 dogs.

Methods: Sera specimens were collected from client-owned dogs that had a clinical suspicion for coccidioidomycosis. Animals were classified as *Coccidioides* antibody-positive (n = 36) based on a positive AGID or *Coccidioides* antibody-negative (n = 12) based on a negative AGID. The performance of the LFA assay was determined by comparing results to AGID results.

Results: The LFA assay demonstrated agreement in 32 of 36 *Coccidioides* antibody-positive specimens and 12 of 12 *Coccidioides* antibody-negative specimens, resulting in a positive percentage agreement of 88.9% (95% confidence interval [CI], 74.7-95.6%) and negative percentage agreement of 100% (95% CI, 75.8-100%) as compared to AGID. A receiver operator characteristic curve was constructed, and the area under the curve was 0.944 (CI, 0.880-1.000).

Conclusion and Clinical importance: This LFA is a rapid alternative to the traditional AGID. The LFA provides excellent predictive value for positive results. Positive agreement was lower in dogs with low AGID titers; therefore, confirmatory testing is recommended if a high index of suspicion exists.

KEYWORDS
diagnostic test, fungal, lateral flow, veterinary

1 | INTRODUCTION

Coccidioidomycosis, or Valley Fever, is a systemic mycosis caused by the fungal pathogens *Coccidioides immitis* or *Coccidioides posadasii*. These organisms are endemic in arid regions including California,

Abbreviations: AGID, agar gel immunodiffusion; AUC, area under the curve; CF, complement fixation; EIA, enzyme immunoassay; LFA, lateral flow assay; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operator curve; TP, tube precipitin.

Arizona, New Mexico, Texas, and northern Mexico.[1] The dimorphic fungi exist in the soil as mycelium and can lead to infection in a wide range of mammals when the arthroconidia become aerosolized in dust and are inhaled. Once inhaled, spherules form and establish infection that can lead to a wide range of clinical presentations. Coccidioidomycosis most commonly is characterized by respiratory infection that can range from subclinical to severe disease.[2] Disseminated infection occurs in approximately 25% of individuals and can involve the skeletal system, central nervous system, eyes, skin, lymphatic system, and pericardium.[3]

Cases of coccidioidomycosis have increased dramatically in the Southwest United States over the past decade, with a record number of cases in humans diagnosed in 2018 (the most recent year with data available).[4] Case numbers in veterinary medicine are not widely available for comparison, but an increase in newly diagnosed cases of coccidioidomycosis in dogs was noted at our institution with a peak in 2018 (unpublished data). The increase in coccidioidomycosis cases has been attributed to climate changes, increased population in endemic regions, increased soil disturbances and construction activities, and increases in disease awareness and testing.[5]

Detection of anti-*Coccidioides* antibodies provides the laboratory basis for diagnosis of coccidioidomycosis in most cases. Organism detection by cytology, histopathology, or culture identification of fungal organisms is considered the gold standard diagnostic methods. These methods however are invasive and insensitive, and fungal culture poses a risk to laboratory personnel. The serologic reference standard in dogs is the agar gel immunodiffusion (AGID) assay. This assay's sensitivity and specificity at selected institutions approaches 100%.[6,7] However, the AGID performance varies among institutions, and false positives and false negatives have been reported in other geographical locations.[8,9] The AGID can detect immunoglobulin M (IgM) against the protein tube precipitin (TP) antigen or immunoglobulin G (IgG) against the protein complement fixation (CF) antigen.[10] Performance of the AGID is complex, labor-intensive, expensive, and incubation times of up to 1 week are required to provide results in some cases.

Rapid antibody detection assays have been developed, including enzyme immunoassays (EIA) and an immunochromatographic lateral flow assay (LFA). A commercially available EIA (MiraVista Labs, Indianapolis, Indiana) was evaluated in dogs compared with AGID and determined to have a sensitivity of 89.2% and specificity of 97.2%.[11] Although more rapid than AGID, EIAs are not performed at the point of care and result turnaround times can still exceed 48 hours at reference laboratories. Alternatively, LFAs can be performed with minimal training and laboratory equipment at the point of care and return results in approximately 30 minutes. The sona *Coccidioides* antibody LFA (IMMY, Norman, Oklahama) provides qualitative detection of antibodies against TP and CF antigens from *Coccidioides* according the manufacturer. The sona *Coccidioides* antibody LFA has been evaluated in a cohort of dogs residing in Arizona.[12] The LFA results were compared to AGID results submitted to 1 of several reference laboratories, and an overall agreement of 87.5% was noted.[12] Here, we aim to assess the diagnostic performance of the sona *Coccidioides* LFA as compared to a standardized AGID performed in a single reference laboratory in dogs suspected of having coccidioidomycosis and residing in a wider geographic area.

