

# Validation of an Enzyme-Linked Immunosorbent Assay That Detects *Histoplasma capsulatum* Antigenuria in Colombian Patients with AIDS for Diagnosis and Follow-Up during Therapy

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We validated an antigen capture enzyme-linked immunosorbent assay (ELISA) in Colombian persons with AIDS and proven histoplasmosis and evaluated the correlation between antigenuria and clinical improvement during follow-up. The sensitivity of the *Histoplasma capsulatum* ELISA was 86%, and the overall specificity was 94%. The antigen test successfully monitored the response to therapy.

**H**istoplasmosis is a disease caused by the fungus *Histoplasma capsulatum* and is most frequently diagnosed in the American continent. In persons living with HIV/AIDS (PLWHA), an infection often develops into a clinical form called progressive disseminated histoplasmosis (PDH), which has a high mortality if not treated early (1, 2). PDH symptoms are nonspecific and may be similar to those of other infectious diseases, thus complicating diagnosis and treatment (1, 3). In Colombia and other countries in the Americas where the disease is endemic and where access to highly active antiretroviral therapy (HAART) is limited, histoplasmosis is a major cause of mortality (up to 30%) in PLWHA (4–9).

Detection of circulating *Histoplasma* antigens in urine specimens by an antigen capture enzyme-linked immunoabsorbent assay (ELISA) is highly sensitive (95%), but this test is not generally available outside the United States (10, 11). To make such a test

available to resource-challenged countries, a similar *Histoplasma* antigen capture ELISA was developed at the Centers for Disease Control and Prevention (CDC) (12) and was validated in a cohort of AIDS patients in Guatemala, demonstrating a sensitivity of 81%

