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# Comparison of Four Assays for the Detection of Cryptococcal Antigen

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We compared the performance of four assays for the detection of cryptococcal antigen in serum samples ( $n = 634$ ) and cerebrospinal fluid (CSF) samples ( $n = 51$ ). Compared to latex agglutination, the sensitivity and specificity of the Premier enzyme immunoassay (EIA), Alpha CrAg EIA, and CrAg lateral flow assay (LFA) were 55.6 and 100%, 100 and 99.7%, and 100 and 99.8%, respectively, from serum samples. There was 100% agreement among the four tests for CSF samples, with 18 samples testing positive by each of the assays.

Cryptococcal disease is rare in the United States with an estimated incidence of 0.4 to 1.3 cases per 100,000 people; however, the incidence is much higher in the immunosuppressed population (e.g., HIV/AIDS) where it can reach 7 cases per 1,000 people (2). Importantly, the disease continues to have a stunning impact on individuals with HIV/AIDS in sub-Saharan Africa where more than 1 million cases of cryptococcal meningitis are diagnosed each year.

Due to the high morbidity and mortality associated with cryptococcal infection, especially in the developing world, there is a need for rapid and accurate laboratory tests to identify infected persons. Traditionally, the laboratory diagnosis of cryptococcosis has been established using fungal culture, direct microscopy of clinical samples, or detection of cryptococcal antigen in serum or cerebrospinal fluid (CSF). Fungal culture demonstrates high sensitivity and specificity for *Cryptococcus* spp., but recovery of the organism from clinical samples may take several days and accurate identification of *Cryptococcus* spp. from culture isolates requires trained laboratory personnel. Direct microscopy of clinical samples offers a rapid diagnostic approach but lacks sensitivity and specificity. Therefore, detection of cryptococcal antigen, which is rapid, sensitive, and specific, has become a mainstay in the diagnosis of this infection (1, 4, 5).

Common laboratory methods for the detection of cryptococcal antigen include latex agglutination (LA) test and enzyme immunoassay (EIA), both of which are available in commercial, FDA-approved formats that have demonstrated comparable performance (1, 8). The latex agglutination test has been considered the gold standard method in past studies (1, 3), but this approach is manual and subjective and has a low testing throughput. To overcome the limitations of LA test for cryptococcal antigen screening, many reference laboratories in the United States have implemented EIAs, which allow for automation and an objective interpretation of results. EIA screen-reactive samples are then tested by LA test to determine an endpoint titer, which is used to determine disease severity and monitor a patient's response to therapy. Recently, a novel lateral flow assay (LFA) (Immuno-Mycologics [IMMY], Norman, OK) was developed; this assay allows for the detection of cryptococcal antigen in <15 min. This assay has gained FDA approval for serum and CSF samples and offers promise as a point-of-care test in both the United States and resource-limited settings (3, 6).

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In order to evaluate the performance of available methods for the detection of cryptococcal antigen, we tested serum samples ( $n = 634$  [632 prospective, 2 archived]) and CSF samples ( $n = 51$  [all archived]) by each of the following assays: Premier EIA (Meridian Biosciences, Cincinnati, OH), Cryptococcal Antigen Latex Agglutination System (CALAS; Meridian Biosciences), CrAg LFA (IMMY), and Alpha CrAg EIA (IMMY). Each of these methods is FDA approved, except the Alpha CrAg EIA, which was labeled "for investigational use only" at the time of this evaluation but is currently under review at the FDA. All testing was performed in a blinded fashion according to the manufacturers' package inserts. Statistics were calculated using GraphPad QuickCalcs (GraphPad Software, Inc., La Jolla, CA) with categorical data analysis to assess confidence intervals of proportion, overall percent agreement, and kappa ( $\kappa$ ) coefficients.

Compared to LA, which is currently used in our laboratory to confirm screen-reactive samples and provide an endpoint titer, the Premier EIA, Alpha CrAg EIA, and CrAg LFA demonstrated sensitivities of 55.6% (5/9), 100% (9/9), and 100% (9/9), respectively, for serum samples (Table 1). The Premier EIA showed a specificity of 100% (625/625), while the Alpha CrAg EIA and LFA yielded a specificity of 99.7% (623/625) and 99.8% (624/625), respectively. Interestingly, one sample was interpreted as negative by the LA test but was positive by both the Alpha CrAg EIA and LFA; however, this sample showed 1+ reactivity by latex at the screening dilution, but this did not meet the criteria ( $\geq 2+$  agglutination) to be considered positive. There was one additional sample that was positive by the Alpha CrAg EIA but negative by all other tests (Table 1).

