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Evaluation of a *Histoplasma* antigen lateral flow assay for the rapid diagnosis of progressive disseminated histoplasmosis in Colombian patients with AIDS

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Summary

Background: Progressive disseminated histoplasmosis (PDH) is an important cause of mortality in persons living with HIV (PLHIV), especially in countries where patients have limited access to antiretroviral therapies and diagnostic testing.

Objective: A lateral flow assay (LFA) to detect *Histoplasma capsulatum* antigen in serum developed by MiraVista® was evaluated.

Methods: We tested 75 serum samples: 24 from PLHIV and culture-proven PDH and 51 from PLHIV with other fungal and bacterial infections as well as people without HIV. LFA devices were read manually (read by eye) and by an automated reader.

Results: When the LFA was read manually, sensitivity was 96% and specificity was 90%. When an automated reader was used, sensitivity was 92% and specificity was 94%. The Kappa index comparing manual and automated reader was 0.90. Cross-reactions were observed principally in samples from patients with proven diagnosis of paracoccidioidomycosis.

Conclusions: The MiraVista® Diagnostics *Histoplasma* antigen LFA had high analytical performance and good agreement between manual and automated reader. This LFA allows *Histoplasma* antigen testing with minimal laboratory equipment and infrastructure requirements.

1 | INTRODUCTION

Histoplasmosis is an important cause of mortality in persons living with HIV (PLHIV) with advanced disease, especially in countries where patients have limited access to antiretroviral therapies and diagnostic testing. In PLHIV, the progressive disseminated form of histoplasmosis (PDH) can be fatal without and in the delay of treatment.[1] Early diagnosis is critical for providing proper treatment; however, diagnosis of PDH can be challenging. Symptoms in PLHIV may lack specificity and be similar to opportunistic infections, especially infections caused by *Mycobacterium* species, complicating diagnosis and treatment of PDH, especially in geographic regions where tuberculosis and other opportunistic infections are frequent.[2-5]

Laboratory diagnosis by culture can take weeks and serology may be falsely negative early in infection or as a result of immunosuppression in these patients.[6,7] Recently, an in-house Western blot assay has reported high sensitivity to diagnose PDH in HIV (90% sensitivity), but this assay is not commercially available.[8]

Detection of *Histoplasma* antigen in patient specimens improves sensitivity and timeliness of diagnosis, but the current method by enzyme immunoassay must be performed by highly trained personnel in specialty laboratories.[9] Recently, the development of the lateral flow technology has provided a method that is simple to use

and can be performed in a setting closer to the patient. In this study, a lateral flow assay (LFA) developed by MiraVista® Diagnostics was evaluated for the detection of *Histoplasma* antigen in serum.

2 | MATERIALS AND METHODS

2.1 | Study patient specimens

All specimens used in the current study were obtained from an earlier prospective study conducted from May 2008 to August 2011 at the Hospital La María in Medellín, Colombia.[9,10] Briefly, patients presenting with at least three of the following symptoms were enrolled: fever, pancytopenia, weight loss, the presence of skin or mucosal lesions, and pulmonary involvement by radiography. Any patient who had previously received amphotericin B or itraconazole or who had a diagnosis of histoplasmosis prior to the enrolment period were excluded from the study.

All enrolled patients were tested by culture and special fungal stains using at least one of the following sample types: blood, tissue, sterile fluid, or respiratory. Additionally, immunodiagnostics assays, including immunodiffusion and complement fixation for fungal pathogens (*Histoplasma spp*, *Paracoccidioides spp* and *Aspergilus spp*) and a polyclonal antibody EIA developed at CDC for the detection of *Histoplasma* urinary antigens were also performed.[10] Final diagnosis was established based on laboratory results and review of patients' clinical records. Specimens were stored at −80°C until time of analysis.

For the current study, a total of 75 serum samples were blinded for evaluation: 24 from patients with culture-proven PDH and 45 from patients with other infections, including *Mycobacterium* disease (n = 24), cryptococcosis (n = 10), *Pneumocystis* pneumonia (n = 3), paracoccidioidomycosis (n = 2), aspergillosis (n = 1), candidiasis (n = 1), salmonellosis (n = 2) and toxoplasmosis (n = 2). Histoplasmosis and non-histoplasmosis cases were classified based on laboratory results and review of clinical records. We also tested serum samples from six people without HIV who lived in histoplasmosis endemic areas (Figure 1).

