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Low *Cryptococcus* Antigen Titers as Determined by Lateral Flow Assay Should Be Interpreted Cautiously in Patients without Prior Diagnosis of Cryptococcal Infection

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ABSTRACT Detection of *Cryptococcus* antigen (CrAg) is invaluable for establishing cryptococcal disease. Multiple different methods for CrAg detection are available, including a lateral flow assay (LFA). Despite excellent performance of the CrAg LFA, we have observed multiple cases of low-titer (≤1:5) positive CrAg LFA results in patients for whom cryptococcosis was ultimately excluded. To investigate the accuracy of low-titer positive CrAg LFA results, we performed chart reviews for all patients with positive CrAg LFA results between June 2014 and December 2016. During this period, serum and/or cerebrospinal fluid (CSF) samples from 3,969 patients were tested with the CrAg LFA, and 55 patients (1.5%) tested positive. Thirty-eight of those patients lacked a history of cryptococcal disease and were the focus of this study. Fungal culture or histopathology confirmed *Cryptococcus* infection for 20 patients (52.6%), and CrAg LFA titers in serum and CSF samples ranged from 1:5 to ≥1:2,560. For the 18 patients (47.4%) without culture or histopathological confirmation, the CrAg LFA results were considered true-positive results for 5 patients (titer range, 1:10 to ≥1:2,560), due to clinical improvement with targeted therapy and decreasing CrAg LFA titers. The remaining 13 patients had CrAg LFA titers of 1:2 (n = 11) or 1:5 (n = 2) and were ultimately diagnosed with an alternative condition (n = 11) or began therapy for possible cryptococcosis without improvement (n = 2), leading to an overall CrAg LFA false-positive rate of 34%. We recommend careful clinical correlation prior to establishing a diagnosis of cryptococcal infection for patients with first-time positive CrAg LFA titers of 1:2.

KEYWORDS *Cryptococcus*, antigen, lateral flow assay

Cryptococcus species are encountered worldwide and are most often associated with causing opportunistic invasive fungal disease in immunosuppressed individuals, particularly patients with HIV/AIDS. While the global burden of cryptococcal meningitis in this patient population remains high, with nearly 1 million cases annually, the use of highly active antiretroviral therapy (HAART) has reduced the incidence of cryptococcal meningitis in the United States to less than 1.3 cases per 100,000 individuals (1, 2). Cryptococcal infections can also lead to significant morbidity and death among patients with defective cellular immunity, solid organ transplant recipients, and individuals receiving prolonged high-dose corticosteroid therapy (3). Certain species of *Cryptococcus* have also been associated with significant disease in otherwise healthy individuals without underlying comorbidities (4). Prompt identification of cryptococcal disease and initiation of targeted antifungal therapy are essential for patient survival,

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although the mortality rate for acute cryptococcal meningoencephalitis remains approximately 20% in HIV/AIDS patients (5).

Members of the *Cryptococcus* genus have recently undergone taxonomic revision, with the multiple *Cryptococcus neoformans* and *Cryptococcus gattii* serotypes and molecular types now classified as seven unique species (6). While fungal culture remains the reference method for detection of these encapsulated yeast, isolation from clinical specimens requires several days of incubation, which may delay the diagnosis. Identification of *Cryptococcus* yeast by histopathology is also an important diagnostic approach. However, the invasive procedures necessary to collect preferred specimens (e.g., lung tissue) for histopathology are often contraindicated for severely ill patients (7). Direct microscopy, particularly of cerebrospinal fluid (CSF), is also routinely performed but is hampered by low sensitivity, primarily for patients with low fungal burdens (8). Due to these limitations, detection of *Cryptococcus* antigen (CrAg) in serum and CSF samples has emerged as an invaluable tool for the diagnosis of cryptococcal disease.