The serum data were also analyzed by comparing the performance of each individual test to a "consensus of the test panel," which was defined as at least 3 of 4 tests being in agreement. A "consensus" result was able to be determined for 633 of 634 (99.8%) samples. The single sample that did not show a consensus result was positive by the Alpha CrAg EIA and LFA but negative by the Premier EIA and LA tests. This sample was excluded from the

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TABLE 1 Comparison of three cryptococcal antigen assays to the latex agglutination test using serum specimens ( $n = 634$ )<sup>a</sup>

Assay and result	No. of samples with the following Meridian LA test result:		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Agreement (%) (95% CI)	Kappa
	Positive	Negative				
<b>Premier EIA</b>						
Positive	5	0	55.6 (26.6, 81.2)	100 (99.3, 100)	99.4 (98.3, 99.8)	0.71
Negative	4	625				
<b>Alpha CrAg EIA</b>						
Positive	9	2 <sup>b</sup>	100 (65.5, 100)	99.7 (98.8, 99.9)	99.7 (98.8, 99.9)	0.90
Negative	0	623				
<b>CrAg LFA</b>						
Positive	9	1 <sup>c</sup>	100 (65.5, 100)	99.8 (99.0, 100)	99.8 (99.0, 99.9)	0.95
Negative	0	624				

<sup>a</sup> LA, latex agglutination; EIA, enzyme immunoassay; LFA, lateral flow assay; 95% CI, 95% confidence interval.

<sup>b</sup> One of these two samples showed 1+ reactivity by the Meridian latex agglutination assay upon screening but was interpreted as negative according to the package insert's requirement for 2+ reactivity.

<sup>c</sup> This sample showed 1+ reactivity by the Meridian latex agglutination assay upon screening but was interpreted as negative according to the package insert's requirement for 2+ reactivity.

analysis summarized in Table 2. The results showed excellent correlation ( $\kappa \geq 0.9$ ) between the LA test and the Alpha CrAg EIA and LFA tests (Table 2).

We were able to compare the performance of the four assays using 51 archived CSF samples in addition to serum samples. Among the 51 CSF samples, 18 were positive and 33 were negative by our routine screening test, the Meridian EIA. These CSF samples were subsequently tested by the LA test, IMMY EIA, and IMMY LFA, and the results showed 100% agreement among positive (18/18) and negative (31/31) samples for each assay.

The findings outlined in this report demonstrate that the Alpha CrAg EIA and LFA assays may be more sensitive than the Premier EIA for the detection of cryptococcal antigen in serum samples (100% versus 55.6%, respectively). This is likely due to the differ-

ent capture antibodies used by the Meridian and Immuno-Mycologics assays. An earlier study by Percival et al. (7) demonstrated that the monoclonal antibody used in the IMMY assays has increased reactivity for certain serotypes of *Cryptococcus neoformans*, especially serotype C (*C. neoformans* var. *gattii*), which is most prevalent in Papua New Guinea and Northern Australia and has recently caused outbreaks in the northwest region of the United States. These data have important implications for laboratories using the Premier EIA for screening purposes, which suggest this EIA may have lower sensitivity compared to those of the LA test, LFA, and the Alpha CrAg EIA. It is important to note that our group previously compared the performance of the Meridian LA test and EIAs and found the percent positive agreement to be much higher than reported in this study (97.9% versus 55.6%) (1).

TABLE 2 Comparison of four cryptococcal antigen assays to a consensus of the test panel using serum specimens ( $n = 633$ )<sup>a</sup>

Assay and result	No. of samples with consensus of test panel results <sup>b</sup>		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Agreement (%) (95% CI)	Kappa
	Positive	Negative				
<b>Meridian LA</b>						
Positive	9	0	100 (65.5, 100)	100 (99.3, 100)	100 (99.3, 100)	1.0
Negative	0	624				
<b>Premier EIA</b>						
Positive	5	0	55.6 (26.6, 81.2)	100 (99.3, 100)	99.4 (98.3, 99.8)	0.71
Negative	4	624				
<b>Alpha CrAg EIA</b>						
Positive	9	1	100 (65.5, 100)	99.8 (99.0, 99.9)	99.8 (99.0, 99.9)	0.95
Negative	0	623				
<b>CrAg LFA</b>						
Positive	9	0	100 (65.5, 100)	100 (99.3, 100)	100 (99.3, 100)	1.0
Negative	0	624				

<sup>a</sup> LA, latex agglutination; EIA, enzyme immunoassay; LFA, lateral flow assay; 95% CI, 95% confidence interval.

<sup>b</sup> A consensus of the test panel results was defined as at least 3 of 4 cryptococcal antigen results being in agreement. There was one sample that did not yield a consensus result; therefore, only 633 sera were included in this analysis.

