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Authors
Reagan, Krystle L
McHardy, Ian
Thompson, George R
et al.

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STANDARD ARTICLE

Evaluation of the clinical performance of 2 point-of-care cryptococcal antigen tests in dogs and cats

Krystle L. Reagan[1] | Ian McHardy[2] | George R. Thompson III[3] | Jane E. Sykes[4]

[1]Veterinary Medical Teaching Hospital, School of Veterinary Medicine, 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

[4]Department of Medicine & Epidemiology, University of California-Davis, Davis, California

Correspondence
Krystle L. Reagan, 1 Garrod Drive, Davis, CA.
Email: kreagan@ucdavis.edu

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Abstract

Background: Point-of-care (POC) *Cryptococcus* antigen assays may provide veterinarians with a more rapid, patient-side diagnosis when compared with traditional laboratory-based latex agglutination tests.

Objective: To determine the sensitivity and specificity of 2 POC lateral flow cryptococcal serum antigen tests, CrAg LFA (Immy, Norman, OK) and the CryptoPS (Biosynex, Strasbourg, France) for diagnosis of cryptococcosis in dogs and cats, using the cryptococcal antigen latex agglutination system (CALAS) as the reference standard.

Animals: 102 serum samples from 51 dogs and 40 cats.

Methods: Specimens were classified as CALAS-positive (n = 25) or CALAS-negative (n = 77). The sensitivity and specificity of each POC assay was calculated by comparing the results to the serologic reference standard results.

Results: The CrAg LFA assay correctly classified 23/25 CALAS-positive specimens and 69/74 CALAS-negative specimens resulting in a sensitivity of 92.0% (confidence interval [CI], 75.0%-98.6%) and specificity of 93.2% (CI, 85.1%-97.1%). The CryptoPS assay correctly classified 8/10 tested CALAS-positive specimens and 56/59 tested CALAS-negative specimens resulting in a sensitivity of 80.0% (CI, 49.0%-96.5%) and specificity of 94.9% (CI, 86.1%-98.6%).

Conclusion and Clinical Importance: The POC assays appear to be a sensitive and specific alternative to the traditional CALAS assay with more rapid turnaround times, which may result in earlier diagnosis and treatment.

KEYWORDS
Cryptococcus, diagnostic test, fungal

1 | INTRODUCTION

Cryptococcus is an emerging pathogen and cryptococcosis is the most common systemic fungal disease in the domestic cat.[1] Severe disease with dissemination to the central nervous system can occur in dogs and people.[1,2] Rapid and reliable diagnostic tests are required to allow early and appropriate treatment recommendations to be made.

The genus *Cryptococcus* contains over 19 species. *Cryptococcus neoformans* and organisms that belong to the *Cryptococcus gattii* species complex cause the majority of disease in cats, dogs, and people.[3] The organism has a complex polysaccharide capsule and appears as narrow-based budding yeasts on cytologic examination. The fungus is found worldwide with most cases in dogs and cats being reported

Abbreviations: AUC, area under the curve; CALAS, cryptococcal antigen latex agglutination system; LFA, lateral flow assay; ROC, receiver operator curve.

from the western United States, British Columbia in Canada, South America, and Australia.[4,5]

Infection is thought to follow inhalation of basidiospores into the nasal cavity or lungs after which the organism disseminates hematogenously to other organs.[6] Cryptococcosis in cats typically is characterized by the presence of upper respiratory tract signs, nodular or ulcerative cutaneous lesions, chorioretinitis, or neurologic disease arising from meningoencephalitis.[3] The majority of affected dogs have disseminated *C. neoformans* infections and show signs that include weight loss, lethargy, anorexia, neurologic signs, gastrointestinal or respiratory signs, and nasal or cutaneous lesions.[6]

The reference standard for diagnosis of cryptococcosis is fungal culture; however, diagnosis is also commonly obtained by cytology or histopathology. These diagnostic tests frequently rely on invasive procedures for specimen collection and there is often a substantial lag time between specimen submission and the reporting of results. Use of India ink stain on cerebrospinal fluid (CSF) specimens can aid in the rapid cytologic identification of this organism by highlighting the capsule, but organisms are only identified on cytologic examination of the CSF in 60%-80% of animals with cryptococcal meningitis and 44%-52% of affected people.[5,7,8] Biopsy specimens of affected lesions can be obtained for histopathology, and identification is confirmed with Mayer's mucicarmine stain or immunohistochemistry.[9]