Multiple assays have been developed and FDA approved for detection of the capsular glucoronoxymannan polysaccharide of *Cryptococcus* species, including a CrAg latex agglutination system (CALAS) (Meridian Bioscience, Cincinnati, OH) and a CrAg lateral flow assay (LFA) (IMMY, Norman, OK). The CrAg LFA offers a number of advantages over the CALAS, including enhanced sensitivity for detection of CrAg and a rapid turnaround time of approximately 15 min; in addition, the assay does not require pronase pretreatment of serum samples (9–11). Although both assays provide endpoint CrAg titers, these semiquantitative results are not directly comparable between the tests, due to the different methodologies used.

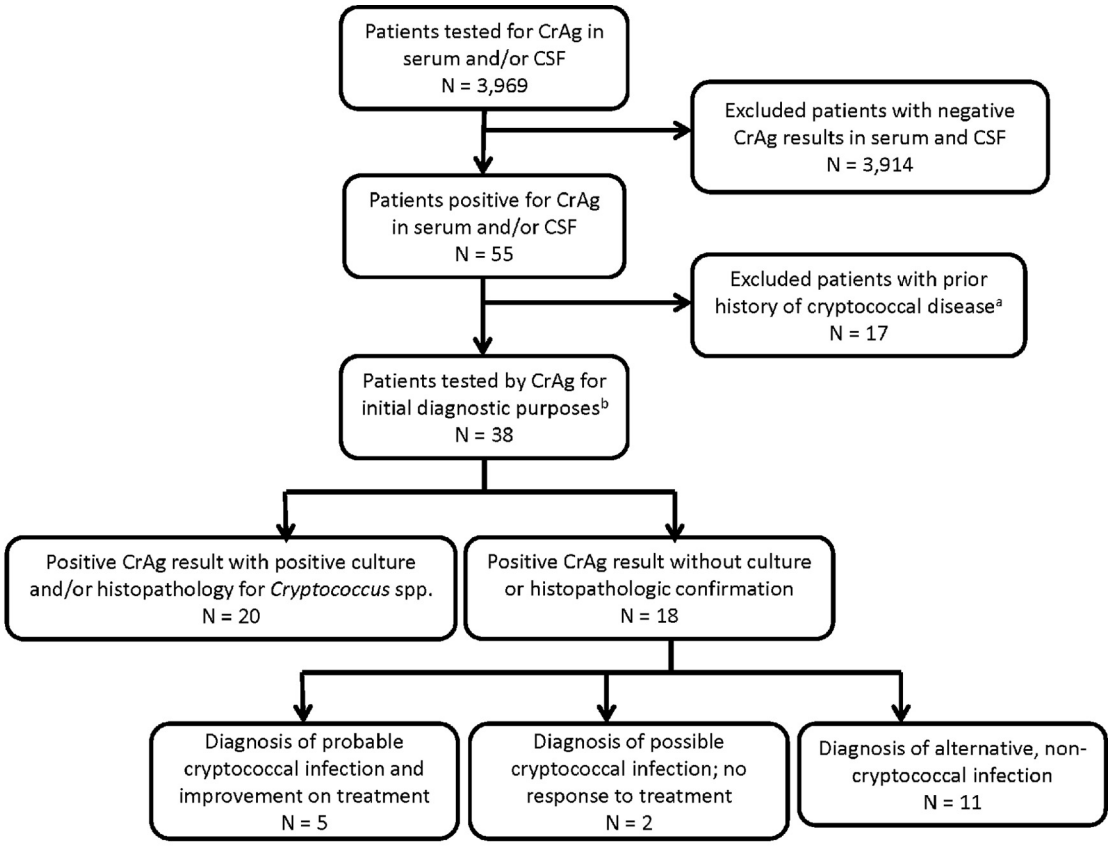
The CrAg LFA was implemented in our laboratory in 2014, replacing the CALAS for detection of CrAg in both serum and CSF samples. Since transitioning to the CrAg LFA, we have observed a number of patients with low CrAg titers ($\leq$1:5) for whom the results did not correlate with the clinical presentation or final diagnosis. In an effort to investigate the accuracy of these low-titer positive CrAg LFA results, we performed chart reviews for all patients with positive CrAg LFA results between June 2014 and December 2016.

