Multicenter validation of commercial antigenuria reagents to diagnose progressive disseminated histoplasmosis in people living with HIV/AIDS in two Latin American countries

Running Title: Validation of a *Histoplasma* antigenuria ELISA

Diego H. Cáceres*1,2, Blanca E. Samayoa*3,4, Narda G. Medina3,5, Angela M. Tobón1,6, Brenda J. Guzmán5, Danicela Mercado3,5, Angela Restrepo1, Tom Chiller2, Eduardo E. Arathoon3,5 and Beatriz L. Gómez*1,7

(*/¥) These authors contributed equally

1) Medical and Experimental Mycology Group, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia. 2) Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, United States. 3) Asociación de Salud Integral, Guatemala City, Guatemala. 4) Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala, Guatemala City, Guatemala. 5) Clínica Familiar “Luis Ángel García,” Hospital General San Juan de Dios, Guatemala City, Guatemala. 6) Hospital La María, Medellín, Colombia. 7) School of Medicine and Health Sciences, Universidad del Rosario, Bogota, Colombia.

**Corresponding author:** Beatriz L. Gómez, PhD

Professor, School of Medicine and Health Sciences, Universidad del Rosario and Corporación para Investigaciones Biológicas, Medellín, Colombia

Phone: +57 320 720 9189

E-mail: beatrizlgomez@hotmail.com, beatriz.gomez@urosario.edu.co
Abstract

Histoplasmosis is an important cause of mortality in patients with AIDS, especially in countries with limited access to antiretroviral therapies and diagnostic tests. However, many disseminated infections in Latin America go undiagnosed. A simple, rapid method to detect *Histoplasma capsulatum* infection in endemic regions would dramatically decrease time to diagnosis and treatment, reducing morbidity and mortality. The aim of this study was to validate a commercial monoclonal *Histoplasma* galactomannan (HGM) ELISA (Immuno-Mycologics [IMMY], Norman, Oklahoma, USA) in two cohorts of people living with HIV/AIDS (PLHIV). We analyzed urine samples from 589 people (466 from Guatemala and 123 from Colombia), including 546 from PLHIV and 43 from non-PLHIV controls. Sixty-three of these people (35 from Guatemala and 28 from Colombia) had confirmed histoplasmosis by isolation of *H. capsulatum*. Using the standard curve provided by the quantitative commercial test, sensitivity was 98% (95% CI, 95-100) and specificity was 97% (95% CI, 96-99) (cutoff=0.5 ng/mL). Semi-quantitative results, using a calibrator of 12.5 ng/mL of *Histoplasma* galactomannan to calculate an EIA Index Value (EIV) of the samples, showed a sensitivity of 95% (95% CI, 89-100%) and specificity of 98% (95% CI, 96-99%) (cutoff ≥2.6 EIV). This relatively simple-to-perform commercial antigenuria test showed a high performance, with reproducible results in both countries, suggesting that it can used to detect progressive disseminated histoplasmosis in PLHIV in a wide range of clinical laboratories in countries where histoplasmosis is endemic.
Introduction:

Histoplasmosis is a disease caused by the thermally dimorphic fungus *Histoplasma capsulatum*. This disease is most frequently diagnosed in the American continents, although it is also diagnosed in certain Asian and African countries (1-5). Histoplasmosis is highly endemic in some regions of Central and South America, including Guatemala and Colombia. This fungal infection often affects people with impaired immunity, including people living with HIV/AIDS (PLHIV) (1,4). Among PLHIV, progressive disseminated histoplasmosis (PDH) is a major cause of mortality (13% to 48%) (6-13). PDH symptoms are nonspecific, and they may be indistinguishable from other infectious diseases, especially disseminated tuberculosis, thus complicating diagnosis and treatment (7,14,15). The nonspecific nature of the presenting symptoms coupled with an uncertain exposure history can create diagnostic challenges (3,4).