The cryptococcal antigen latex agglutination system (CALAS) is a quantitative serologic test that detects *Cryptococcus* polysaccharide capsule antigen and has been shown to be sensitive and specific for the diagnosis of cryptococcosis in veterinary patients.[5,10,11] This test has been applied to both serum and CSF specimens, and is considered to be among the most accurate diagnostic assays for the diagnosis of cryptococcal infections in both humans and animals.[10,12,13] Trained laboratory personnel are required to perform the CALAS assay, which requires serial dilutions of patient serum or CSF, prolonged incubation periods, and experience with interpretation of results. These factors often lead to a lag time of several days before the results are reported to the practitioner.

Other rapid antigen detection assays have been developed including antigen enzyme immunoassay (EIA) and immunochromatographic lateral flow assays (LFAs). These have been validated for use in human medicine for the diagnosis and monitoring of cryptococcosis with high sensitivity (93%-100%) and specificity (93%-98%).[12,14-16] The commercially available EIA kits are automated using spectrophotometric methods, but require specialized training and equipment to perform. Alternatively, LFAs are rapid, requiring <15 minutes to obtain results, can be performed in a practice setting, and have had good agreement with the CALAS in humans.[13,14,17] Studies in people also have shown improved sensitivity of a *Cryptococcus* LFA (Immy, Norman, CA) when compared with the CALAS.[18] Assays may consist of a dipstick test strip or cassette with a membrane to which monoclonal antibodies to cryptococcal antigen are affixed. Antigen present in biologic specimens binds to the monoclonal antibodies and can be detected using a conjugate antibody that generates a colorimetric product.

We aimed to determine the diagnostic performance of 2 commercially available immunochromatographic LFA point-of-care (POC) assays for detection of cryptococcal antigen in dogs and cats, the CrAg LFA (Immy, Norman, OK) and the CryptoPS (Biosynex, Strasbourg, France), as compared to the serologic reference standard CALAS. The former is a nonquantitative dipstick test and the latter is a semi-quantitative cassette-based immunoassay.

2 | MATERIALS AND METHODS

2.1 | Animals

Sera from client-owned dogs and cats were obtained both prospectively and from stored specimens if a CALAS was ordered by the attending veterinarian and the assay was performed at the diagnostic laboratory at our institution. Any animals that required additional blood samples to be collected for the study were enrolled in a protocol approved by our Institutional Animal Care and Use Committee (protocol 20154). Dogs and cats were classified as CALAS-positive if they had a positive CALAS, with or without identification of *Cryptococcus* by fungal culture. Control animals (*Cryptococcus* antigen negative) were included if they had: (1) clinical signs suggestive of cryptococcosis (upper respiratory tract disease, ulcerative cutaneous lesions, chorioretinitis, neurological signs) but a negative CALAS and an alternate diagnosis that explained the clinical signs; (2) a definitive diagnosis of another systemic fungal disease; or (3) been previously treated for cryptococcosis and now tested negative using the CALAS. All serum specimens were collected from patients by routine venipuncture. With the exception of serum specimens from controls that had a diagnosis of coccidioidomycosis, sera were submitted for CALAS at the time of patient evaluation (ie, immediately) and remaining sera was stored at −20°C for up to 5 years until POC assays were performed. The serum specimens from dogs with a diagnosis of coccidioidomycosis as determined by positive *Coccidioides* serology by gel immunodiffusion at a single laboratory (Coccidioidomycosis Serology Laboratory, University of California, Davis, CA) were stored at −20°C for up to 4 weeks before CALAS and POC testing was performed. Information from the medical record or diagnostic laboratory submission forms was collected including clinical diagnosis, diagnostic tests performed and whether cytological or histopathological evidence of *Cryptococcus* infection was present. Complete medical records were not available for patients that had serum submitted to the diagnostic laboratory from veterinarians that practiced outside of our institution (38 CALAS-negative controls).

2.2 | CALAS

The CALAS was performed according to the manufacturer's protocol in a single veterinary diagnostic laboratory at our institution. The procedure included a pronase step (CALAS, IMMY, Norman, OK). Titers of ≥1:2 were considered positive.