**TABLE 3** Correlation of reciprocal endpoint titers for cryptococcal antigen-positive serum samples by the latex agglutination test and a lateral flow assay

Serum sample	Reciprocal endpoint titer by <sup>a</sup> :	
	LA	CrAg LFA
1	256	160
2	8	10
3	16	80
4	1,024	2,560
5	Negative <sup>b</sup>	10
6	8	20
7	2	10
8	4	10
9	64	160
10	8	20

<sup>a</sup> LA, latex agglutination; LFA, lateral flow assay.

<sup>b</sup> This sample showed 1+ reactivity by the Meridian latex agglutination assay upon screening but was interpreted as negative according to the package insert's requirement for 2+ reactivity.

This is likely due to differences in study design, with our initial study including positive sera that were selected on the basis of known reactivity by the LA test compared to the current study where the vast majority of sera (632/634) were tested prospectively.

In addition to the high sensitivity and specificity of the CrAg LFA test, this assay was found to be rapid and amenable to high-volume testing. Timing studies showed that 20 sera could be tested by the CrAg LFA in ~17 min compared to 50 min by Premier EIA, 60 min by the Alpha CrAg EIA, and 70 min by the LA test. Furthermore, expensive instrumentation is not required to perform testing by the CrAg LFA. Despite these advantages, it is important to note that similar to the LA test, there is subjectivity in the interpretation of results of CrAg LFA, and the results are not directly interfaced to the laboratory information system. Also, we observed a difference in the endpoint titers between the LA test and LFA, with the LFA reciprocal endpoint being at least twice that of the LA test in 8 of 10 positive patients (Table 3). Therefore, clinical laboratories and health care providers should be aware that endpoint titers may not correlate for different methods, and patients should be monitored by the same assay (e.g., LA test or LFA).

There are several limitations of this study. First, we were unable to correlate our results to other laboratory tests (e.g., culture) or clinical data (e.g., exposure history, clinical signs) because samples were submitted to our reference laboratory without this information. Second, the CSF data may not reflect the true performance

characteristics of the evaluated tests for this source, since CSF samples were archived and selected on the basis of the results of our routine screening assay, the Meridian EIA. Therefore, it is possible that the Meridian EIA may have lower sensitivity for detecting cryptococcal antigen in CSF samples, as we observed in serum samples. Current studies are under way to determine the performance characteristics of EIA, LA test, and LFA using prospectively collected CSF samples.

Despite these limitations, this study is the first to directly compare the Alpha CrAg EIA and LFA tests to latex agglutination and the Premier EIA. The results show that the Alpha CrAg EIA and LFA assays detected four additional positive serum samples compared to the Premier EIA, and these four samples were also positive by the LA test. The LFA is rapid (20 samples in ~17 min) and can be adapted to high-throughput laboratories. Importantly, the LFA may also offer a low-complexity method for resource-limited settings that are in need of an accurate diagnostic assay for cryptococcal disease.

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## REFERENCES

- Babady NE, et al. 2009. Evaluation of three commercial latex agglutination kits and a commercial enzyme immunoassay for the detection of cryptococcal antigen. *Med. Mycol.* 47:336–338.
- CDC. 5 January 2012 posting date. *C. neoformans* cryptococcosis: cryptococcosis statistics. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA. <http://www.cdc.gov/fungal/cryptococcosis-neoformans/statistics.html>.
- Jarvis JN, et al. 2011. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. *Clin. Infect. Dis.* 53:1019–1023.
- Jaye DL, Waites K, Parker B, Bragg S, Moser SA. 1998. Comparison of two rapid latex agglutination tests for detection of cryptococcal capsular polysaccharide. *Am. J. Clin. Pathol.* 109:634–641.
- Kiska DL, Orkiszewski DR, Howell D, Gilligan PH. 1994. Evaluation of new monoclonal antibody-based latex agglutination test for detection of cryptococcal polysaccharide antigen in serum and cerebrospinal fluid. *J. Clin. Microbiol.* 32:2309–2311.
- Lindsley MD, et al. 2011. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clin. Infect. Dis.* 53:321–325.
- Percival A, Thorkildson P, Kozel TR. 2011. Monoclonal antibodies specific for immunorecessive epitopes of glucuronoxylomannan, the major capsular polysaccharide of *Cryptococcus neoformans*, reduce serotype bias in an immunoassay for cryptococcal antigen. *Clin. Vaccine Immunol.* 18:1292–1296.
- Tanner DC, et al. 1994. Comparison of commercial kits for detection of cryptococcal antigen. *J. Clin. Microbiol.* 32:1680–1684.