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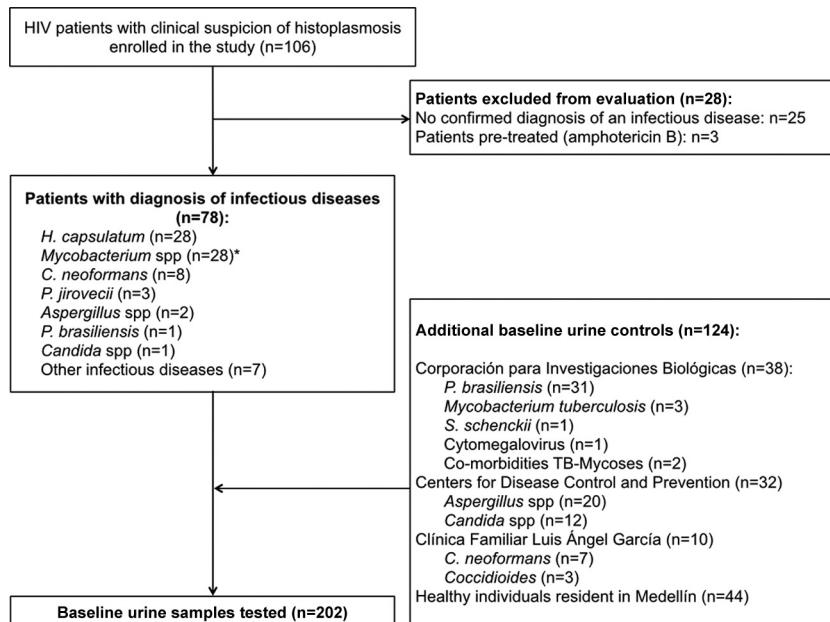
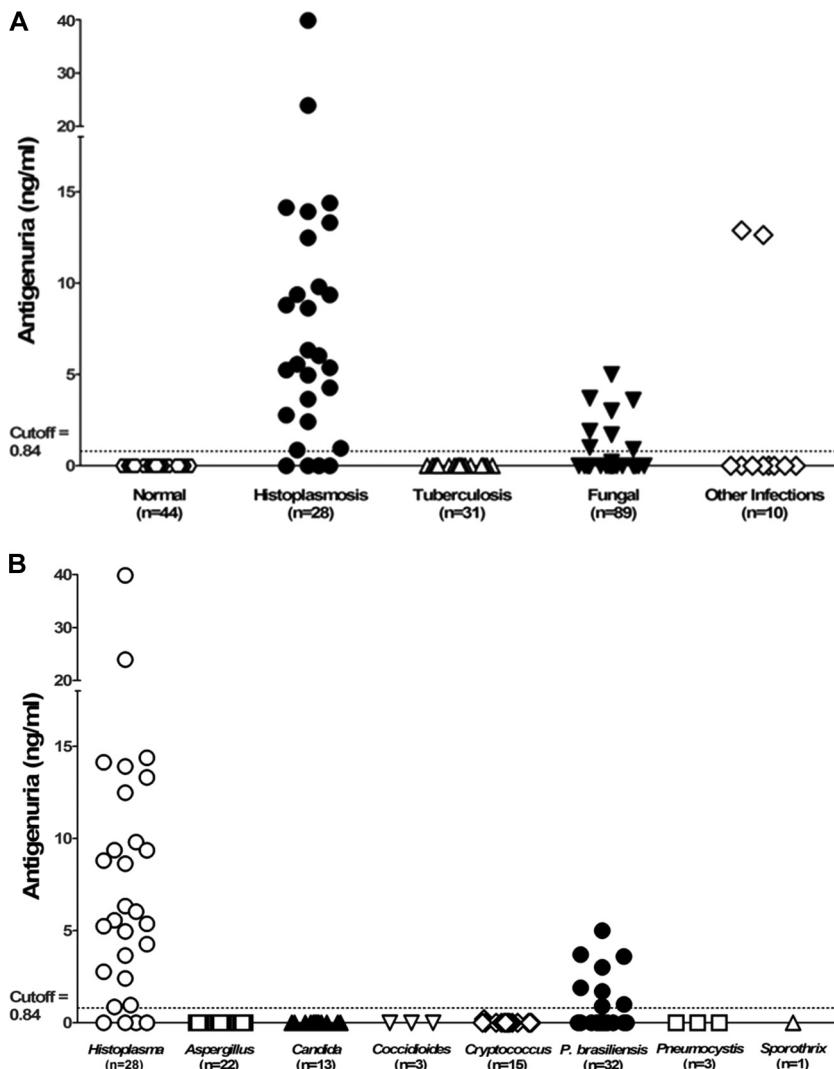


FIG 1 Study subjects and urine samples analyzed during the validation of the *Histoplasma capsulatum* antigen capture ELISA. Of the 28 *Mycobacterium* sp. infections, 23 were identified as *M. tuberculosis* and 1 was identified as *M. avium*-*Mycobacterium intracellulare*; 4 could not be identified to the species level.



**FIG 2** Results of the *H. capsulatum* antigen capture ELISA. Comparison of antigenuria in culture-proven histoplasmosis patients, persons with other diseases, and healthy controls ( $n = 202$ ) (A) and antigenuria of patients with proven fungal infections ( $n = 117$ ) (B).

and a specificity of 95% (7). The aims of our study were to validate the CDC antigen capture ELISA in a Colombian cohort of PLWHA with proven PDH and to determine whether decreases in antigenuria over time correlated with clinical improvements.

A prospective study was conducted from May 2008 to August 2011 at the Hospital La María in Medellín, Colombia. Patients who were enrolled presented with at least three of the following symptoms: fever, pancytopenia, weight loss, the presence of skin or mucosal lesions, and pulmonary involvement by radiography. Patient selection is described in Fig. 1. Enrolled patients were diagnosed with histoplasmosis according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group/National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) definitions (13). A diagnosis was considered proven if *H. capsulatum* could be isolated from any of the following sample types: blood, tissue, sterile fluid, or respiratory. Patients who had previously received amphotericin B or itraconazole or who had a diagnosis of histoplasmosis prior to the enrollment period were excluded from the study.