2.3 | CrAg LFA POC assay

If sufficient serum volume was available, the CrAg LFA POC assay was given priority for additional testing after the CALAS. The CrAg

LFA POC is a qualitative assay and was performed by a single individual (KR) according to the manufacturer's instructions. Briefly, 40 μL of patient serum was mixed with 1 drop of the supplied LFA diluent in a microcentrifuge tube. The tip of the CrAg LFA strip was immersed in the serum/diluent mixture and incubated at room temperature for 10 minutes, after which the result was interpreted immediately. The test was considered valid only if the positive control line appeared. The presence of a test line indicated a positive result.

2.4 | CryptoPS POC assay

The CryptoPS POC assay was performed on any serum specimen for which adequate volume was available after the CrAg LFA POC assay had been performed. Because the CryptoPS assay required a lower volume of serum than the CrAg LFA POC assay, it was also performed on specimens that did not have adequate specimen volume for the CrAg LFA POC assay. The CryptoPS POC assay was performed by a single individual (KR) according to the instructions provided by the manufacturer. This test is a semi-quantitative assay that includes 2 test result lines (T1 and T2). The appearance of a line at T1 represents a positive result (limit of detection, 25 ng/mL of capsular antigen), and the appearance of both T1 and T2 lines represents a strong positive result. The limit of detection of capsular antigen at the T2 line is 2.5 ng/mL. Briefly, the test cassette was placed on a horizontal surface and 20 μL of serum was placed into the sample well of the cassette. Three drops of the supplied diluent then were added to the cassette sample well. The cassette was incubated for 10 minutes at room temperature, and then immediately interpreted. The test was considered valid only if a positive control line was present at 10 minutes.

2.5 | Statistical analysis

Data were analyzed using statistical software (Prism, GraphPad, San Diego, CA). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated with a 95% CI as compared to the reference standard CALAS or cytological diagnosis. Results were used to create receiver operator curves (ROC) and the area under the curve (AUC) was calculated for each diagnostic test. The ROC curves were compared using the DeLong method using the pROC R package (R version 3.5.1, Vienna, Austria). Sensitivity and specificity of each test was compared using a Fisher's exact test.

3 | RESULTS

3.1 | Dogs and cats

One hundred two serum specimens were collected from 51 dogs and 40 cats. For the CALAS-positive animals, there were 5 serum specimens from 3 dogs, and 20 serum specimens from 11 cats. More serum specimens were tested than animals because positive specimens collected at multiple time points (baseline and during treatment) were available from 1 dog and 5 cats. The 3 CALAS-positive dogs consisted of 2 dogs with central nervous system (CNS) cryptococcosis that both had *Cryptococcus* organisms identified on CSF cytology and 1 dog that had a final diagnosis of *Coccidioides* osteomyelitis based on concurrent radiographic changes, positive *Coccidioides* serology and positive response to treatment with no evidence of *Cryptococcus* infection and a CALAS titer of 1:8. The 11 CALAS-positive cats included 4 cats with CNS involvement, 4 with nasal involvement, 1 with mediastinal masses, 1 with disseminated disease that involved peripheral lymph nodes and 1 with a periocular abscess. For 6 of these 11 cats, the diagnosis of cryptococcosis also was supported by identification of the organism using culture, cytological or histopathological examination of tissue or body fluid specimens. Three of the remaining 5 cats had progressive decreases in their CALAS titer (titers 1:128 to 1:2, 1:256 to 1:2, and 1:128 to negative) after initiation of antifungal treatment. The remaining 2/5 CALAS-positive cats without culture or cytological confirmation of cryptococcosis had initial CALAS titers of 1:1024 and 1:2048. The CALAS titers for dog and cat CALAS-positive samples are shown in Figure 1.