2 | MATERIALS AND METHODS

2.1 | Sera specimens

Sera specimens from client-owned dogs were collected both prospectively and from stored specimens submitted to the UC Davis Coccidioidomycosis Laboratory for *Coccidioides* antibody testing. If sufficient volume of serum remained after AGID, the specimens were stored at −80°C until further analysis. A cohort was chosen for LFA analysis using convenience sampling. Complete medical records were not available for patients that had serum submitted to the UC Davis Coccidioidomycosis Laboratory from veterinarians that practiced outside of our institution.

2.2 | Agar gel immunodiffusion performance

The AGID assays were performed as previously described by a single laboratory, the UC Davis Coccidioidomycosis Laboratory (Davis, California).[10] Samples were placed in a well within the agar plate, and the corresponding purified antigen (TP or CF) placed in an opposing well. The plates were incubated for up to 96 hours and monitored daily for development of an antigen-antibody precipitation line. If a precipitation line was noted, quantitative immunodiffusion was performed to determine the IgG titer.

2.3 | Lateral flow assay performance

The LFA was performed according to the manufacturer's instruction by a single investigator (KR). The kit was brought to room temperature for 30 minutes before testing. The specimen was diluted 1:441 in specimen diluent using microcentrifuge tubes. Next, 100 μL of the diluted specimen was placed into a flat-bottom 96-well plate. The LFA test strip tip was inserted into the well containing the specimen. The plate then was incubated at room temperature for 30 minutes. Concurrently, a positive control specimen (manufacturer supplied) and negative control (specimen diluent only) were assayed. Test results were recorded as negative (red control line present), positive (red control and test lines present), or invalid (absence of control line regardless of test line presence).

2.4 | Statistical analysis

Data were analyzed using statistical software (Prism, GraphPad, San Diego, California). Positive percentage agreement, negative percentage agreement, positive predictive value (PPV), and negative predictive value (NPV) were calculated with 95% confidence interval (CI) as

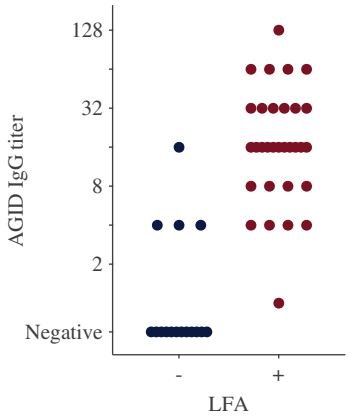

FIGURE 1 Results of lateral flow assay (LFA) and agar gel immunodiffusion (AGID). Scatterplot indicating results of LFA and corresponding AGID titer for all dogs with an available immunoglobulin G titer (n = 47). The serum AGID titer is represented in a logarithmic scale on the *y*-axis. Results of the LFA are represented on the *x*-axis and stratified into negative (–, blue dots) and positive (+, red dots)

compared to the serologic reference standard AGID. Results were used to create receiver operator characteristic (ROC) curves, and the area under the curve (AUC) was calculated for the LFA. Agreement between the LFA and AGID results was assessed with a Cohen's kappa statistic.

3 | RESULTS

3.1 | Dogs

Serum specimens from 48 dogs were prospectively and retrospectively collected from dogs that were suspected to have or previously been diagnosed with coccidioidomycosis. Specimens were submitted to the UC Davis Coccidioidomycosis Laboratory from 6 states; 25 from California; 19 from Texas; and 1 each from Hawaii, Minnesota, Nevada, and Washington. Dogs ranged in age from 6 months to 14 years with a median age of 5 years.