The median CD4 count for the 28 patients with proven histo-

plasmosis and AIDS was 84 cells/ $\mu$ l (range, 3 to 570 cells/ $\mu$ l). Fifty patients were diagnosed with other infections: mycobacterial (tuberculosis [TB] and not TB) ( $n = 28$  patients), other fungal infections ( $n = 15$ ), and other infections ( $n = 7$ ), which included parasitic infection ( $n = 2$ ), viral infection ( $n = 1$ ), bacterial infection ( $n = 1$ ), and confirmed TB and coinfections ( $n = 2$  with cryptococcosis and 1 with toxoplasmosis). Additionally, we analyzed a total of 124 urine samples that were not part of the Hospital La María cohort, which included 80 patients with positive cultures for other infectious diseases and 44 samples from healthy individuals from Medellín, Colombia.

Patients were evaluated clinically at the time of diagnosis and at each one of the follow-up visits (weeks 1, 2, 4, 8, 16, 24, 36, and 48). Patients received 1 year of specific antifungal treatment in agreement with consensus recommendations (amphotericin B deoxycholate [0.7 to 1.0 mg/kg of body weight daily] for 2 weeks, followed by itraconazole solution [600 mg for 3 days and then 400 mg daily]) (14). Responses to therapy were measured according to the scoring system of Restrepo et al. (15; see also supplemental material).