There were 77 CALAS-negative (control) serum specimens from 48 dogs and 29 cats. Of the 48 dogs that were CALAS-negative, 35 had a diagnosis of an alternative infectious disease. Of these 35 dogs, 34 had coccidioidomycosis based on positive antibody serology and 1 had hepatic algal disease that was diagnosed using histopathology. Four additional dogs had CNS disease with CSF cytology performed in

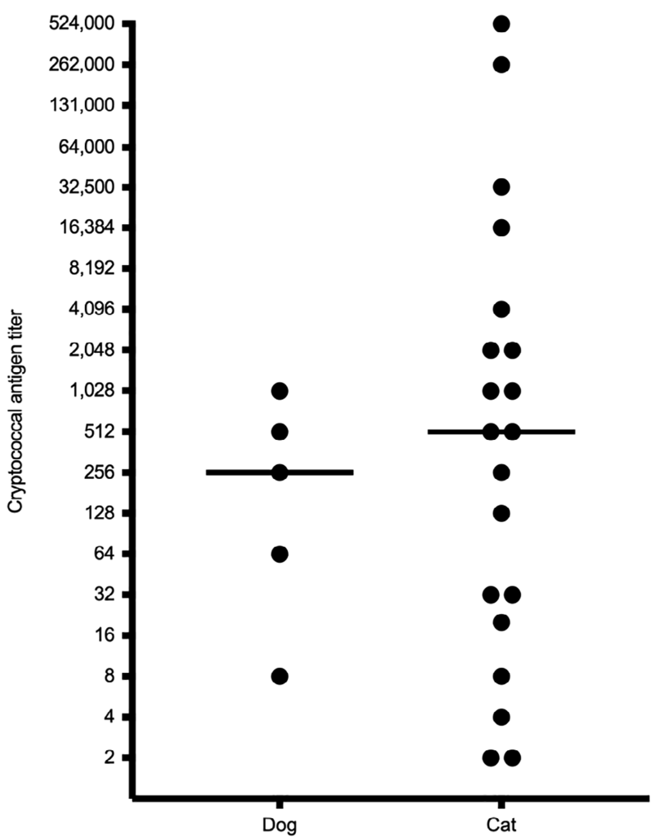

FIGURE 1 CALAS titers for sera classified as CALAS-positive. Scatterplot indicating the serum cryptococcal antigen titer as determined by CALAS all CALAS-positive dogs (n = 5) and cats (n = 21). The reciprocal titers on a logarithmic scale are present on the y-axis. The horizontal line represents the median titer

all dogs and magnetic resonance imaging (MRI) in 3/4 dogs. Diagnoses in these 4 dogs were steroid-responsive meningitis and arteritis (1) and inflammatory multifocal CNS disease (3). Two CALAS-negative dogs were diagnosed with optic neuritis that was suspected to be immune-mediated. Two CALAS-negative dogs had thoracic disease; 1 had dynamic airway disease based on videofluoroscopic examination and the other had idiopathic tracheobronchial lymphadenomegaly and also had negative *Coccidioides* serology. Two CALAS-negative dogs had been treated for cytologically confirmed cryptococcosis (both with ocular and CNS involvement) and now were in clinical remission. One CALAS-negative dog had CNS histiocytic sarcoma. One dog did not have medical records available for review.

Of the 29 CALAS-negative cats, 16 had upper respiratory tract signs that prompted CALAS testing. Eight of the 16 cats with upper respiratory signs had an open diagnosis that was suspected to be chronic rhinitis when CALAS testing was negative, however no confirmatory testing such as computed tomography (CT) or rhinoscopy was performed. One of the 16 was diagnosed with nasopharyngeal carcinoma based on rhinoscopy and nasal biopsy. Three had suspected nasal neoplasia based on CT findings (1), facial deformity (1), or absent nasal airflow (1); however, no biopsy specimens were obtained from these cats to confirm this diagnosis. One cat had chronic lymphoplasmacytic rhinitis based on rhinoscopy and biopsy. Two cats had nasal sporotrichosis as determined by fungal culture and the remaining cat had nasal aspergillosis diagnosed based on rhinoscopy, nasal biopsy, and fungal culture.

Of the remaining 13 CALAS-negative cats without respiratory signs, 4 had confirmed or suspected neoplasia; 2 with pulmonary masses and 2 with lymphoma based on cytologic examination of a laryngeal mass and a lesion on the nasal planum. Three had CNS disease including 1 cat each with idiopathic vestibular disease (based on normal contrast CT and CSF analysis), idiopathic inflammatory myelopathy (based on MRI and CSF analysis), and an extra-axial left parietal lobe mass (visualized using MRI). Ocular disease was described in 3 cats; 2 with idiopathic cranial uveitis and 1 with a retinopathy suspected to be secondary to ivermectin toxicosis. Two cats did not have medical records available for review and the final diagnosis was unknown. One cat had previous nasal cryptococcosis that was in remission based on clinical signs.