Thirty-six dogs were categorized as *Coccidioides* antibody-positive based on positive AGID results. Quantitative IgG titers were obtained in 35 of 36 of the *Coccidioides* antibody-positive dogs and ranged from 1:1 to 1:128 (Figure 1). Twelve dogs were classified as *Coccidioides* antibody-negative. The control dogs included dogs for which the attending veterinarian had a clinical suspicion of coccidioidomycosis, but ultimately an alternative diagnosis was established. These included 2 dogs with multisystemic autoimmune disease; 1 of these dogs had marked mixed inflammation of the cerebrospinal fluid (CSF), keratoconjunctivitis sicca, and generalized lymphadenopathy with reactive lymphoid morphology noted on cytology that responded favorably to immunosuppression. The other dog with multisystemic autoimmune disease had multifocal sterile draining tracts with pyogranulomatous inflammation and soft tissue opacity pulmonary nodules that responded favorably to immunosuppression. Two of the control dogs were diagnosed with respiratory disease: 1 with tracheobronchomalacia based on thoracic fluoroscopy and the other with pulmonary fibrosis based on histopathology of tissues obtained at necropsy. Two dogs were diagnosed with alternative infectious diseases: 1 with cryptococcosis of the central nervous system and 1 with babesiosis. Three of the control dogs were diagnosed with malignancy: 1 was diagnosed with intermediate to large cell T-cell lymphoma involving the mediastinal lymph node based on cytology and PCR for antigen receptor rearrangements; 1 was diagnosed with osteosarcoma based on biopsy of an osteolytic lesion after thoracic limb amputation; and 1 was diagnosed with metastatic apocrine gland anal sac adenocarcinoma based on cytology of a retroperitoneal mass. One control dog was diagnosed with granulomatous meningoencephalomyelitis based on magnetic resonance imaging and CSF assessment. Panuveitis was diagnosed in 1 control dog that responded favorably to topical anti-inflammatory treatment and doxycycline. The remaining dog had a polyostotic lesion suspected to be secondary to previous septic arthritis.

TABLE 1 Summary of results of the agar gel immunodiffusion (AGID) and lateral flow assay (LFA) for specimens illustrating number of dog specimens that were *Coccidioides* antibody-positive and antibody-negative as determined by the 2 methodologies

	AGID	
	Positive	**Negative**
LFA		
Positive	32	0
Negative	4	12

3.2 | IMMY sona *Coccidioides* LFA performance

The LFA was performed, and a valid test result was recorded for all 48 serum specimens. All 12 *Coccidioides* AGID antibody-negative specimens had negative LFA results (Table 1). Thirty-two of the 36 of the *Coccidioides* AGID antibody-positive specimens tested positive using the LFA, whereas 4 had negative LFA results. Four specimens had discordant results with negative LFA and positive AGID (Figure 1). Three of these dogs had AGID IgG titers of 1:4, and 1 had an AGID IgG titer of 1:16. Overall, 44 of 48 (91.7%) of the observed results agreed between the LFA and the AGID. Cohen's kappa statistic was 0.80 (95% CI, 0.61-0.98).

Compared to the AGID, the LFA has a positive percentage agreement of 88.9% (95% CI, 75%-96%) and a negative percentage agreement of 100% (95% CI, 76%-100%). An ROC curve was constructed and the AUC was 0.94 (95% CI, 0.88-1.00). The PPV in this population was 100% (95% CI, 89.3%-100%) and the NPV was 75% (95% CI, 50%-90%).

4 | DISCUSSION

We found a high degree of agreement between the LFA for detecting *Coccidioides* antibodies compared to AGID in dogs suspected of

having coccidioidomycosis. In this population, the LFA demonstrated concordance with the AGID and had a positive percentage agreement of 89% and 100% negative percentage agreement. These results indicate the LFA may serve as a useful rapid screening test, but additional testing may be indicated if a high index of suspicion remains in the face of a negative LFA result.

Our objective was to compare the results of the LFA to the results of the AGID rather than determine the true sensitivity and specificity of the LFA, which would require confirmation of fungal organisms by light microscopy or fungal culture. Organism detection methods are not often pursued clinically, because doing so requires invasive and expensive diagnostic procedures such as the collection of tissue biopsy specimens. The AGID has been established as the serologic reference standard for diagnosing coccidioidomycosis in dogs and is relied upon heavily to confirm clinical suspicion of disease.[13,14] The AGID can have variable results among reference laboratories, but at our institution, sensitivity approaches 100%.[6,7,9] False-negative results have been reported in other geographic locations utilizing other reference laboratories.[8]

The LFA in our population had a high negative percentage agreement (100%), with no false-positive results observed. A positive percentage agreement of 89% was observed, with 4 negative LFA results recorded in dogs with positive AGID titers. High negative percentage agreement and PPV (100%) indicate that the LFA could be utilized as a screening test in dogs suspected to have coccidioidomycosis, and a positive LFA result would indicate the presence of *Coccidioides* antibodies, whereas a negative LFA may not rule out the presence of antibodies. In a dog with concurrent clinical signs and laboratory findings, the availability of a positive LFA result would allow for rapid initiation of antifungal treatment, whereas negative tests may need to be repeated using a more sensitive test if a high index of suspicion remains.