RESULTS

Study population. Between 1 June 2014 and 31 December 2016, the CrAg LFA was performed with 4,627 serum or CSF samples collected from 3,969 patients. Among those patients, 3,914 (98.6%) were negative for CrAg in all specimens and were excluded from this study (Fig. 1). Based on chart reviews, 17 (30.9%) of the 55 patients with positive CrAg LFA results in serum and/or CSF samples had been previously diagnosed with cryptococcal infections and testing had been ordered to monitor responses to treatment; therefore, those patients were also excluded from the study. The remaining 38 patients (69.1%) with positive CrAg LFA results lacked a history of cryptococcal infection, and CrAg testing had been ordered for initial diagnostic purposes. Those 38 patients were the focus of this study, and in-depth chart reviews were performed by one of the authors (E.S.T.).

Among the 38 patients, *Cryptococcus neoformans* was isolated in culture or identified by histopathology (to the genus level) for 20 patients (52.6%), confirming the positive CrAg LFA results (Fig. 1). Of the 18 patients (47.4%) without culture or histopathological evidence of *Cryptococcus* infection, 5 were diagnosed with probable cryptococcal infections and showed improvement with targeted antifungal treatment. Two of the remaining 13 patients were diagnosed with possible cryptococcal infections but did not show improvement with antifungal treatment, and an alternative diagnosis was established for the remaining 11 patients (Fig. 1).

CrAg LFA titers for patients with confirmed *Cryptococcus* infections. A definitive diagnosis of cryptococcal infection, based on isolation of *C. neoformans* in culture and/or identification by histopathology, was established for 20 of the 38 patients tested with the CrAg LFA for initial diagnostic purposes (Table 1). Those patients ranged in age



[a] CrAg ordered to monitor treatment response and disease progression.
[b] No prior history of cryptococcal disease

FIG 1 Summary of patients for whom serum and/or CSF samples were tested with the CrAg LFA between 1 June 2014 and 31 December 2016.

from 30 to 88 years (median, 58 years) and were predominantly male ($n = 17$). *C. neoformans* was recovered most frequently from CSF ($n = 10$), followed by respiratory fluid ($n = 4$), blood ($n = 2$), tissue ($n = 3$), and urine ($n = 1$), and was identified by histopathology in a single case. The median time to culture positivity was 2 days (range, 1 to 7 days). Among the 10 patients with confirmed cryptococcal meningitis, the CrAg LFA titers for CSF samples ranged from 1:20 to ≥1:2,560 (median, 1:1,280). Eight of those patients also had a paired serum sample drawn near the time of CSF sample collection (typically within 24 h), and CrAg LFA titers for the paired serum samples ranged from 1:2 to ≥1:2,560 (median, 1:160). Among the 10 patients for whom *C. neoformans* was detected from sources other than CSF, serum CrAg LFA titers ranged from 1:5 to ≥1:2,560 (median, 1:40). Sufficient specimen volume was available to perform CALAS testing for 18 patients and, with the exception of a single case, the CALAS results were positive for all specimens that had corresponding CrAg LFA titers of ≥1:20. Notably, the CALAS results were negative for all specimens collected from 2 patients with culture-confirmed cryptococcal infections (Table 1, patients 1 and 3).