3.2 | CrAg LFA POC results

The CrAg LFA POC assay was performed on all 25 CALAS-positive serum specimens (Figure 2). Two CALAS-positive specimens tested negative with the CrAg LFA POC assay (Table 1). One was the previously mentioned dog with *Coccidioides* osteomyelitis. The other was a cat with CNS cryptococcosis that had a serum CALAS titer of 1:4096. A serum specimen obtained from this patient 1 month before that time had a CALAS titer of 1:2048 and a positive CrAg LFA POC result.

The CrAg LFA POC assay was performed on all 48 dog CALAS-negative specimens and 27 of 29 CALAS-negative specimens from cats (serum sample size was insufficient in 2 cats). Five CALAS-negative specimens tested positive with the CrAg LFA POC (Table 1). Two specimens were from animals with a previous diagnosis of cryptococcosis that had been treated with antifungal drugs and were in clinical remission (a cat with nasal cryptococcosis and a dog with ocular and CNS cryptococcosis). Both of these animals had infection confirmed at the time of initial diagnosis by fungal culture. The 3 remaining CALAS-negative, CrAg LFA-positive specimens were from 2 dogs with coccidioidomycosis (1 with pericardial effusion and suspected *Coccidioides* pericarditis based on positive *Coccidioides* serology and positive response to treatment and 1 dog with a positive *Coccidioides* titer of 1:16 for which medical records were not available for review) and the cat with idiopathic vestibular disease.

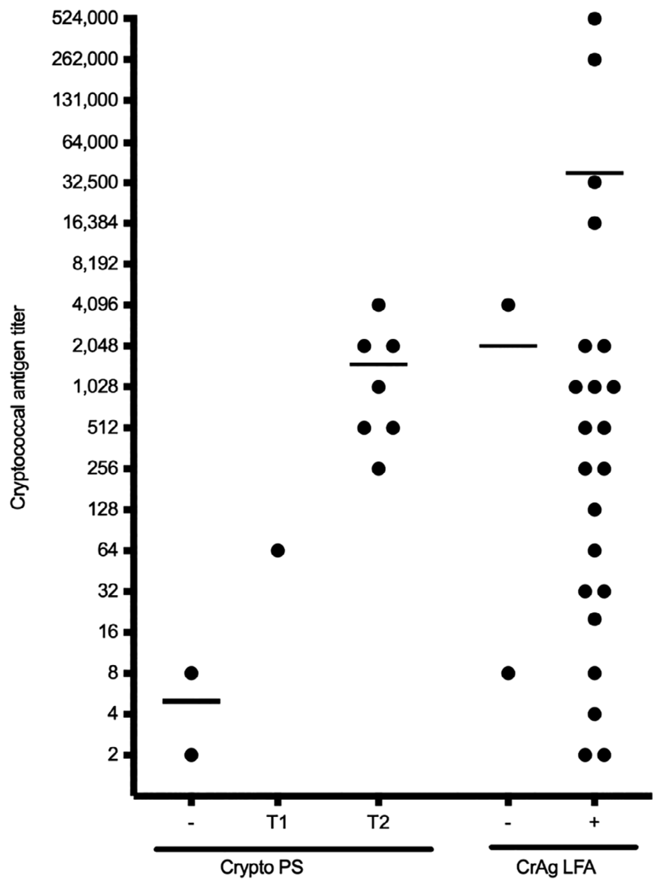

FIGURE 2 Results of CryptoPS and CrAg LFA assays for sera classified as CALAS-positive. Scatterplot indicating results of CryptoPS and CrAg LFA in all CALAS-positive animals. The serum cryptococcal antigen titer is represented in a logarithmic scale on the y-axis. Results of the point-of-care testing are represented on the x-axis and stratified into Crypto PS negative (–), positive (T1) or strong positive (T2), and CrAg negative (–) or positive (+). The horizontal line represents the median titer

When compared with the results of the CALAS assay, the CrAg LFA POC assay had a sensitivity 92.0% (95% CI, 75.0%-98.6%) and specificity of 93.2% (95% CI, 85.1%-97.1%). A ROC curve was constructed and had an AUC of 0.93 (95% CI, 0.86-0.99). The PPV in this population was 82.1% (95% CI, 64.4%-92.1%) and the NPV was 97.2% (95% CI, 90.3%-99.5%).