2.2 | MiraVista® Diagnostics lateral flow assay (LFA) for detection in serum of *Histoplasma* antigen (MVista® *Histoplasma* Ag LFA)

The antigen LFA, kindly provided by Dr Joseph Wheat from MiraVista Diagnostic Laboratories, is a dipstick sandwich immunochromatographic assay that uses a rabbit polyclonal antibody that recognises a *H capsulatum* galactomannan antigen. Specimens were processed according to the manufacturer's instructions.

2.3 | Serum specimens

In order to increase assay accuracy, serum specimens were first pretreated (extraction) to dissociate immune-complexes in the specimen and improve antigen detection according to the manufacturer instructions described as follows. (a) In a microfuge tube, 300 µL of serum and 100 µL of 4% EDTA (provided in the LFA kit) were mixed and vortexed. (b) The mix was boiled in water bath at 100°C for 3 minutes. (c) After boiling, the mix was centrifuged at 8000-10 000 × *g* for 10 minutes. (d) After centrifugation, 100 µL of sample supernatant was placed in the LFA device sample reservoir and allowed to flow at room temperature for 30 minutes. Final results were interpreted both by human eye (manually), and using an automated reader (opTricon cube-reader; opTricon GmbH). This cube-reader is suitable for use in the quantitative and qualitative evaluation of the LFA, and interpretation of results is based on colour intensity of device bands. Positive results were interpreted as the presence of two lines (test

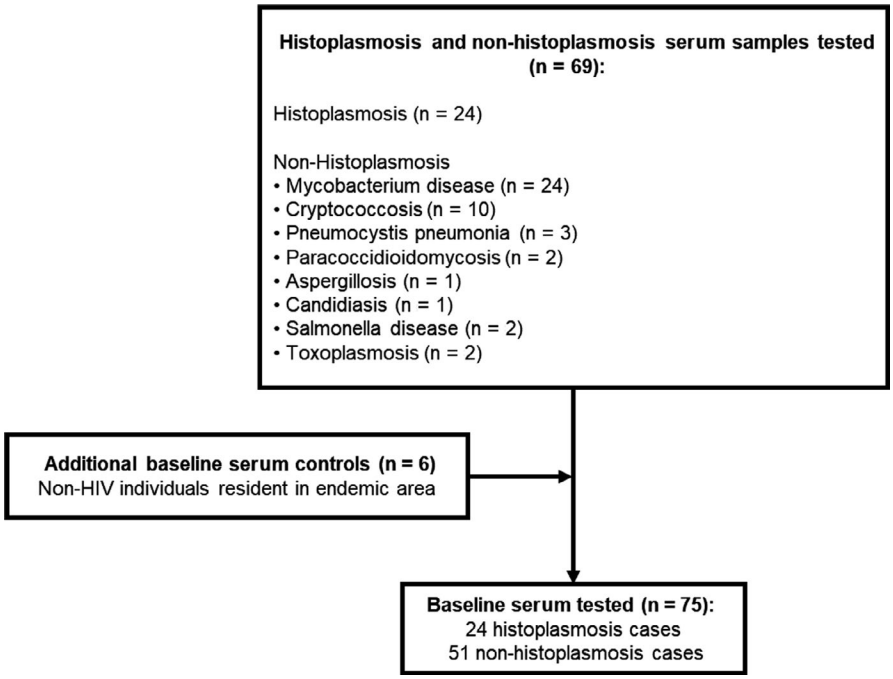

FIGURE 1 Study subjects and serum samples tested during the evaluation of the MiraVista® *Histoplasma* Ag lateral flow assay

TABLE 1 Analytical performance of the MiraVista® *Histoplasma* Ag lateral flow assay: comparison of human eye and automated reader interpretation

A						
	Manual reading			Automated reader		
	Culture			Culture		
		+	−		+	−
Histoplasma Ag LFA	+	23	5	+	22	3
	−	1	46	−	2	48

B		
	% (CI 95%)	
***Histoplasma* Ag LFA**	**Human eye**	**Automated reader**
Sen	96 (79-100)	92 (73-99)
Spe	90 (79-97)	94 (84-99)
Acc	92 (83-97)	93 (85-98)
PPV	82 (67-91)	88 (71-96)
NPV	98 (87-99)	96 (86-99)
Kappa	0.90 (0.80-1.00)	

Abbreviations: 95% CI, confidence interval; Acc, accuracy; NPV, negative predictive value; PPV, positive predictive value; Sen, sensitivity; Spe, specificity.

line and control line). Negative results were interpreted as the presence of the control line alone. No presence of lines, or only the presence of the test line, was interpreted as invalid results.