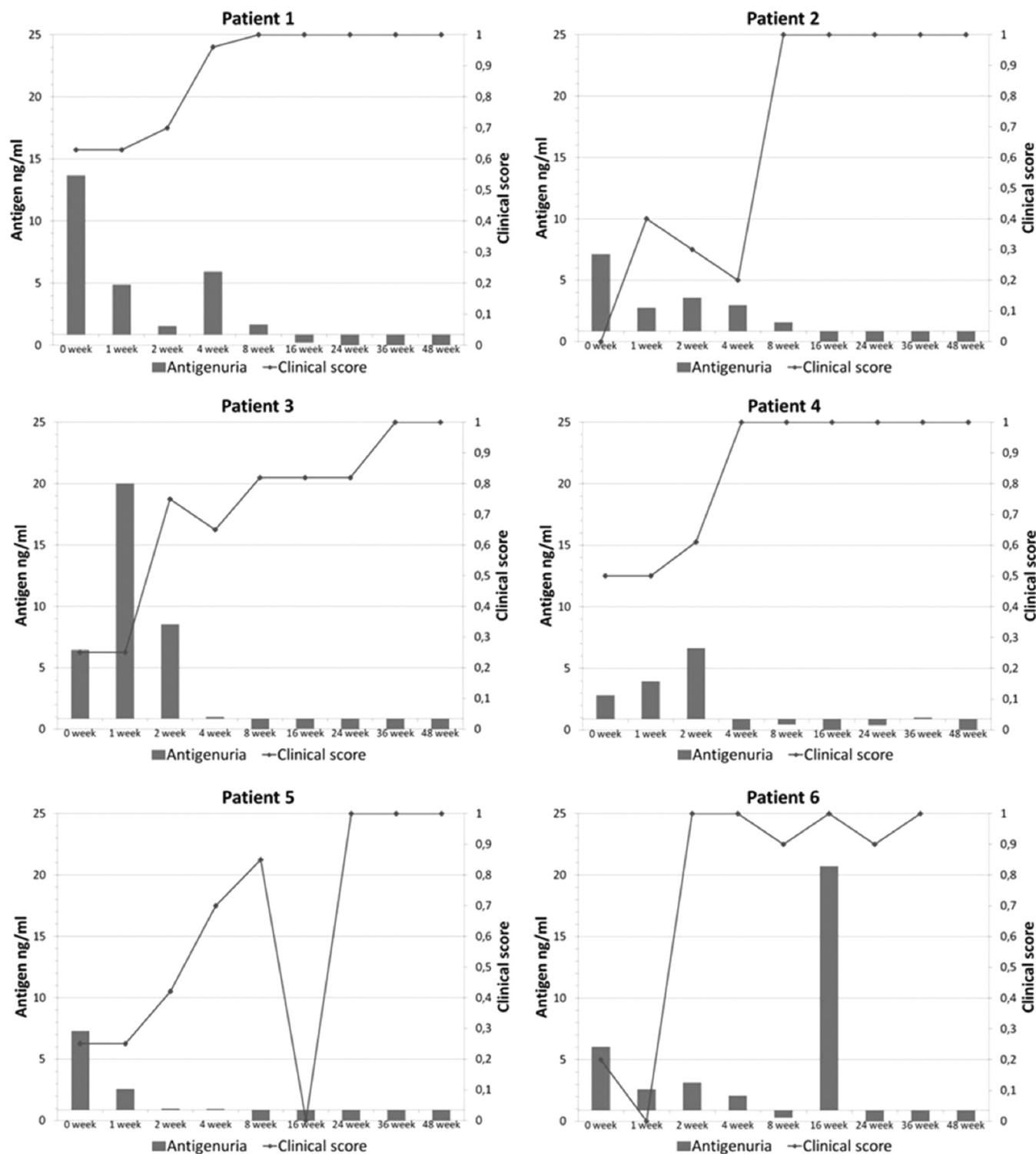


FIG 3 Results of the follow-up using antigenuria and a clinical score system in 6 patients with PDH and AIDS during treatment (48 weeks).

Urine samples from all patients were used for the assay validation. The ELISA antigen detection test uses a polyclonal antibody that recognizes an *H. capsulatum* polysaccharide antigen (HPA), as previously described (7).

Results are shown in Fig. 2A and B. The cutoff point for this assay was 0.84 ng/ml *H. capsulatum* antigen. The area under the

curve (receiver operating characteristic [ROC] area) was 0.90 (95% confidence interval, 0.83 to 0.96). The sensitivity was 86% (95% CI = 71 to 100%), and the overall specificity (for healthy controls and persons with other diseases) was 94% (95% CI, 91 to 98%). In the group of 89 urine samples from patients with other mycoses, the specificity was 91% (95% CI, 84 to 97%), with cross-