3.3 | CryptoPS POC results

The CryptoPS POC assay was performed on 10/25 CALAS-positive serum specimens (Figure 2). The assay was negative for 2 of the

TABLE 1 Discordant test results

Species	Clinical diagnosis	CALAS titer	CrAg LFA	CryptoPS	Classification
Dog	Coccidioidomycosis	1:8	–	–	FP CALAS
Cat	CNS cryptococcosis	1:4096	–	T2+	FN CrAg LFA
Cat	CNS cryptococcosis	1:2	+	–	FN CryptoPS
Dog	Coccidioidomycosis	–	+	T1+	Not determined
Dog	Cocci. osteomyelitis	–	–	T1+	FP Crypto PS
Dog	Cocci. osteomyelitis	–	+	–	FP CrAg LFA
Cat	Vestibular disease	–	+	NT	FP CrAg LFA
Cat	Nasal cryptococcosis	–	+	T1+	FN CALAS
Dog	CNS cryptococcosis	–	+	–	Not Determined

Abbreviations: –, negative test; +, positive test; FN, false-negative; FP, false positive; NT, not tested; T1+, weak positive; T2+, strong positive.

10 CALAS-positive specimens (Table 1), 1 from the dog with *Coccidioides* osteomyelitis (CALAS titer 1:8, negative CrAg LFA POC assay), and 1 from a cat being monitored during treatment for CNS cryptococcosis (CALAS titer 1:2, positive CrAg LFA POC). The cat had an initial serum CALAS titer of 1:256, however the serum sample volume was not sufficient to use the CryptoPS POC assay. Seven of the 8 positive CryptoPS POC results were strong positives with both T1 and T2 lines present, and 1 dog had a positive result with only the T1 line present.

The CryptoPS POC assay was performed on 42/48 CALAS-negative specimens from dogs and 17/29 CALAS-negative specimens from cats. Three CALAS-negative specimens were positive for the T1 line but not for the T2 line (Table 1). Two were from dogs with a diagnosis of coccidioidomycosis, which consisted of the dog with pericardial effusion and suspected *Coccidioides* pericarditis (positive CrAg LFA POC assay) and the dog with *Coccidioides* osteomyelitis of the right scapula based on radiographic changes, positive *Coccidioides* serology (1:32) and positive response to treatment (negative CrAg LFA POC assay). The other specimen was from a cat that had been treated for nasal cryptococcosis and was in clinical remission (positive CrAg LFA POC assay).

When compared with the CALAS, the sensitivity of the CryptoPS POC assay was 80.0% (95% CI, 49.0%-96.5%) and the specificity was 94.9% (95% CI, 86.0%-98.6%). An ROC curve was constructed and the AUC was 0.87 (95% CI, 0.72-0.99). In this population, the PPV was 72.7% (95% CI, 43.4%-90.3%) and NPV was 96.6% (95% CI, 88.3%-99.4%).

3.4 | Comparison of POC assays

When compared to each other, no statistically significant differences were noted in sensitivity ($P = .6$) or specificity ($P = .9$) between the 2 POC assays. Additionally, no statistically significant difference was observed between the ROC curves generated for the 2 POC assays ($P = .5$).

4 | DISCUSSION

Our study evaluated the performance of 2 POC cryptococcal antigen tests on sera collected from dogs and cats. The results of these POC assays were compared to those of the CALAS, the serologic reference standard for cryptococcosis for dogs and cats. The CALAS has been previously established as an accurate diagnostic tool in dogs and cats with a sensitivity of 95%-98% and specificity of 100% when compared to diagnosis by fungal culture or microscopic identification of *Cryptococcus* organisms in tissue fluids or biopsy specimens.[5,10,11] In this population, the CrAg LFA POC assay had a sensitivity of 92% and specificity of 93% whereas the CryptoPS POC assay had a sensitivity of 80% and specificity of 95% when compared to the CALAS results, with no significant differences in performance noted between the assays.

