

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

Cryptococcal meningitis primarily occurs in AIDS patients and especially in those with CD4 cell counts less than 100 cells/ μ L, and continues causing high mortality and morbidity, especially in resource-limited countries¹. In Colombia, as in other Latin American countries, access to antiretroviral therapy (ART) may be limited. As a result, cryptococcosis and other opportunistic infections (OIs) are major causes of mortality in people living with HIV and AIDS (PLWHA)¹. Early and rapid diagnosis of cryptococcosis reduces the risk of mortality and morbidity and the development of severe disease by facilitating prompt and timely treatment². Detection of cryptococcal antigen in serum and cerebrospinal fluid (CSF) has proven to be a highly sensitive (93-100%) and specific (93-98%) method for diagnosing cryptococcosis³. In this study, we compared two cryptococcal antigen tests, one dipstick sandwich immunochromatographic assay developed by IMMY[®] (Immuno-Mycologics, Norman, Oklahoma, USA) and a Cryptococcal Antigen Latex Agglutination System (CALAS[®]) developed by Meridian Bioscience (Cincinnati, Ohio, USA).

A retrospective validation study of the CrAg[®] LFA test was conducted in the Medical Mycology laboratory of a private research institution, the *Corporación para Investigaciones Biológicas* (CIB), in *Medellín*, Colombia. Serum and CSF samples from patients with or without diagnosis of cryptococcosis were randomly selected from a collection of biological samples stored in the CIB's biobank. All samples were coded and analyzed blindly. Diagnosis of cryptococcosis was based on the EORTC/MSG definitions⁴. A case was considered proven if *Cryptococcus* was isolated from blood, tissue, sterile fluids or respiratory specimens, or if there was a positive antigen test in serum or CSF using CALAS[®]⁴. Sensitivity and specificity of the CrAg[®] LFA were calculated using microbiological criteria for diagnosing cryptococcosis. The capacity to perform the diagnosis of cryptococcosis

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between CrAg[®] LFA and CALAS[®] was compared, and the ability to quantify *Cryptococcus* antigens in CSF was also evaluated for each test⁵. 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).

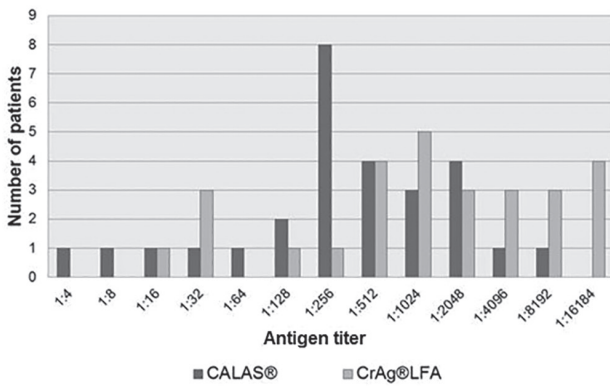


Figure 1 - Reactivity against *Cryptococcus* antigen: Comparison of the CrAg[®] LFA and the CALAS[®] tests. Analytical sensitivity of CrAg[®] LFA (light gray bars) and CALAS[®] (dark gray bars) are shown. The vertical axis shows the number of patients and the horizontal axis shows the final antigen titer

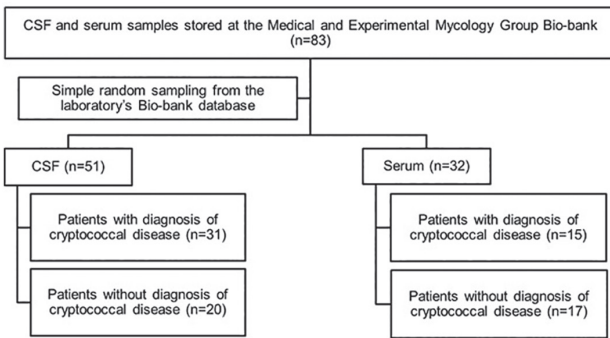


Figure 2 - Flowchart of serum and CSF samples analyzed during the validation of the CrAg[®] LFA. Study flowchart of analyzed samples stored at the Medical and Experimental Mycology Group biobank. A total of 83 samples were tested, corresponding to 51 CSF and 32 sera

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 world⁶⁻¹⁰. 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 reported⁹.

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 study⁶⁻¹⁰.

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[®]

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 [®]	
		+	-			+	-
CrAg [®] LFA	+	31	0	CrAg [®] LFA	+	15	0
	-	0	20		-	0	17
kappa index= 1.0; 95% CI = 1.0-1.0				kappa 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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