Evaluation of a Cryptococcal antigen Lateral Flow Assay in serum and cerebrospinal fluid for rapid diagnosis of cryptococcosis in Colombia

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ABSTRACT

A Lateral Flow Assay to detect cryptococcal antigen (CrAg® LFA) in serum and cerebrospinal fluid for the rapid diagnosis of cryptococcosis was evaluated. A retrospective validation was performed. Sensitivity and specificity of the CrAg® LFA was 100%. High concordance (kappa index=1.0) between Cryptococcal Antigen Latex Agglutination System (CALAS®) and CrAg® LFA was observed. CrAg® LFA showed higher analytical sensitivity for detecting low concentrations of cryptococcal antigen.

KEYWORDS: Cryptococcosis. Cryptococcus. Antigen. Diagnosis. Point-of-care. Lateral Flow Assay. Immunochromatographic assay. Latex agglutination system.
between CrAg® LFA and CALAS® was compared, and the ability to quantify Cryptococcus antigens in CSF was also evaluated for each test\(^5\). Positive antigen samples were diluted initially 1:4, followed by a two-fold serial dilution up to 1:16,184 (dilutions are shown in Figure 1). CrAg® LFA and CALAS® were performed in parallel using the same sample’s dilutions. A total of 51 CSF and 32 serum samples from 83 patients were tested. Of these, 31 CSF and 15 sera were from proven cryptococcal cases and the remaining samples were from patients who did not have cryptococcosis (Figure 2).

Sensitivity and specificity of CrAg® LFA in sera were both 100% (95% confidence interval [95% CI] = 97-100%). For the CSF, both sensitivity and specificity were also 100% (95% CI = 98-100% for sensitivity, and 95% CI = 97-100% for specificity). In the two types of sample (serum and CSF), the concordance between CrAg® LFA and CALAS® was 1.0 (kappa index, 95% CI = 1.0-1.0) (Table 1). When we compared the capacity to detect different Cryptococcus antigens levels between CrAg® LFA and CALAS® in CSF, CrAg® LFA demonstrated higher sensitivity to detect lower concentrations of antigen (Figure 1).

Results of this study showed that both serum and CSF specimens tested with the CrAg® LFA were 100% sensitive and specific. This is consistent with the reported sensitivity and specificity of this LFA in other laboratories around the world\(^6-10\). The CrAg® LFA was also able to detect lower concentrations of antigens in CSF as compared with the CALAS® assay, a finding that has been previously reported\(^9\).

The CrAg® LFA test is a simple technique that can be easily performed in any laboratory; additionally, storage and incubation of reagents are performed at room temperature, and the specimen does not require pre-treatment with pronase or any other additional step.

A limitation of this study was that we were unable to access complete information from the clinical records on associated complications and mortality. Additionally, the number of samples that were used in this study was small. For the CrAg® LFA, two independent technicians performed the readings and no discordant results were found, although, for the latex agglutination test, only one technician with extensive training and expertise performed the readings. These diagnostic methods are supported by multiple validations with results similar to those observed in this study\(^6-10\).

In summary, the study presented here evaluated the CrAg® LFA test for detection of cryptococcosis using serum and CSF in a specialized laboratory in Medellín, Colombia. This test is relatively cheap and readily available, requires minimal laboratory steps and can be completed in less than 15 minutes using basic supplies. It shows high sensitivity, specificity, and concordance with currently CrAg tests used in the diagnosis of cryptococcosis. Additionally, the CrAg®

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**Table 1 - Analysis of concordance between CrAg® LFA and CALAS®**

| A. Results in CSF samples (n=51) | B. Results in serum samples (n=32) |
|----------------------------------|---------------------------------|
| **CALAS®**                       | **CALAS®**                      |
| +                                | +                               |
| -                                | -                               |
| +                                | 31                              | +                                |
| -                                | 0                               | -                                |
| CrAg®LFA                         | 0                               | CrAg®LFA                         |
| -                                | 20                              | -                                |
| kcal index= 1.0; 95% CI = 1.0-1.0 | kcal index= 1.0; 95% CI = 1.0-1.0 |

CSF: cerebrospinal fluid, 95% CI: 95% confidence interval, +: positive, -: negative
LFA demonstrated higher analytical sensitivity to detect low concentrations of cryptococcal antigen compared with the CALAS® assay. This test could significantly reduce the time to diagnose and to treat cryptococcosis.

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CONFLICT OF INTERESTS

The authors report no conflict of interests.

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REFERENCES

1. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009;23:525-30.
2. Kaplan JE, Vallabhaneni S, Smith RM, Chideya-Chihota S, Chehab J, Park B. Cryptococcal antigen screening and early antifungal treatment to prevent cryptococcal meningitis: a review of the literature. J Acquir Immune Defic Syndr. 2015;68 Suppl 3:331-9.
3. Pfeiffer CD, Wong B. Diagnostic immunology. In: Hospenthal DR, Rinaldi MG, editors. Diagnosis and treatment of human mycoses. 2nd ed. New York: Humana Press; 2015. p.45-64.
4. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46:1813-21.
5. Orozco LC. Medicación en salud: diagnóstico y evaluación de resultados: un manual crítico más allá de lo básico. Santander: Universidad Industrial de Santander; 2010. Validación de criterio o de la sensibilidad específica para predecir la calidad de las probabilidades; p.115-57.
6. Lindsley MD, Mekha N, Baggett HC, Surinthong Y, Autthateinchai R, Sawatwong P, et al. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. Clin Infect Dis. 2011;53:321-5.
7. Jarvis JN, Percival A, Bauman S, Pelfrey J, Meintjes G, Williams GN, et al. 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. 2011;53:1019-23.
8. McMullan BJ, Halliday C, Sorrell TC, Judd D, Sleiman S, Marriott D, et al. Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory. PLoS One. 2012;7:e49541.
9. Escandón P, Lizarazo J, Agudelo CI, Chiller T, Castañeda E. Evaluation of a rapid lateral flow immunoassay for the detection of cryptococcal antigen for the early diagnosis of cryptococcosis in HIV patients in Colombia. Med Mycol. 2013;51:765-8.
10. Huang HR, Fan LC, Rajbanshi B, Xu JF. Evaluation of a new cryptococcal antigen lateral flow immunoassay in serum, cerebrospinal fluid and urine for the diagnosis of cryptococcosis: a meta-analysis and systematic review. PLoS One. 2015;10:e0127117.