Conventional laboratory methods used for diagnosis of histoplasmosis, such as cultures and histopathological analysis, have low sensitivity (~50%), antibody tests also have poor sensitivity (30-70%) in immunocompromised persons. Molecular tests, which so far are all ‘in house’ tests developed by different groups and reference laboratories, have shown promising results, but none of these tests are commercially available and they need more extensive validation in order to be used more widely (3,4,16-18).

Detection of circulating *Histoplasma* antigen in urine by antigen capture ELISA has proven highly sensitive (95%), but this test is offered as a service by a private laboratory in the United States and has not been widely available in most Latin American countries (18,20-22). There is a commercially available, FDA cleared ELISA for *Histoplasma* antigen detection in urine (ALPHA *Histoplasma* Antigen EIA, IMMY®), but previous reports demonstrated low analytical performance for this kit (44% sensitivity).
An in-house double polyclonal antibody sandwich ELISA, developed at CDC, uses a polyclonal antibody (CDC polyclonal ELISA, HPA) to detect *Histoplasma* antigens. It was used in Guatemala and later in Colombia, with a reported sensitivity of 81% and 86%, respectively (10, 24). However, the reagents for this test are no longer available, and no further distribution was planned in the regions where histoplasmosis is endemic.

Since those assays were designed, a commercial *Histoplasma* antigen single monoclonal antibody sandwich ELISA (IMMY monoclonal ELISA, HGM) has also recently been developed by IMMY® (Immuno-Mycologics, Norman, Oklahoma, USA). The reagents have been evaluated in two laboratories in the United States, and showed high performance to diagnose histoplasmosis (sensitivity of 91% and specificity 96%) (25, 26). The aims of our study were 1) to evaluate the analytical performance of this commercial ELISA for detection of *Histoplasma* antigens in urine samples, using the standard curve provided in the kit, and 2) to evaluate this test as a semi-quantitative ELISA, in order to reduce expenses associated with the use of this test in laboratories outside USA.

**Results**

Evaluation of the analytical performance of the IMMY monoclonal ELISA (HGM)

*Quantitative antigen-capture ELISA (using a 7-point standard curve):*

Manufacturer-recommended cutoff criteria considered a concentration ≥0.5 ng/mL as a positive result. The IMMY monoclonal ELISA (HGM) test detected 62 of 63 culture-proven histoplasmosis cases (98% CI 95%, 95-100%), with an overall specificity of 97% (95% CI, 95-99%). In the Guatemalan cohort, a sensitivity of 100% (CI 95%, 99-100%) was observed and 96% (CI 95%, 88-100%) in the Colombia cohort. In addition, this test showed high specificity 97% (CI 95%, 96-99%) in Guatemala and 95% (CI 95%, 90-
100%) in Colombia. All ROC values were higher than 95% (Figure 1A). Predictive values and Kappa index were also calculated and are summarized in Table 1 and 2.

Discrepant results against culture using the quantitative IMMY monoclonal ELISA (HGM): Urine samples from 16 patients with negative fungal cultures were tested positive by IMMY ELISA (Table 3). 11 were from Guatemalan patients. Those patients presented with the following clinical and laboratory findings: four patients with positive urinary CDC polyclonal ELISA (HPA) (samples 1 to 4), two patients with history of PDH with negative CDC polyclonal ELISA (HPA) below cutoff (samples 5 and 6), and five patients were severely ill (median CD4 cell count of 51 cells/mm$^3$) with symptoms of PDH but with negative fungal culture and negative CDC polyclonal ELISA (HPA) (samples 7 to 11). Among these five patients, one had acute renal failure and co-infection with *M. tuberculosis* (sample 11). The other discrepant results were from two Colombian patients with bacterial disease who had a positive urinary CDC polyclonal ELISA (HPA) and positive antibody test for histoplasmosis (samples 13 and 14). Three samples presented cross reactivity, all were from patients with diagnosis of paracoccidioidomycosis (samples 15, 16 and 17). Finally, a false negative result was observed in a Colombian patient with culture-proven PDH (sample 18).