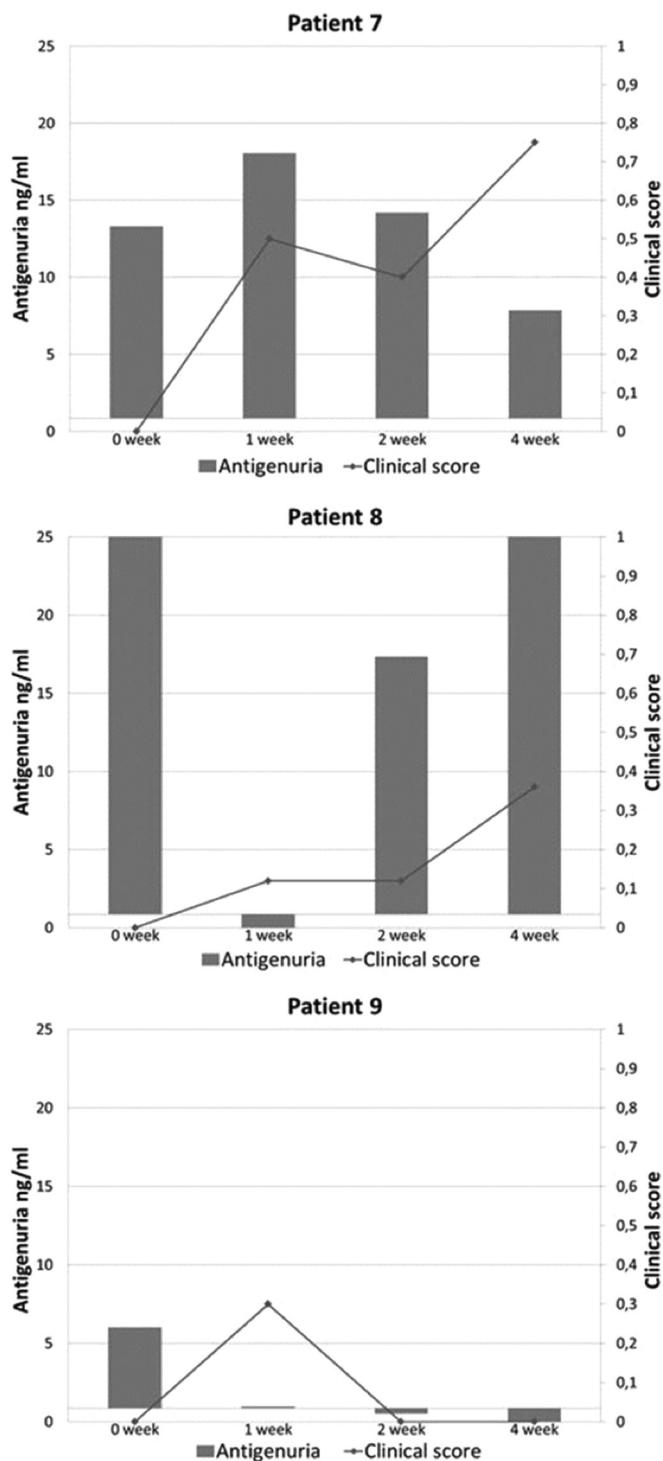


FIG 4 Results of the follow-up using antigenuria and a clinical score system in 3 patients with PDH and AIDS who died during the first month of treatment.

reactions noted in urine samples from patients with paracoccidioidomycosis (PCM) (Fig. 2A and B). Prior studies of *H. capsulatum* antigenuria tests have reported cross-reactivity with urine samples from patients with PCM (Scheel et al., 28% [7] and Wheat et al., 88% [16]), but it is important to note that PCM is diagnosed much less frequently than histoplasmosis in PLWHA (17). For serology details, see the supplemental material.

We were able to follow 9 of the 28 culture-proven histoplasmosis patients, 6 of them for 48 weeks and 3 for 2 months (until their deaths; see below). In the first 6 patients, urinary antigen was measurable at diagnosis, as well as during the first 4 weeks at variable concentrations, and all showed a marked decrease in antigen concentration after treatment. In 4 of these patients (patients 1 to 4), we observed concomitant and progressive gains in the clinical score as antigenuria decreased (Fig. 3); the remaining 2 patients (patients 5 and 6) had comorbidities such as TB, *Pneumocystis carinii* pneumonia (PCP), and *Salmonella* species infections, and we noted a poor response in the clinical score despite the decreases in antigenuria during the course of treatment.

The remaining 3 patients with culture-proven histoplasmosis presented with comorbidities and died after 8 weeks of treatment: 2 patients (patients 7 and 8) had persistent elevated *H. capsulatum* urinary antigen concentrations accompanied by an insignificant increase in the clinical score. Patient 7 was diagnosed with cerebral toxoplasmosis and sepsis with *Escherichia coli* infection, and patient 8 had bacterial pneumonia and septic shock. The third patient (patient 9) was diagnosed with cytomegalovirus (CMV) infection and pneumonia with *Enterococcus* species infection, and the *H. capsulatum* urinary antigen concentration gradually decreased but the clinical response worsened despite the continuous administration of specific antifungal treatment (Fig. 4). Comorbidities recorded in the group of fatal cases are presented in Table S1 in the supplemental material. A detailed discussion of these and other results is included in the supplemental material.

There was excellent correlation with the reported sensitivity and specificity of this test as validated in Guatemala (7). The high sensitivity and specificity of the antigen capture ELISA in the diagnosis of histoplasmosis in PLWHA has been demonstrated in a Colombian laboratory. The ELISA can be performed in less than 1 day, and the materials required for the test are inexpensive. This technique is robust and highly reproducible and significantly reduces the time to diagnosis of PDH. Additionally, this antigen test has potential for use to monitor and evaluate antifungal therapy responses in patients with AIDS and PDH, especially in those who are severely immunosuppressed.

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