2.4 | Statistical analysis

Calculation of the analytical performance of the assay was done using 2 × 2 tables comparing LFA results vs culture-proven cases. We also calculated the assay sensitivity, specificity, accuracy, and positive and negative predictive values, with their respective 95% confidence intervals (95% CI).[11] Concordance analysis was performed to evaluate the agreement between results interpreted manually and by the automated reader, by calculating the Kappa index (K) and its respective 95% CI (10). Analyses were conducted using STATA 3.1 software and EPIDAT 8.0.

2.5 | Ethics

The samples were obtained under the terms agreed by and with the full approval of the ethical committees of the Centers for Disease Control and Prevention (CDC), Corporación para Investigaciones Biológicas (CIB) and Hospital La Maria IRB Number 7250, designed for the investigation of newer rapid methodologies for the diagnosis of histoplasmosis. All patients enrolled in the study signed an informed consent form. All clinical information from the participants in the study was anonymised and entered in an electronic MICROSOFT ACCESS® database, using an alphanumerical code.

3 | RESULTS

3.1 | Evaluation of the analytical performance of the MVista® *Histoplasma* Ag LFA

When read manually, the LFA displayed a sensitivity of 96% (23 of 24 serum samples) and a specificity of 90% (46 of 51 serum samples). Using an automated reader to obtain the result, the sensitivity was 92% (22 of 24 serum samples) and specificity was 94% (48 of 51 serum samples). The Kappa index comparing manual and automated reader results was 0.90 (95% CI: 0.80-1.00; Table 1).

Discrepant results were observed in six serum samples that were read manually. Of these specimens, five resulted in false-positive results. Two were from patients with paracoccidioidomycosis (Table 2, samples 1 and 2), and two were from patients with *Salmonella* bloodstream infections (Table 2, samples 3 and 4). The fifth false-positive result corresponded to a patient with toxoplasmosis and TB (Table 2, sample 5), who lacked clinical or epidemiological evidence of PDH. One specimen yielded a false-negative result from a patient with culture-proven PDH (Table 2, sample 6).

Using the automated reader, five culture-discrepant results were observed. Of these specimens, three resulted in false-positive results which included one of the two paracoccidioidomycosis patients (Table 2, sample 1) and the two patients with *Salmonella* bloodstream infections that were also identified as positive by manual reading (Table 2, samples 3 and 4). False-negative results were reported in two culture-proven PDH cases, including the sample reported as

TABLE 2 Characteristics of serum samples with culture-discrepant results using the MVista® *Histoplasma* Ag LFA

		Histoplasma Ag LFA		
Sample #	**Diagnosis**	**Manual reading**	**Automated reader**	**Clinical and laboratory findings**
Cross-reactivity				
1	Paracoccidioidomycosis	P	P	Cross-reactivity with *Paracoccidioides brasiliensis* antigens
2	Paracoccidioidomycosis	WP	N	Cross-reactivity with *Paracoccidioides brasiliensis* antigens
Discrepant negative culture				
3	*Salmonella* infection	P	P	CDC HPA positive (12.6 ng/mL), HGM positive (83.7 ng/mL) and ID positive (M band)
4	*Salmonella* infection	P	P	CDC HPA positive (12.9 ng/mL), HGM positive (53.8 ng/mL) and ID positive (M band)
5	Toxoplasmosis and TB	WP	N	Negative fungal cultures and stains, negative fungal serology, negative CDC HPA and negative HGM
False-negative				
6	PDH	N	N	Diagnosis of PDH by culture
7	PDH	WP	N	Diagnosis of PDH by culture

Note: Antigenemia result interpretation: P, positive result; N, negative result; WP, weak positive.
Abbreviations: #, number; CDC HPA, Histoplasma urinary antigen CDC polyclonal ELISA; HGM, Histoplasma urinary antigen IMMY monoclonal ELISA; ID, immunodiffusion; PDH, progressive disseminated histoplasmosis.

negative by manual reading (Table 2, sample 6), and an additional sample that had been reported as weak positive by manual reading (Table 2, sample 7).

4 | DISCUSSION

In this report, we describe the successful evaluation of a lateral flow assay (LFA), the first point of care assay developed for the rapid diagnosis of PDH in PLHIV. The LFA displayed excellent sensitivity of 96% with a specificity of 90%. The one false-negative result was from a patient with proven PDH by culture who had received trimethoprim/sulphamethoxazole (TMP/SMX) for the treatment of *Pneumocystis* pneumonia. TMP/SMX has been used for the treatment of other endemic mycoses (eg, paracoccidioidomycosis) and has displayed activity against *H capsulatum* in in vitro studies.[12] Therefore, it is possible that this medication reduced the patient's antigenemia below the detection limit of the LFA.