When we compared the quantitative results of the antigen CDC polyclonal ELISA (HPA) against the quantitative results of the IMMY monoclonal ELISA (HGM), we observed that antigen concentrations using the IMMY monoclonal ELISA (HGM) were higher than those obtained using the CDC polyclonal ELISA (HPA), with 12 times higher concentration.
Semi-quantitative antigen-capture ELISA (using a calibrator): In an evaluation of the analytical performance of the six points of the standard curve as a calibrator (0.8 to 25 ng/mL), all points demonstrated the same accuracy (97%) to diagnose histoplasmosis. Based on our results, we selected the antigen concentration standard of 12.5 ng/mL as calibrator (Table 4). Using this calibrator, we determined as a cutoff an EIA Index Value (EIV) greater or equal to 2.6 EIV. This choice had a sensitivity of 95% (95% CI, 89-100%), specificity of 98% (95% CI, 96-99%) and accuracy of 97% (95% CI, 96-99%). Independent analyses from the Guatemalan and Colombian laboratories showed comparable results. Detailed results for all calibrators, combined and per country, are described in Table 4. ROC analysis yielded an area under the curve (AUC) that corresponded to a test accuracy higher than 95% (Figure 1B).

Discrepant results with semi-quantitative methodology: We used the point of the standard curve with concentration of 12.5 ng/mL as a calibrator, and a cutoff of 2.6 EIV. We observed positive results in 9 of the 13 patients with culture discrepant results using the quantitative methodology (Table 3, samples 1 to 3 and 8 to 11 and 13 and 14). Cross reactivity was observed in the same three paracoccidioidomycosis patients (samples 15 to 17). Three false negative results were observed, for the Colombian patient with negative quantitative ELISA (sample 18), and two with positive quantitative ELISA (samples 12 and 19). Findings are summarized in Table 3.

Discussion

In this study, we report the successful multicenter validation of commercial reagents (IMMY monoclonal ELISA [HGM]) with high analytical performance and reproducibility for rapid detection of urinary Histoplasma antigen in two Latin America laboratories, using a 7-point standard curve (quantitative) or an alternative calibrator.
(semi-quantitative). The semi-quantitative method could offer similar results to those obtained using a quantitative test but at a lower cost (seven ELISA wells in the quantitative test for the standard curve versus two ELISA wells in the semi-quantitative test for calibrator). However, modification of protocols requires approval by the test manufacture. This assay uses urine, an easily obtained clinical specimen, and the ELISA can be performed in <3 hours. These reagents were shown to be robust and highly reproducible, and significantly reduced the time to diagnosis of PDH.

The quantitative and semi-quantitative IMMY monoclonal ELISA (HGM) presented high agreement with the fungal culture (97% and 98%, respectively). The quantitative methodology was able to identify as positive 62/63 (98%) urines from patients with PDH, and the semi-quantitative methodology detected 60/63 (95%) culture-proven case urines. These results showed a higher analytical performance than those previously reported using the CDC polyclonal ELISA (HPA) with a sensitivity of 81% and 86% and a specificity of 95% and 94%, in Guatemala and Colombia, respectively (10,24). Variabilities in quantitative antigen concentrations between the assays are likely due to different detection antibodies, CDC ELISA used a polyclonal antibody and IMMY ELISA used a monoclonal antibody. These commercial reagents were shown to be robust and they provide an easy method to implement in clinical laboratories with capacity to perform ELISA tests. In addition, our results are consistent with a previous report of the evaluation of the same reagents in the United States (sensitivity of 91% and specificity 96%), indicating that these commercial reagents can be successfully used in different laboratories (25).