The CrAg LFA POC assay performance has been assessed in dogs, cats, and koalas in Australia and performance was similar to that reported here, with a sensitivity of 92% and 100% in cats and dogs, respectively and specificity of 81% and 84% in cats and dogs, respectively.[19,20] However, the results in our study differ from those found in people, where the CrAg LFA POC assay has a sensitivity of 100% whereas the CALAS had a sensitivity 91% in the same population when diagnosis of cryptococcosis was confirmed by culture, histopathologic, or molecular diagnosis.[14] The CryptoPS POC assay has a sensitivity of 100% and specificity of 98% in people when compared to the EIA.[21] These differences may be related to the choice of reference standard or because of differences in *Cryptococcus* infections between people and animals. Additionally, the CALAS performance may vary regionally because performance is based on prevalence of circulating molecular types and the extent to which these molecular types shed capsular antigen into body fluids.

Our objective was to compare the results of the POC assays to the results of the CALAS, not to determine the true sensitivity and specificity of these POC assays, which would require confirmation of cryptococcosis at minimum by light microscopic identification of the fungus and ideally by fungal culture. However, doing so would require collection of CSF or tissue specimens, which is invasive and expensive. In addition, fungal culture itself is costly relative to CALAS testing (> $200 per specimen at our institution compared with $50 for CALAS testing). Financial limitations therefore can affect the application of fungal culture as a reference standard in animals. Because of the established high sensitivity and specificity of the CALAS, the results of a CALAS assay generally can be relied upon in a clinical setting.[5,10,11] Nevertheless, we carefully scrutinized records for confirmation of the diagnosis whenever possible in order to attempt to determine whether discordant test results reflected inaccurate performance of the CALAS or POC assays. Eight of the 14 animals categorized as CALAS-positive had cryptococcosis confirmed with fungal culture or microscopic identification of *Cryptococcus* organisms on tissue or body fluid examination. Animals without a confirmed diagnosis of cryptococcosis in which the diagnosis was felt to be highly likely consisted of 3 cats that responded clinically to antifungal treatment in association with a subsequent decrease in the CALAS titer, and 2 cats with high CALAS titers and compatible clinical signs. Based on another study at our institution, the sensitivity of the CALAS was 100% in cats with titers >1:200.[5] In the present study, 1 dog with *Coccidioides* osteomyelitis had a positive CALAS (1:8) and negative POC tests, and thus likely represented a false positive CALAS assay.

One dog and 1 cat with a history of cryptococcosis that were in clinical remission had negative CALAS results but still had positive results on 1 (dog) or both (cat) POC assays. Antifungal treatment was discontinued in both of these animals after the negative CALAS results were obtained. No clinical signs recurred and CALAS remained negative at 1.5 years (dog) and 8 months (cat) after the last positive POC assay result. Similar findings have been reported in koalas where the CALAS became negative several months before the POC assay becoming negative, indicating that the POC tests may have increased sensitivity for antigenemia during treatment.[20] In another cat treated for CNS cryptococcosis, the CryptoPS had become negative when the CALAS remained weakly positive (1:2). Therefore, use of a combination of CALAS and POC assays may maximize sensitivity for detection of fungal antigen. Additional studies are required to determine whether or not discontinuation of treatment in animals that are CALAS-negative but positive using POC assays is premature and results in increased likelihood of clinical relapse.

Three dogs that were diagnosed with coccidioidomycosis had negative CALAS results but positive results on ≥1 of the POC assays. One of these dogs had *Coccidioides* osteomyelitis based on concurrent radiographic changes, positive *Coccidioides* titers and a positive response to treatment. One dog with pericardial effusion was suspected to have *Coccidioides* pericarditis based on positive serology and positive response to treatment. Complete medical records were not available for review for the third dog. The apparent false positive test results may represent the presence of shared (cross-reactive) antigens on these 2 fungal organisms as has been documented previously when false positive *Cryptococcus* serum immunofluoresence results were noted in serum specimens from people with coccidioidomycosis.[22] Cross-reactivity between *Trichosporon* and *Aspergillus* antigen and the CrAg LFA POC assay also has been documented in people, and dogs with cryptococcosis can test positive on *Aspergillus* galactomannan testing.[18,23,24]

One cat with cryptococcosis and a CALAS titer of 1:4096 had a negative CrAg LFA POC test and a positive CryptoPS POC test. Interestingly, serum from this cat collected at a previous time point when the CALAS was 1:2048 tested positive using both POC assays. The CrAg LFA POC false-negative result may have been caused by the prozone effect, as noted in people with high antigenemia.[25] With the prozone effect, fungal antigen binds in large quantities to the colloidal gold-labeled antibody in the assay, in turn preventing it from binding to the antigen complexed with immobilized antibody, leading to a false-negative.[26] However, in our study 4 other CALAS-positive specimens had higher CALAS titers with positive CrAg LFA POC results, indicating that other mechanisms also may have contributed to this false-negative result.