CrAg LFA titers for patients without confirmed cryptococcal infections. *Cryptococcus* spp. were not recovered in culture or identified by histopathology for 18 (47.4%) of the 38 patients with positive CrAg LFA results who were tested for initial diagnostic purposes (Table 2). Those patients ranged in age from 33 to 88 years (median, 61 years) and were predominantly male ($n = 11$). Among those 18 patients, the CrAg LFA result was considered to be a true-positive result for 5 patients (27.8%) (patients 1 to 5), based on (i) clinical and radiological features consistent with cryptococcal infection, (ii) observed decreases in serial CrAg LFA titers, and (iii) documented clinical improvement with anticryptococcal therapy. Four (80%) of those 5 patients were diagnosed with

TABLE 1 CrAg LFA and CALAS titers for patients with first-time diagnoses of cryptococcal disease confirmed by culture or histopathology ($n = 20$)

Patient no.	Age (yr)/sex	Source for fungal culture	Time to growth (days)	Source for CrAg assay[a]	CrAg LFA titer	CALAS titer
1	88/M	CSF	4	CSF	1:20	Negative
				Serum	ND	ND
2	70/M	CSF	2	CSF	≥1:2,560	≥1:4,096
				Serum	1:160	1:32
3	67/M	Bronchial wash	7	Serum	1:5	Negative
4	78/F	Bronchial wash	1	Serum	1:40	1:16
5	73/M	Blood	2	Serum	≥1:2,560	≥1:4,096
6	78/M	Blood	2	Serum	≥1:2,560	ND
7	76/M	CSF	2	CSF	≥1:2,560	1:2,048
				Serum	≥1:2,560	1:1,024
8	57/M	CSF	2	CSF	≥1:2,560	1:128
				Serum	1:160	1:32
9	50/M	BAL fluid	3	Serum	1:20	1:4
10	72/M	CSF	2	CSF	≥1:2,560	1:512
				Serum	1:640	1:64
11	71/M	CSF	7	CSF	≥1:2,560	1:1,024
				Serum	ND	ND
12	61/M	CSF	2	CSF	1:20	1:1
				Serum	1:20	1:4
13	63/M	Urine	7	Serum	1:40	1:8
14	39/M	CSF	2	CSF	1:640	1:64
				Serum	1:2	Negative
15	48/F	Calf tissue	5	Serum	≥1:2,560	1:1,024
16	58/F	CSF	3	CSF	1:160	1:16
				Serum	1:160	1:64
17	43/M	CSF	2	CSF	1:320	1:128
				Serum	≥1:2,560	1:128
18	58/M	Lung tissue	2	Serum	1:20	1:16
19	52/M	Lung tissue[b]	NA	Serum	1:10	1:2
20	30/M	Sputum	2	Serum	≥1:2,560	ND

[a]CrAg, *Cryptococcus* antigen; ND, not done; NA, not applicable; BAL, bronchoalveolar lavage; LFA, lateral flow assay.
[b]Organisms consistent with *Cryptococcus* spp. were observed in the histopathological assessment of a lung biopsy specimen, using both Gomori methenamine silver (GMS) and mucicarmine stains.

probable cryptococcal pulmonary infections and had CrAg LFA titers ranging from 1:10 to 1:1,280. Interestingly, the CALAS results were negative for 2 of those 4 patients. The fifth patient was found to be HIV positive at the time of presentation ($CD4^+$ cell count, 48 cells/mm^3) and was diagnosed with cryptococcal meningitis, with a CrAg LFA titer of ≥1:2,560 in CSF.

Two of the 18 patients were diagnosed with possible *Cryptococcus* infections (Table 2, patients 6 and 7). The first patient was status post liver transplant in 2004 and presented with fever, rigors, and anorexia. This patient was diagnosed with possible cryptococcal granulomatous hepatitis, based on liver biopsy findings showing granulomas with central necrosis (negative for fungal organisms) and a CrAg LFA serum titer of 1:2. CALAS results for this specimen and repeat CrAg LFA results for a separate serum specimen (collected 2 days later) were both negative. The second patient presented with fever and tremors, and imaging studies revealed a few punctate lung nodules; the CrAg LFA titer was 1:5 in serum, and the CALAS results were negative. Both patients began fluconazole therapy without clinical improvement, and an alternative diagnosis was not established for either patient.