We observed, in the quantitative analysis, 16 discrepant results between positive urinary antigen test and negative cultures. Cross-reactions were observed in three
samples from patients with paracoccidioidomycosis (PCM). Prior studies of *H. capsulatum* antigenuria tests have reported cross-reactivity of 28% and 88% with urine from patients with PCM (10,27,28). It is important to note that PCM is diagnosed much less frequently than histoplasmosis in PLHIV and there are several other immunological tests that help in the diagnosis of PCM (2,17). The remaining 13 discrepant results were from 11 samples from patients from Guatemala, where it is possible that the IMMY monoclonal ELISA (HGM) correctly identified circulating antigen in 7 patients (64%): four patients presented with a positive CDC polyclonal ELISA (HPA), and three patients with a previous diagnosis of PDH and had low adherence to fungal therapy. The remaining two were from the Colombian group, those patients presented with a positive serological test and positive CDC polyclonal ELISA (HPA), but with negative fungal culture. As previously described, fungal culture was less sensitive than antigen test (29). Finally, one false negative was identified, for a patient treated with trimethoprim/sulfamethoxazole as PCP prophylaxis. This medication is also used for the treatment of paracoccidioidomycosis, so it is possible that the medication could have lowered the fungal burden of these patients, subsequently reducing their levels of circulating *H. capsulatum* galactomannan antigen (30). As reported previously by Wheat et al, the use of antifungal treatment decreases circulating antigen (31). Another consideration is that histoplasmosis may cause proteinuria, and it could limit the detection of the antigen. Further studies could evaluate the follow up and antigen concentrations levels during antifungal therapy to better establish this relation as well as renal failure and its influence on clinical results (32).

The semi-quantitative methodology presented fewer discrepant results against negative fungal culture (four less), compared with the quantitative test, but it is important
to mention that the semi-quantitative methodology was slightly less sensitive in detecting urinary *Histoplasma* antigen in culture-proven cases, compared with the quantitative methodology, three vs one false negative respectively. We also mention that these samples had low concentration of antigen by the quantitative methodology, leading semi-quantitative analysis to classify those as negative.

Prevalence of histoplasmosis in the overall cohort was 11%, 8% in Guatemala and 23% in Colombia, but stratified analysis of the analytical performance shows similar results between countries. The differences in prevalence may be a result of differences between study sites, as the Guatemala study site forms part of one of the largest HIV programs in the country, whereas the Colombia study site is involved only in a local HIV program in the city of Medellin. We observed high negative predictive values using the IMMY monoclonal ELISA (HGM) kit (close to 100%), making this test a good tool to discard PDH diagnoses in patients with negative results. On the other hand, we observed a positive predictive value of 79% (CI 95%: 70-89), and this value was affected principally by false positive results as a consequence of cross reactivity in patients with diagnoses of paracoccidioidomycosis and a history of PDH. Finally, it is important to highlight that clinical decisions should be based on the correlation of the clinical, epidemiological and laboratory findings presented by the patients at the time of diagnosis.

Our study also has some limitations inherent to retrospective analyses (access to patient’s medical information). We have not evaluated the IMMY monoclonal ELISA (HGM) in non-HIV immunocompromised patients who may also be at risk to develop PDH. Serum specimens were not tested in this study, and further evaluation of this assay with sera appears to be warranted. The laboratories included in this study have
expertise implementing ELISA tests for *H. capsulatum* detection, and it is necessary to provide training and technical support to those laboratories interested in performing this methodology for the first time.

As final conclusion, the high sensitivity and specificity of the commercial antigen capture ELISA for the diagnosis of histoplasmosis in PLHIV has been demonstrated in two laboratories in Latin America. The semi-quantitative and quantitative and ELISA showed similar results, this finding could decrease the cost of the test, facilitating its use in developing countries. This assay uses urine, an easily obtained clinical specimen. ELISA tests can be performed in less than 3 hours, and this technique is robust and highly reproducible and significantly reduces the time to diagnosis of PDH.