Our results show similar overall agreement to a study assessing this LFA in dogs residing in Arizona.[12] A small number of negative LFA tests with positive AGID results were noted in both studies, and all but 1 discordant result was associated with AGID titers ≤1:4. This observation suggests that the LFA may not be as sensitive when *Coccidioides* antibody titers are low, such as early in an infection.[12] However, in our study, 5 patient specimens were determined to have AGID titers ≤1:4 and had corresponding positive LFA results.

In our study, no positive LFA with negative AGID discordant results was noted, which differs from a previous study that found that 15% of the dogs with negative AGID results had positive LFA results.[12] In the previous study, 2 of the discordant results were from dogs that previously were diagnosed with coccidioidomycosis and were receiving antifungal treatment. The other 2 discordant results were from dogs that had clinical disease highly suspicious for coccidioidomycosis, and the attending clinicians recommended convalescent AGID titers to assess for seroconversion that was not pursued by the clients.[12] The cause of this difference in discordant results with a positive LFA and negative AGID is unknown. The AGID assay performance in the previous study was not standardized. Therefore, differences in assay performance among reference laboratories may have been present, making comparisons between the studies difficult.

Our results show similar overall agreement to preliminary studies conducted in people diagnosed with coccidioidomycosis.[15-17] However, a more recent study assessing the LFA performance on specimens from people early in the course of infection only had a sensitivity of 30% to 40% compared to EIA or AGID.[18] The chronology of clinical signs was not assessed in our study, and determination of LFA performance in dogs early in the course of coccidioidomycosis should be further evaluated.

The major limitation of the AGID assay is the turnaround time between collection of patient specimens and availability of diagnostic results. One study conducted in a reference laboratory for human medicine determined that implementation of LFA screening decreased turnaround time from up to 10 days to <24 hours.[17] Indeed, in our study, the LFA provided rapid results with minimal laboratory equipment or technical expertise. Performance of the LFA requires a laboratory pipette to dilute the specimen and a microcentrifuge tube, into which the diluted serum specimen is placed, and the test strip tip is immersed before incubation. Interpretation of the LFA occurs after a 30-minute incubation period and does not require any specialized equipment.

Our study's main limitation was dependence on the AGID to categorize dogs based on the presence of *Coccidioides* antibody rather than identification of fungal organisms using cytologic or microbiologic methods to diagnose coccidioidomycosis definitively. Reaching a definitive diagnosis in that manner requires invasive and expensive diagnostic tests that are not often pursued clinically. Furthermore, dogs enrolled in our study were at different timepoints in the course of their disease. Assessing the LFA performance at the time of diagnosis and during the treatment period may provide further information, including sensitivity early in infection and utility of the LFA as a treatment monitoring tool. Additionally, the control dogs in our study were dogs for which the attending veterinarians had clinical suspicion of systemic mycosis, but only 1 of which was diagnosed with an alternate fungal infection (cryptococcosis). It would be valuable to assess this assay's specificity in animals with another systemic mycosis, such as histoplasmosis, which also is endemic in our geographic area.

In conclusion, the LFA has a positive percentage agreement of 89% and negative percentage agreement of 100% compared to the AGID. This assay may allow for the swift initiation of treatment, decrease the need for more invasive and costly diagnostic testing, and improve antimicrobial stewardship by preventing empirical antifungal treatment while waiting for diagnostic results. Further assessment of this assay is warranted early in the course of coccidioidomycosis and to determine its utility in therapeutic monitoring.

ACKNOWLEDGMENT

No funding was received for this study.