For the remaining 11 patients who were positive by the CrAg LFA but were negative for *Cryptococcus* spp. by culture and/or histopathology, cryptococcal disease was excluded and an alternative diagnosis (either infectious or noninfectious) was ultimately established (Table 2). For 10 of those 11 patients, the CrAg LFA endpoint titer was 1:2; the 11th patient showed a titer of 1:5 in CSF. CALAS testing was performed in 10 of those cases, and the results were negative in each of them. Serial CrAg LFA testing was performed for 7 of the 11 patients, and results either were negative ($n = 5$), remained unchanged at a titer of 1:2 ($n = 1$), or were repeated at a titer of 1:2 and

TABLE 2 Review of data for patients tested by the CrAg LFA without culture or histopathological confirmation of cryptococcal disease ($n = 18$)[a]

Patient no.	Age (yr)/ sex	Presenting symptom(s)	Comorbidity/ immunosuppression	Radiological findings	Culture source	CrAg LFA titer (source)	CALAS titer (source)	Final diagnosis	Antifungal treatment initiated/response?	Repeat CrAg LFA titer (days between tests)[b]
1	33/M	Chronic dry cough	S/p kidney Tx (2006); tacrolimus and mycophenolate	Bilateral innumerable small lung nodules	Blood, CSF	1:10 (S)	Neg. (S)	Probable cryptococcal pulmonary infection	Fluconazole/yes	1:10 (3)
2	80/F	Dyspnea, weight loss, fatigue	Idiopathic pulmonary fibrosis	Ground glass lung opacities	Blood, CSF	1:10 (S)	Neg. (S)	Probable cryptococcal pulmonary infection	Fluconazole/yes	1:2 (96), 1:2 (111)
3	60/F	SOB, fever, HA, chronic cough	S/p kidney Tx (2010), DM2; tacrolimus	Bilateral nodular lung opacities	Blood	1:1,280 (S)	1:256 (S)	Probable cryptococcal pneumonia	Fluconazole and flucytosine/yes	1:640 (13), 1:80 (153)
4	74/M	Cough, weight loss, fatigue, fever	Autoimmune hemolytic anemia; high-dose prednisone	Cavitary lesion in right upper lobe	Blood	1:40 (S)	ND	Probable cryptococcal pulmonary infection	Fluconazole/yes	1:40 (30), 1:20 (123), neg. (10 mo)
5	53/M	Severe HA, neck pain, fever, photophobia	Newly diagnosed with HIV (48 $CD4^+$ cells/mm^3)	None	CSF	≥1:2,560 (CSF)[c]	≥1:4,096 (CSF)	Cryptococcal meningitis	Amphotericin B and flucytosine/yes	≥1:2,560 (CSF, 34)
6	68/M	Fever, rigors, anorexia	S/p liver Tx (2004), IBD; tacrolimus	None	Blood, CSF	1:2 (S)	Neg. (S)	Possible cryptococcal granulomatous hepatitis	Fluconazole/no	Neg. (2)