**Materials and methods**

*Settings and study design:* We analyzed retrospectively residual urine samples collected from patients seen at two HIV clinics, one in Guatemala City, Guatemala, (Clínica Familiar “Luis Angel García”/Hospital General San Juan de Dios) and the other in Medellín, Colombia (Hospital La María). These urine samples were collected using the same study protocol (CDC protocol 4250), and were tested using the CDC polyclonal ELISA (HPA) (10,24). Samples were collected from 2008 to 2014 and stored at -80ºC. We included 589 urine samples from the two endemic regions were analyzed (Guatemala n=466 and Colombia n=80). Of the 546 samples from PLHIV, 63 were cases confirmed to have histoplasmosis by isolation of *H. capsulatum* from clinical samples (35 from Guatemala and 28 from Colombia). Of the remaining 483 patients infected with HIV, in 371 were just reported the infection with HIV, and 112 patients were diagnosed with other infections: *Mycobacterium* disease was diagnosed in 87 patients, 65 of them with *M. tuberculosis* infection, and 22 with other mycobacterial infection.
Other fungal infections were diagnosed in 21 patients: cryptococcosis (n=12), *Pneumocystis* pneumonia (n=3), paracoccidioidomycosis (n=3), aspergillosis (n=2) and candidiasis (n=1). Bacterial infections by *Salmonella enterica* were diagnosed in 2 patients, parasitic infection by *Toxoplasma* and viral infection by CMV in 1 patient each. Finally, we added to the analysis a total of 43 urine samples from non-HIV controls.

Patients' samples are described in Figure 2.

**Patient diagnoses:** A case of PDH was considered proven if *H. capsulatum* was isolated from any of the following samples: blood, tissue, sterile fluids or respiratory specimens (33). Blood and bone marrow cultures were tested using commercial methodologies, and samples were processed according to the manufacturers’ instructions. Biopsies, respiratory samples and other body fluids were cultured on Mycosel™ (BD), Sabouraud Dextrose agar™ (BD) and on tubes with Lowenstein-Jensen medium™ (BD) and *Mycobacterium* growth indicator tube (MGIT). We differentiated between *M. tuberculosis* and non-*M. tuberculosis* mycobacteria (NTM) using commercial molecular tests. Final patient diagnoses were made based on laboratory and clinical criteria.

**Enzyme-linked immunosorbent assay (ELISA) for detection of Histoplasma antigen (IMMY monoclonal ELISA [HGM]):** The ELISA antigen detection test is based on the use of a monoclonal antibody that recognizes a *H. capsulatum* galactomannan antigen (HGM). Urine samples were processed according to the manufacturer’s instructions, using a seven-point antigen standard curve, with a range of concentration from 0.4 to 25 ng/mL. Optical densities were read at 450 nm, and concentration of *Histoplasma* antigen was calculated based on a 7-point calibration curve, generated using a 4-parameter equation. As an alternative, we evaluated six of the seven points of
the antigen standard curve (0.8 to 25 ng/mL) in order to identify which was the best cut-off calibrator. The EIA index value (EIV) for each sample was calculated by dividing the mean optical density (OD) of the sample by the mean OD of the calibrator, and the result of the division was multiplied by 10 (multiplication factor). The EIV calculation is summarized in the following equation:

\[
\left( \frac{\text{Average blanked OD of specimen}}{\text{Average blanked OD of calibrator}} \right) \times 10 = \text{EIA index value (EIV)}
\]

**CDC polyclonal ELISA (HPA):** The ELISA antigen detection test is based on the use of a polyclonal antibody that recognizes a *H. capsulatum* polysaccharide antigen (HPA), as previously described (10,34).

**Evaluation of the analytical performance of the IMMY monoclonal ELISA (HGM):**

All ELISA tests were performed independently in Guatemala and Colombia by different laboratory technicians. All samples were coded, and analyzed in a double-blind design. All analyses were then compared to verify the reproducibility of the results obtained. Calculation of the analytical performance of the test was done using 2x2 tables comparing ELISA results versus culture-proven cases. We also calculated the test sensitivity, specificity, accuracy, and positive and negative predictive values, with their respective 95% confidence intervals (95% CI). ELISA Receiver Operating Characteristic (ROC) curves were used to determine the ideal test cutoff value (35). Kappa values and their respective 95% confidence intervals (95% CI) were calculated by a concordance analysis in order to evaluate the agreement between the methods used (35). Analyses were conducted using STATA 3.1 and EPIDAT 8.0.