CONFLICT OF INTEREST DECLARATION

Diagnostic test strips were a generous donation from IMMY; however, they were not involved in study design, the acquisition of data, the preparation of this manuscript, or the decision to publish the results.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Krystle L. Reagan https://orcid.org/0000-0003-3426-6352

REFERENCES

1. Brown J, Benedict K, Park BJ, et al. Coccidioidomycosis: epidemiology. *Clin Epidemiol*. 2013;5:185.
2. Shubitz LF, Butkiewicz CD, Dial SM, Lindan CP. Incidence of Coccidioides infection among dogs residing in a region in which the organism is endemic. *J Am Vet Med Assoc*. 2005;226:1846-1850.
3. Davidson AP, Shubitz LF, Alcott CJ, Sykes JE. Selected clinical features of Coccidioidomycosis in dogs. *Med Mycol*. 2019;57:S67-S75.
4. Surveillance and Statistics Section Infectious Diseases Branch, Division of Communicable Disease Control Center for Infectious Diseases, California Department of Health. *Epidemiologic Summary of Coccidioidomycosis in California*. Sacramento, California: California Department of Health; 2018.
5. Cooksey GS, Nguyen A, Knutson K, et al. Notes from the field: increase in Coccidioidomycosis—California, 2016. *MMWR Morb Mortal Wkly Rep*. 2017;66:833-834.
6. Johnson LR, Herrgesell EJ, Davidson AP, Pappagianis D. Clinical, clinicopathologic, and radiographic findings in dogs with coccidioidomycosis: 24 cases (1995–2000). *J Am Vet Med Assoc*. 2003;222:461-466.
7. Heinritz CK, Gilson SD, Soderstrom MJ, Robertson TA, Gorman SC, Boston RC. Subtotal pericardectomy and epicardial excision for treatment of coccidioidomycosis-induced effusive-constrictive pericarditis in dogs: 17 cases (1999–2003). *J Am Vet Med Assoc*. 2005;227:435-440.
8. Graupmann-Kuzma A, Valentine BA, Shubitz LF, Dial SM, Watrous B, Tornquist SJ. Coccidioidomycosis in dogs and cats: a review. *J Am Anim Hosp Assoc*. 2008;44:226-235.
9. Johnson JE, Jeffery B, Huppert M. Evaluation of five commercially available immunodiffusion kits for detection of *Coccidioides immitis* and *Histoplasma capsulatum* antibodies. *J Clin Microbiol*. 1984;20:530-532.
10. Pappagianis D, Zimmer BL. Serology of coccidioidomycosis. *Clin Microbiol Rev*. 1990;3:247-268.
11. Holbrook ED, Greene RT, Rubin SI, et al. Novel canine anti-Coccidioides immunoglobulin G enzyme immunoassay aids in diagnosis of coccidioidomycosis in dogs. *Med Mycol*. 2019;57:800-806.
12. Schlacks S, Vishkautsan P, Butkiewicz C, Shubitz L. Evaluation of a commercially available, point-of-care Coccidioides antibody lateral flow assay to aid in rapid diagnosis of coccidioidomycosis in dogs. *Med Mycol*. 2020;58:328-332.
13. Shubitz L. Comparative aspects of coccidioidomycosis in animals and humans. *Ann New York Acad Sci*. 2007;1111:395-403.
14. Pappagianis D. Serologic studies in coccidioidomycosis. *Seminars in Respiratory Infections*; 2001;16:242-250.
15. Maddox S, Doherty B, Pelfrey J, et al. Rapid detection of anti-Coccidioides antibodies using the sōna Coccidioides Ab lateral flow assay. In: 7th International Coccidioidomycosis Symposium, Stanford, CA 2017.
16. Contreras D, Li Q, Garner O. Evaluation of Sōna Lateral Flow Assay for the Rapid Detection of *Coccidioides immitis*. In: ASM Microbe, San Francisco, CA 2019.
17. Candelaria W. Implementation of the sōna Coccidioides Antibody Lateral Flow Assay in the Clinical Laboratory Proves to Reduce Cost and Decrease Turnaround Time When Compared to Send out Immunodiffusion and Complement Fixation Testing. In: ID Week 2019, Washington, DC 2019.
18. Donovan FM, Ramadan FA, Khan SA, et al. Comparison of a novel rapid lateral flow assay to enzyme immunoassay results for early diagnosis of Coccidioidomycosis. *Clin Infect Dis*. 2020. https://doi.org/10.1093/cid/ciaa1205.

How to cite this article: Reagan KL, McHardy I, Thompson GR III, Sykes JE. Clinical performance of a point-of-care *Coccidioides* antibody test in dogs. *J Vet Intern Med*. 2021;35:965–969. https://doi.org/10.1111/jvim.16087