Five false-positive antigen-discrepant results were observed in culture-negative samples. These discrepant results included two cross-reactions observed in samples from patients with paracoccidioidomycosis, this cross-reaction has been reported before by MiraVista® Diagnostics (88%).[13] However, the clinical and epidemiological profile of patients with paracoccidioidomycosis differs from patients with histoplasmosis. Other immunological and conventional laboratory assays, like direct microscopic observation, are also available to facilitate the diagnosis of paracoccidioidomycosis.[7,14] Two other discrepant results were observed in two patients with salmonellosis who lacked positive culture for *H capsulatum*. However, these patients had clinical and epidemiological evidence of PDH, as well as positive urinary antigen measured by two different ELISAs and positive serology (one patient with complement fixation titres of 1:8, and both patients with immunodiffusion M band) for histoplasmosis.[9,10] It is possible that these two patients truly had PDH despite lack of culture confirmation. If these patients were also considered to be histoplasmosis cases, the specificity of the LFA would increase to 94% and the sensitivity to 96%. Finally, a weak false-positive MiraVista® *Histoplasma* Ag LFA result was observed in a patient without evidence of PDH.

As part of this study, an evaluation of an automated reader was also included to determine if such a device would increase the sensitivity of the assay; that is, detecting the presence of a positive band that was undetectable by manual reading. However, instead of increasing sensitivity, an increase in specificity was observed when using the automated reader, possibly by calling negative results more reliably than manual reading (seeing weak positive results that were not). When using the automated reader, a sensitivity of 92% with a specificity of 94% was observed. The sensitivity decreased, due to a second false-negative PDH patient, which was reported as negative by the automated reader, but had been reported as weak positive by manual reading.

The specificity of the assay, however, increased from 90% to 94% after using the automated reader. This result was due to a decrease from five to three false-positive, antigen-discrepant results as reported by the automated reader. The reduction of discrepant results was due to the automated reader calling two specimens

negative that were called weakly positive when read manually; possibly due to over-reading the results. It has been reported that visual LFA interpretation may be affected by factors like age, visual accuracy and previous experience of the person who read the device. An inclusion of an automatic device could reduce risk of non-accurate results.[15] The two patients, described earlier, with salmonellosis who were positive for Histoplasma antigen by LFA, but lacked positive culture for *H capsulatum*, continued to be called positive by automated reader, providing more evidence that these might be true *Histoplasma* antigen positive cases. It is possible that these two patients truly had PDH despite lack of culture confirmation. If these patients were also considered to be histoplasmosis cases, the specificity of the LFA would increase to 98% and the sensitivity increased to 92% by as read by the automated reader.[9,10]

This study presents several limitations. Ideally, a larger sample size would be desirable. However, the 75 patients (24 histoplasmosis and 51 non-histoplasmosis patients) included in this study were all confirmed by laboratory assays and review of clinical records, which took over 3 years to accumulate during the original prospective study. Another limitation is the patient cohort did not include patients diagnosed with blastomycosis, coccidioidomycosis and talaromycosis due to the lack of endemicity of these diseases in Colombia. Finally, while this LFA provides rapid lifesaving results in patients with PDH, further investigation is necessary to evaluate the capacity of this LFA for the diagnosis of non-progressive clinical forms of histoplasmosis.

The development and use of an LFA for the diagnosis of histoplasmosis provides *Histoplasma* antigen testing with minimal laboratory equipment and infrastructure requirements. Based on the results presented here, this new method is a viable option for rapid diagnosis of PDH. LFA provides highly sensitive results in <1 hour, being faster and more sensitive for PDH than other immunological assays, such as antigen ELISA (3-5 hours; >90% sensitivity), detection of antibody by immunodiffusion and complement fixation (2 days; ~70% sensitivity). Other conventional microbiological methods, including staining smear (1-2 hours; 25%-75% sensitivity), histopathologic examination (1-2 days; ~75% sensitivity) and culture (2-4 weeks; ~75% sensitivity) require obtaining specimens that require more invasive procedures, are less sensitive and/or require days to weeks for a result.

Finally, as this assay obtains FDA and/or CE approval for in vitro diagnostic use, these labels will facilitate assay accessibility in many laboratories worldwide. An assay that does not require a cold chain and provides prompt diagnosis of PDH will impact public health by allowing early treatment initiation, thereby reducing mortality.

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DECLARATION OF INTEREST

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AUTHOR CONTRIBUTIONS

BLG, AMT, DHC, TC, MDL. Conceived the ideas; DHC, BLG, AMT and MDL. Collected the data; DHC and MDL analysed the data; DHC and MDL led the writing.

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