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Declaration of interest: The authors report no conflicts of interest. ELISA reagents were donated by IMMY, Norman, Oklahoma, USA. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the CDC.
References


https://www.ncbi.nlm.nih.gov/pubmed/?term=Disseminated+histoplasmosis+in+HIV-
V-
infected+patients+in+South+America%3A+a+neglected+killer+continues+on+its+
rampage


https://www.ncbi.nlm.nih.gov/pubmed/?term=Clinical+and+laboratory+profile+of+
persons+living+with+human+immunodeficiency+virus%2Facquired+immune+defi
ciency+syndrome+and+histoplasmosis+from+a+Colombian+hospital.


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a Prospective Cohort of Human Immunodeficiency Virus/ Acquired Immune Deficiency Syndrome Patients with Histoplasmosis in Guatemala


Based Approach to Diagnosis and Management. Seminars in respiratory and critical care medicine


reactivity in Histoplasma capsulatum variety capsulatum antigen assays of urine samples from patients with endemic mycoses


https://www.ncbi.nlm.nih.gov/pubmed/?term=Exploratory+study+of+proteins+in+urine+of+patients+with+histoplasma+antigenuria


Figure 1: Results of the *H. capsulatum* quantitative (A) and semi-quantitative (B) antigen 2 capture ELISA (IMMY). Comparison of antigenuria in culture-proven histoplasmosis patients, persons with other diseases, and healthy controls.

Figure 2: Study subjects and urine samples analyzed during the validation of the commercial IMMY *Histoplasma capsulatum* antigen capture monoclonal ELISA.
*All infectious diseases were co-infections with HIV in these patient cohorts.
546 HIV patients with clinical suspicion of histoplasmosis enrolled in the study

Colombia patients (n=80):
- Histoplasmosis (n=28)
- *Mycobacterium* disease (n=31) ≠
- Cryptococcosis (n=8)
- *Pneumocystis* pneumonia (n=3)
- Paracoccidioidomycosis (n=3)
- Aspergillosis (n=2)
- Candidiasis (n=1)
- *Salmonella* disease (n=2)
- Toxoplasmosis (n=1)
- *Cytomegalovirus* disease (n=1)

Guatemala patients (n=466):
- Histoplasmosis (n=35) ≠
- HIV/AIDS (n=371)
- *Mycobacterium* disease (n=56) ≠
- Cryptococcosis (n=4)

Addition baseline urine controls
(n=43)
- Non-PLHIV controls resident in endemic area (Medellin, Colombia) (n=43)

Baseline urines tested (n=589):
- 63 histoplasmosis cases
- 526 non histoplasmosis cases

(≠) Three patients were co-infected with *Mycobacterium* (*M. tuberculosis* n=2; *M. non tuberculosis* n=1).

(*) Of the n=87, 65 were identified as *M. tuberculosis*, and 22 were *M. non tuberculosis*.

(≠) (*) Of the n=87 with *Mycobacterium* disease, 4 present co-infection with *Cryptococcus* (n=2), *Toxoplasma* and *Clostridium* (n=1, each).
Table 1. Evaluation of the analytical performance of the quantitative IMMY monoclonal ELISA (HGM)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined (n=589)</td>
<td>98 (95-100)</td>
<td>97 (95-99)</td>
<td>97 (96-99)</td>
<td>79 (70-89)</td>
<td>100 (99-100)</td>
</tr>
<tr>
<td>Guatemala (n=466)</td>
<td>100 (99-100)</td>
<td>97 (96-99)</td>
<td>98 (96-99)</td>
<td>76 (63-90)</td>
<td>100 (99-100)</td>
</tr>
<tr>
<td>Colombia (n=123)</td>
<td>96 (88-100)</td>
<td>95 (90-100)</td>
<td>95 (91-99)</td>
<td>84 (70-99)</td>
<td>99 (97-100)</td>
</tr>
</tbody>
</table>

n: number of samples; CI: Confidence Interval 95%; PPV: positive predictive value; NPV Negative predictive value
Table 2. Analysis of concordance between Quantitative IMMY (HGM) and Culture