It is worth cautioning that whereas POC assays may be convenient for rapid testing of cats and dogs for cryptococcosis, testing large numbers of animals with common conditions such as upper respiratory tract disease in areas with low prevalence may result in poor PPV, and the potential for overdiagnosis of cryptococcosis. In our study population, PPV ranged from 73% for the CryptoPS and 83% for the CrAg, and thus false positive reactions may be frequent in some settings. The diagnosis of cryptococcosis in animals with positive POC test results should be confirmed using additional testing to ensure that animals are not unnecessarily treated with antifungal drugs, which are costly, must be administered for months to years, and have the potential to cause clinically important adverse effects.

The POC assays examined here provide rapid results with minimal requirement for technical expertise. Both tests require a laboratory pipette to add the appropriate volume of serum, and the CrAg LFA POC test requires use of microcentrifuge tubes, into which the serum specimen is placed and the test strip tip is immersed before incubation. The CryptoPS test is a cassette-based test that requires fewer steps and less manipulation of kit components, but both assays were straightforward to use.

The CryptoPS POC assay has the added benefit of being semi-quantitative with a test line that appears at 2.5 ng/mL of capsular antigen and a test line that appears at 25 ng/mL of capsular antigen. A correlation between CryptoPS semi-quantitative results and CALAS titers could not be performed because only 1 CALAS-positive sample had a T1 result whereas all others had a T2 result. Titration protocols are provided by the manufacturer for both POC assays whereby serial dilutions of a patient's serum specimen are reacted with the test kit, and the titer is determined as the highest dilution at which a positive test result is achieved. This may have prognostic value because higher antigen titers are correlated with worse outcomes in human patients.[27] Because of limited specimen volumes, titration protocols were not evaluated in our study.

The main limitation of our study was reliance on CALAS titers to categorize patients as cryptococcal antigen positive or negative, because of the need for exhaustive or invasive diagnostic tests to confirm a diagnosis of cryptococcosis using microbiologic or cytologic methods, which may have been declined by owners. In some situations, it was clear that the results of the POC tests more accurately identified animals with *Cryptococcus* infections than did the CALAS. The availability of a large number of control specimens from animals with a diverse range of confirmed non-*Cryptococcus* diagnoses for which CALAS testing had been performed would have strengthened the study. In addition, the control animals with alternative fungal infections had infections that were endemic in our geographical region (primarily coccidioidomycosis) and specificity of these POC tests may differ in other geographical regions where other fungal infections are endemic, such as blastomycosis and histoplasmosis. Another limitation of the study was the limited availability of sufficient volumes of serum to allow both POC assays to be performed.

The small sample size of patients with cryptococcosis also was a limitation of the study and precluded meaningful statistical analysis of cats and dogs separately. Previous reports have suggested higher sensitivity in dogs (100%) as compared to cats (92%) with the CrAg LFA POC and further evaluation of this finding is warranted.[19] Some of the CALAS-positive specimens in our study were from the same animal at several time points during the course of treatment and therefore were not completely independent of each other. This may have affected the statistical analysis.

In conclusion, the POC assays in our study were technically straightforward to perform and provided rapid results, with CrAg LFAPOC sensitivity of 92% and CryptoPS sensitivity of 80% and specificity approximately 95% for both assays when compared with the CALAS. Use of these assays may result in earlier diagnosis and treatment of animals with cryptococcosis. Additional, prospective studies are recommended that include a diverse range of controls with confirmed diagnoses from different geographic regions, as well as animals being treated for cryptococcosis.

CONFLICT OF INTEREST DECLARATION

Diagnostic test strips were a generous donation from IMMY and Biosynex, however these companies were not involved in the acquisition of data or the preparation of this manuscript.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

IACUC approval for the collection of additional sera from patients for diagnostic assay.

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

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