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Serotype Sensitivity of a Lateral Flow Immunoassay for Cryptococcal Antigen

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To meet the needs of a global community, an immunoassay for cryptococcal antigen (CrAg) must have high sensitivity for CrAg of all major serotypes. A new immunoassay for CrAg in lateral flow format was evaluated and found to have a high sensitivity for detection of serotypes A, B, C, and D.

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With a global burden of more than 1 million cases annually, cryptococcosis has emerged as a leading cause of death in patients with HIV or AIDS, killing an estimated 500,000 people (estimate range, 100,000 to 900,000) each year in sub-Saharan Africa alone (1). Early diagnosis is critical to effective treatment (2). The high burden of cryptococcosis in resource-limited settings underscores the need for an affordable, sensitive, and equipment-free diagnostic and has resulted in the development of the cryptococcal antigen lateral flow assay (CrAg LFA), a rapid point-of-care (POC) immunoassay (3).

The CrAg LFA (Immuno-Mycologics, Inc.) was constructed using monoclonal antibodies reactive with the capsular polysaccharide glucuronoxylomannan (GXM), the primary cryptococcal antigen (CrAg) that is shed during infection. The CrAg LFA has a dipstick format capable of detecting GXM in either serum or cerebrospinal fluid. GXM has variable levels of O-acetylation that contribute to serotype specificity and is classified into four major serotypes—A, B, C, and D (4). Although most cases of cryptococcosis are caused by isolates of serotype A, the need for sensitive diagnostics capable of detecting all four serotypes is emphasized by the report of serotype C infections in HIV patients in sub-Saharan Africa (5) and an increasing number of temperate regions reporting serotype B infections in otherwise healthy individuals (6). Moreover, serotype D is found with a high frequency in clinical cases in Europe (7–9). Several of the leading CrAg immunoassays for cryptococcosis display serotype bias, having a markedly reduced sensitivity for serotype C (10). In this study, the CrAg LFA serotype sensitivity was evaluated using endpoints determined visually and by densitometry scanning to determine the limit of detection, with GXM isolated from strains representing each of the four major serotypes.

The nine representative *Cryptococcus* strains used in this study were selected on the basis of the chemotype and structure reporter groups typical of each of the four major serotypes (10, 11). GXM was isolated from culture supernatant fluids as previously described (10). Assays were performed by inserting CrAg LFA dipsticks into samples (100 μl) containing GXM that was serially diluted 2-fold from 32 ng/ml to 0.25 ng/ml in phosphate-buffered saline (PBS). Assays were allowed to develop for 10 min and then read in a blinded fashion by 4 different individuals instructed to evaluate tests as either positive or negative. The visual limit of detection was defined as the lowest concentration where at least 50% of observers stated the test was positive. Examples of LFAs tested with GXM from serotypes A and C are shown in Fig. 1. Tests were then read via densitometry scanning with an ESE lateral flow

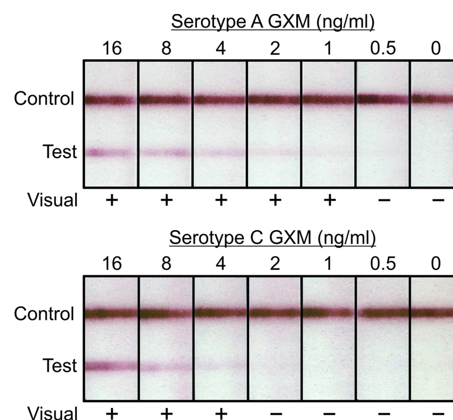

FIG 1 Representative examples of the cryptococcal antigen lateral flow assay (CrAg LFA) tested with serial dilutions of GXM from serotype A strain CN6 and serotype C strain 24066. Shown are results from blinded visual inspection of strips by four independent observers. A positive (+) result is reported when 50% or more of the observers scored the result as positive.

immunoassay reader (Qiagen, Inc.). The ESE reader generates quantitative data by performing a densitometer scan of the dipstick, where test line width (mm) is plotted against signal intensity (mV). Results yielding the calculated area of each peak (mm · mV) were plotted against corresponding concentrations, and the slope was then used to calculate a limit of detection with an endpoint of 50 mm · mV.

The CrAg LFA showed high sensitivity for GXM from all four serotypes (Table 1). Comparing the visual limits of detection, the assay had the highest sensitivity for serotypes A and B, detecting GXM from two strains of each serotype at 1 ng/ml. Sensitivity levels for GXM of three serotype C strains were 16 ng/ml (strain 34), 8 ng/ml (strain 298), and 4 ng/ml (strain 24066). The sensitivity limits for GXM of serotype D strains M0024 and 127 were each 8 ng/ml. Endpoints based on densitometric scanning of strips

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TABLE 1 Limit of detection of CrAg LFA for detection of GXM of different serotypes and comparison to previous report of sensitivity of latex agglutination assay

		Limit of detection (CrAg ng/ml) for:		
		LFA		
CrAg serotype	*C. neoformans* strain	Visual observation[a]	Densitometry[b]	Latex agglutination[c]
A	CN6	1	1.3	24
	MU-1	1	1.0	32
B	184	1	0.94	27
	409	1	1.2	68
C	34	16	27	432
	298	8	4.4	260
	24066	4	2.2	460
D	127	8	10	62
	M0024	8	11	63

[a] Lowest concentration at which 50% of observers considered a test to be positive.
[b] Limit of detection calculated on the basis of densitometry scanning with an electronic reader.
[c] Latex agglutination data are from a previous report of the serotype sensitivity of the Immuno-Mycologics *Cryptococcus* antigen latex agglutination test system (10).

were nearly identical to the visual results for serotypes A and B. Marginal differences were observed for serotype C and D samples. Overall there was a high correlation between limits of detection determined by visual inspection of the strips and limits of detection determined by densitometric scanning (Pearson product-moment correlation coefficient, 0.95; $P = 0.00015$). Despite the strong correlation between endpoints based on visual inspection and densitometric scanning, there was no obvious advantage in sensitivity determined by either method. Because the CrAg LFA is designed as a POC assay to be assessed without the use of equipment, densitometric scanning is not a substitute for direct observation, but rather an added method to experimentally quantify signal levels and validate visual results. However, the ability to electronically capture results of POC testing is consistent with the goal of using such data for patient follow-up, quality control, and public health surveillance (12).

This study demonstrated that the CrAg LFA has high sensitivity for GXM of all four serotypes, with A = B > C > D. The observed sensitivity of the CrAG LFA was greater than we previously reported for currently available CrAg immunoassays in latex agglutination or enzyme immunoassay formats (10). The previously reported serotype sensitivity of the Immuno-Mycologics, Inc., latex agglutination assay (10), one of the most sensitive assays in either the latex agglutination or enzyme-linked immunosorbent assay (ELISA) formats, is shown for comparison purposes in Table 1. The mean increase in sensitivity across CrAg from all four serotypes was 40-fold using the LFA format relative to the Immuno-Mycologics latex agglutination assay. The increase in sensitivity was greatest with CrAg of serotype C strain 24066 (150-fold) and least with the CrAg of the two serotype D strains (7-fold).

The performance and cost effectiveness of this new assay have prompted two recent reports recommending the use of the CrAg LFA to screen severely immunodeficient patients (CD4 < 100 cells/μl) (13, 14). Screening would allow for early diagnosis and treatment of patients with subclinical infections. This study demonstrates the high sensitivity of the CrAg LFA for detection of GXM, further validating its use for prospective screening of patients with HIV or AIDS and those presenting with clinical signs of cryptococcal meningitis.

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