7	73/M	Tremors, fever	Monoclonal gammopathy, microscopic anemia	Few punctate lung nodules	Blood	1:5 (S)	Neg. (S)	Possible subacute cryptococcal infection, Waldenström's macroglobulinemia	Fluconazole/no	ND
8	85/M	Hematuria, back pain	S/p aortic valve replacement	None	Blood, CSF, spinal tissue, urine	1:2 (S)	Neg. (S)	*Pseudomonas aeruginosa* UTI, *Streptococcus bovis* spinal infection	No/NA	1:2 (1)
9	51/F	Fever, abdominal pain	S/p liver Tx (2004); azathioprine and tacrolimus	None	Blood, urine	1:2 (S)	Neg. (S)	Large diffuse B-cell lymphoma	Fluconazole/no	1:2 (2), neg. (75)
10	42/F	Weight loss, fatigue, ARDS at admission	Congenital urinary/ lower GI abnormalities	None	Blood	1:2 (S)	Neg. (S)	ARDS secondary to adrenal insufficiency	No/NA	Neg. (2)
11	61/F	Foot ulcer, hemodynamic instability	Rheumatoid arthritis, DM2, ESRD with hemodialysis; etanercept	None	Blood, hip tissue	1:2 (S)	Neg. (S)	Cellulitis and contiguous osteomyelitis due to *Staphylococcus aureus* and *Streptococcus pyogenes*	No/NA	ND
12	46/M	Fever, hip pain	Total hip arthroplasty	Bilateral lung nodules/opacities	Blood	1:2 (S)	Neg. (S)	*Candida albicans* bloodstream infection	Caspofungin for *C. albicans* infection/yes	ND
13	88/F	Gait disturbance, difficulty swallowing	None	Enlargement of left lateral pons	CSF	1:2 (CSF)[c]	Neg. (CSF)	Glioma	No/NA	ND
14	61/M	SOB, cough	S/p allogeneic PBSC Tx (2015)	Bilateral lung consolidation and septal thickening	Blood, BAL fluid, CSF	1:2 (S)	Neg. (S)	Pulmonary GVHD	Amphotericin B, switched to fluconazole/no	Neg. (3)
15	43/F	Right upper extremity weakness	None	Frontal lobe brain atrophy	CSF	1:5 (CSF), neg. (S)	Neg. (CSF), ND (S)	Corticobasal degeneration	No/NA	Neg. (S, 1)
16	88/M	Progressive neurological symptoms	Coronary artery disease, lumbar fusion	None	Blood, CSF	1:2 (S)	Neg. (S)	Cauda equina nerve root enhancement	No/NA	ND
17	75/M	Fever	Gout, hypothyroidism	Bilateral small lung nodules	Blood	1:2 (S)	ND	Resolution with empiric levofloxacin	No/NA	Neg. (5)
18	60/M	SOB, chronic cough, fatigue, fever	Adenocarcinoma s/p chemoradiation (2016), HCV s/p ledipasvir (2016)	Cavitary lung lesion, ground glass opacities	Blood	1:2 (S)	Neg. (S)	Pneumonia due to *Aspergillus fumigatus* and *Acinetobacter baumannii/ Acinetobacter calcoaceticus*	Fluconazole (discontinued)/no	Neg. (22)