<table>
<thead>
<tr>
<th></th>
<th>A. Combined cohort</th>
<th>B. Guatemala cohort</th>
<th>C. Colombia cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=589)</td>
<td>(n=466)</td>
<td>(n=123)</td>
</tr>
<tr>
<td>Culture</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quantitative IMMY (HGM)</td>
<td>62</td>
<td>35</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Culture</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Quantitative IMMY (HGM)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>510</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>90</td>
</tr>
</tbody>
</table>

K: 0.86 (0.79-0.93)  K: 0.85 (0.76-0.94)  K: 0.87 (0.76-0.97)

n: number of samples; (+) positive result; (-) negative result; K: Kappa index and (Confidence Interval 95%);
Table 3. Characteristics of patient’s samples with discrepant results using the IMMY monoclonal ELISA (HGM)

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Case definition</th>
<th>Quantitative IMMY (HGM) Using standard curve Cutoff: ≥0.5 ng/mL</th>
<th>Semi-quantitative IMMY (HGM) Calibrator: 12.5 ng/mL Cutoff: ≥2.6 EIV</th>
<th>Clinical and laboratory findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Discrepant results against fungal negative culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>HIV infection</td>
<td>9.0 (+)</td>
<td>7.6 (+)</td>
<td>History of PDH, CDC HPA positive (7.6 ng/mL), 22 CD4 cells/µL.</td>
</tr>
<tr>
<td>2</td>
<td>HIV infection</td>
<td>116.4 (+)</td>
<td>19.7 (+)</td>
<td>CDC HPA positive (21.9 ng/mL), 49 CD4 cells/µL, weight of loss, anemia and diarrhea.</td>
</tr>
<tr>
<td>3</td>
<td>HIV infection</td>
<td>16.6 (+)</td>
<td>24.0 (+)</td>
<td>CDC HPA positive (8.1 ng/mL), 53 CD4 cells/µL, pulmonary symptoms and fever.</td>
</tr>
<tr>
<td>4</td>
<td>HIV infection</td>
<td>0.9 (+)</td>
<td>2.5 (-)</td>
<td>CDC HPA positive (9 ng/mL), 14 CD4 cells/µL, fever and pulmonary symptoms.</td>
</tr>
<tr>
<td>5</td>
<td>M. szlugai infection</td>
<td>0.8 (+)</td>
<td>2.4 (-)</td>
<td>History of PDH, CDC HPA negative (0.6 ng/mL), asymptomatic patient with no adherence to treatment.</td>
</tr>
<tr>
<td>6</td>
<td>HIV infection</td>
<td>0.8 (+)</td>
<td>2.5 (-)</td>
<td>History of PDH, CDC HPA negative (0.46 ng/mL), 59 CD4 cells/µL, viral load: 103,571 copies/mL, diarrhea and weight of loss.</td>
</tr>
<tr>
<td>7</td>
<td>HIV infection</td>
<td>0.8 (+)</td>
<td>2.5 (-)</td>
<td>History of PDH, 169 CD4 cells/µL, in treatment with itraconazole for 6 months.</td>
</tr>
<tr>
<td>8</td>
<td>HIV infection</td>
<td>2.2 (+)</td>
<td>4.2 (+)</td>
<td>5 CD4 cells/µL, VL: 227,098 copies/mL, chronic diarrhea, oral candidiasis and wasting syndrome.</td>
</tr>
<tr>
<td>9</td>
<td>HIV infection</td>
<td>1.9 (+)</td>
<td>3.2 (+)</td>
<td>57 CD4 cells/µL, pulmonary symptoms and fever postpartum.</td>
</tr>
<tr>
<td>10</td>
<td>HIV infection</td>
<td>0.9 (+)</td>
<td>2.6 (+)</td>
<td>51 CD4 cells/µL, VL: 171,475 copies/mL, diarrhea, weight of loss, fever, oral candidiasis and wasting syndrome.</td>
</tr>
<tr>
<td>11</td>
<td>TB</td>
<td>5.2 (+)</td>
<td>5.5 (+)</td>
<td>27 CD4 cells/µL, VL: 718,093 copies/mL, co-infection with TB, wasting syndrome, acute renal failure and chronic diarrhea.</td>
</tr>
<tr>
<td></td>
<td>False negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>PDH</td>
<td>0.9 (+)</td>
<td>2.5 (-)</td>
<td>Diagnose of PDH by culture.</td>
</tr>
<tr>
<td></td>
<td>Discrepant results against fungal negative culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Salmonella infection</td>
<td>83.7 (+)</td>
<td>60.2 (+)</td>
<td>CDC HPA positive (12.6 ng/mL) and ID positive (M band).</td>
</tr>
<tr>
<td>14</td>
<td>Salmonella infection</td>
<td>53.8 (+)</td>
<td>38.0 (+)</td>
<td>CDC HPA positive (12.9 ng/mL) and ID positive (M band), 44 CD4 cells/µL.</td>
</tr>
<tr>
<td></td>
<td>Cross reactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Paracoccidioidomycosis</td>
<td>27.3 (+)</td>
<td>21.0 (+)</td>
<td>Cross reactivity with P. brasiliensis antigents.</td>
</tr>
<tr>
<td>16</td>
<td>Paracoccidioidomycosis</td>
<td>18.7 (+)</td>
<td>14.1 (+)</td>
<td>Cross reactivity with P. brasiliensis antigents.</td>
</tr>
<tr>
<td>17</td>
<td>Paracoccidioidomycosis</td>
<td>1.4 (+)</td>
<td>3.0 (+)</td>
<td>Cross reactivity with P. brasiliensis antigents.</td>
</tr>
<tr>
<td></td>
<td>False negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>PDH</td>
<td>0.2 (-)</td>
<td>1.4 (-)</td>
<td>Diagnose of PDH by culture.</td>
</tr>
<tr>
<td>19</td>
<td>PDH</td>
<td>0.5 (+)</td>
<td>1.6 (-)</td>
<td>Diagnose of PDH by culture.</td>
</tr>
</tbody>
</table>