[a]S/p, status post; S, serum; UTI, urinary tract infection; BAL, bronchoalveolar lavage; NA, not applicable; ND, not done; neg., negative; Tx, transplant; ARDS, acute respiratory distress syndrome; GI, gastrointestinal; IBD, irritable bowel syndrome; PBSC, peripheral stem cell; GVHD, graft-versus-host disease; HA, headache; SOB, shortness of breath; DM2, diabetes mellitus type 2; ESRD, end-stage renal disease; HCV, hepatitis C virus.
[b]Indicates the time between the initial CrAg LFA result and the repeat test(s) ordered as part of clinical care.
[c]Serum samples were not evaluated for CrAg.

subsequently became negative ($n = 1$). Targeted anticryptococcal therapy was initiated for 3 of the 11 patients without noted clinical improvement. The 8 patients for whom antifungal treatment was not initiated did not show laboratory or clinical evidence of cryptococcal disease at the time of chart review, performed 5 to 33 months following the initial positive CrAg LFA result. Overall, 13 patients were considered to have false-positive CrAg LFA results, leading to an overall false-positive rate among patients tested for initial diagnostic purposes of 34% (13/38 patients).

DISCUSSION

The aim of this retrospective study was to evaluate the accuracy of low-titer (≤1:5) positive CrAg LFA results for patients without a history of cryptococcal disease. Our review revealed that, for patients with culture- or histopathology-confirmed *Cryptococcus* infections, the first-time positive CrAg LFA titers in CSF or serum ranged from 1:5 to ≥1:2,560. Notably, the lowest CrAg LFA titer observed in CSF from patients with culture-confirmed cryptococcal meningitis was 1:20. In all but one case of confirmed cryptococcal disease outside the central nervous system, the CrAg LFA titers were ≥1:10. We also identified 5 patients without confirmed cryptococcal disease who were diagnosed with probable cryptococcosis based on clinical presentations consistent with cryptococcal pneumonia or meningitis and who responded to antifungal therapy, with concomitant decreases in serial CrAg LFA levels. The initial CrAg LFA titers for those 5 patients ranged from 1:10 to ≥1:2,560, similar to the range observed for patients with confirmed cryptococcal infections. In contrast, all 11 patients with initial CrAg LFA titers of 1:2 and 2 of the 3 patients with first-time titers of 1:5 either had an alternative diagnosis established ($n = 11$) or did not respond to anticryptococcal treatment ($n = 2$). Importantly, none of those 13 patients had developed cryptococcal disease at the time of chart review. Based on these results, we recommend that patients without a history of cryptococcosis who have first-time CrAg LFA titers of 1:2 be evaluated by repeat testing of a new specimen and the results correlated with other clinical and laboratory findings prior to establishing a diagnosis of cryptococcal infection.

Our data support those from previously published studies documenting the enhanced sensitivity of the CrAg LFA over the CALAS (11, 12). Four patients in our cohort with either culture-confirmed *C. neoformans* infections ($n = 2$) or probable cryptococcal pulmonary disease ($n = 2$) were positive by the CrAg LFA (titer range, 1:5 to 1:20) but negative by the CALAS. However, for patients with initial CrAg LFA titers of 1:2, and potentially for patients with first-time CrAg LFA titers of 1:5, results should be evaluated and interpreted with caution. Inaccurate diagnosis of cryptococcal disease based on such low-titer positive CrAg LFA results may lead to missed diagnosis of an alternative condition that is possibly treatable or unnecessary initiation of antifungal therapy. In this study, 5 patients with initial CrAg LFA titers of 1:2 or 1:5 began either fluconazole or amphotericin B therapy without clinical improvement, and alternative diagnoses were ultimately established for 3 of those patients.

Prior studies reported that false-positive CrAg results by the CALAS or other latex agglutination assays may occur for patients with *Capnocytophaga canimorsus* (previously referred to as CDC group DF-2), *Stomatococcus mucilaginosus*, or *Trichosporon* infections, for patients with systemic lupus erythematosus, or for samples transported in anaerobic vials (13–18). Importantly, reports of false-positive results by the CrAg LFA are rare, with only a single study documenting false-positive CrAg LFA results for 2 patients with disseminated *Trichosporon asahii* fungemia (19). None of the 13 patients in our study with suspicious CrAg LFA titers of 1:2 or 1:5 had a *Trichosporon* infection. Interestingly, near the end of this study, the manufacturer issued a recall of three CrAg LFA kit lots due to reduced assay specificity; however, none of those lots was used in our laboratory during the study period. Finally, test accuracy is significantly influenced by the pretest probability of disease, with false-positive results being more likely to occur in low-incidence settings. In this study, we report an overall CrAg positivity rate of 1.4% (55/3,969 patients), with a prevalence of new-onset cryptococcal disease of approximately 0.6% (25/3,956 patients) over the 2.5-year study period. To maintain high

assay specificity, testing with the CrAg LFA should be reserved for patients who are at risk for and present with symptoms consistent with cryptococcal infection.

This study has a number of limitations that should be discussed. First, despite the inclusion of all Mayo Clinic patients who tested positive by the CrAg LFA during a 2.5-year period, only 38 patients met our inclusion criteria; therefore, the conclusions that can be drawn are limited. Second, despite in-depth chart reviews for the 13 patients with low-titer positive CrAg LFA results, a common explanation for the inaccurate CrAg LFA results was not identified. It is important to note, however, the manual and subjective nature of this assay, including both performance and visual assessment for the presence or absence of reactivity with the LFA. In an effort to minimize this, our laboratory routinely repeats samples with initial CrAg LFA endpoint titers of 1:2, in order to confirm the results, before the report is released. Ultimately, additional studies are needed to clarify the cause of low-titer positive CrAg LFA results and to better assess the impact of these results on patient care.

In conclusion, we report that, among patients tested with the CrAg LFA for initial diagnostic purposes, 34% (13/38 cases) of all positive results were considered falsely positive, with semiquantitative titers ranging between 1:2 and 1:5. We show that, with the exception of a single case, all patients with confirmed cryptococcal meningitis or disseminated disease in our study had CrAg LFA titers of $\geq$1:10. For patients with first-time positive CrAg LFA endpoint titers of 1:2, we recommend consideration of repeat CrAg LFA testing with a new specimen and we continue to urge caution in interpretation of low-titer positive results. Correlation of the results with clinical findings and other laboratory data prior to establishing a diagnosis of cryptococcal infection will very likely be required.

MATERIALS AND METHODS

Study design. All patients who were tested with the IMMY CrAg LFA at the Mayo Clinic (Rochester, MN) between June 2014 and December 2016 were identified through query of the laboratory information system. During this time period, all specimens that were positive by the CrAg LFA were frozen and tested with the CALAS (Meridian Bioscience, Inc., Cincinnati, OH) within 1 week after original sample collection. Chart reviews were performed retrospectively for all patients reported as positive by the CrAg LFA, using the European Organization for Research and Treatment of Cancer (EORTC)/Mycoses Study Group (MSG) criteria for invasive fungal infections as guidance (7). Specifically, patient charts were assessed to determine patient demographic characteristics, presentation at the time of testing, reason for CrAg testing (i.e., initial diagnostic purposes versus monitoring of the response to anticryptococcal therapy), immune status, comorbidities, radiological findings, other microbiological laboratory data (e.g., culture, PCR, and/or serological results), final diagnosis, and antifungal treatment. For the purposes of this study, a positive CrAg LFA result was considered true if one of the following criteria was met: (i) a *Cryptococcus* species was recovered in culture from any specimen source, (ii) a *Cryptococcus* species was histopathologically identified in any specimen, or (iii) the patient responded to targeted antifungal therapy with concomitant decreases in serial CrAg LFA titers. This study was approved by the Mayo Clinic Institutional Review Board.

IMMY CrAg LFA. The IMMY CrAg LFA is FDA cleared for use with serum and CSF specimens, and samples were collected, stored, and tested according to the manufacturer's instructions. Briefly, serum and CSF specimens were first screened for the presence of CrAg by preparing a 1:2 dilution of the specimen in specimen diluent. CrAg LFA strips were added to the diluted sample, incubated for 10 min, and visually evaluated for the presence of a test band and a control band. The presence of both bands indicates a positive result; the presence of a single control band indicates a negative result. A semiquantitative procedure was performed for all samples that tested positive at the initial 1:2 screening dilution. Briefly, specimens were diluted to a 1:5 dilution in specimen diluent, followed by serial 2-fold dilutions up to 1:2,560, and were tested as indicated above. The highest dilution that yielded a positive result was reported as the endpoint titer. If the 1:5 dilution tested negative, then the endpoint titer was reported as 1:2.

Meridian CALAS. The Meridian Bioscience CALAS is FDA cleared for use with serum and CSF specimens, and testing was performed according to the manufacturer's instructions. Briefly, serum was treated with pronase, incubated at 56°C for 15 min, and boiled for 5 min. CSF was inactivated by boiling for 5 min. Specimens were cooled prior to use and were screened by the CALAS at a 1:2 dilution for serum or undiluted for CSF. Serum or CSF samples with agglutination reactions of 2+ or stronger were serially diluted, using 2-fold dilutions up to 1:4,096, to determine an endpoint titer.

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