#: number; HGM: Histoplasma galactomannan; EIV: EIA Index Value; PDH: progressive disseminated histoplasmosis; CDC HPA: CDC polyclonal ELISA; ID: immunodiffusion; VL: viral load. All infectious diseases were co-infections with HIV in these patient cohorts. Antigenuria result interpretation: (+) positive result; (-) negative result.
Table 4. Evaluation of the analytical performance of the semi-quantitative IMMY monoclonal ELISA (HGM)

<table>
<thead>
<tr>
<th>Calibrator concentration (Cutoff in EIA Index Value [EIV])</th>
<th>Analytical performance: % (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 ng/mL</td>
<td>25 ng/mL</td>
</tr>
<tr>
<td>(≥1.5 EIV)</td>
<td>(≥2.6 EIV)</td>
</tr>
<tr>
<td>Sen</td>
<td>94 (87-100)</td>
</tr>
<tr>
<td>Spe</td>
<td>97 (96-99)</td>
</tr>
<tr>
<td>Acc</td>
<td>97 (96-98)</td>
</tr>
<tr>
<td>PPV</td>
<td>80 (71-91)</td>
</tr>
<tr>
<td>NPV</td>
<td>99 (98-100)</td>
</tr>
<tr>
<td>Guatemala (n=466)</td>
<td>Sen</td>
</tr>
<tr>
<td></td>
<td>Spe</td>
</tr>
<tr>
<td></td>
<td>Acc</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
</tr>
<tr>
<td>Colombia (n=123)</td>
<td>Sen</td>
</tr>
<tr>
<td></td>
<td>Spe</td>
</tr>
<tr>
<td></td>
<td>Acc</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
</tr>
</tbody>
</table>

n: number of samples; CI: Confidence Interval 95%; Sen: sensitivity; Spe: specificity; Acc: Accuracy; PPV: positive predictive value; NPV Negative predictive value, (*) better performance