Development and Evaluation of an Enzyme-Linked Immunosorbent Assay To Detect *Histoplasma capsulatum* Antigenuria in Immunocompromised Patients[▽]

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Histoplasma capsulatum infection causes significant morbidity and mortality in human immunodeficiency virus-infected individuals, particularly those in countries with limited access to rapid diagnostics or antiretroviral therapies. The fungus easily disseminates in persons with AIDS, resulting in progressive disseminated histoplasmosis (PDH), which can progress rapidly to death if undiagnosed. The availability of a simple, rapid method to detect *H. capsulatum* infection in less developed countries where the infection is endemic would dramatically decrease the time to diagnosis and treatment of PDH. We have developed an antigen-capture enzyme-linked immunosorbent assay (ELISA) to detect PDH antigenuria in infected patients. The assay uses polyclonal antibodies against *H. capsulatum* as both capture and detection reagents, and a standard reference curve is included to quantify antigenuria and ensure reproducibility. We evaluated this assay using specimens collected from patients with AIDS and culture-proven histoplasmosis in a Guatemalan clinic ($n = 48$), from healthy persons ($n = 83$), and from patients with other, nonhistoplasmosis diseases ($n = 114$). The ELISA demonstrated a sensitivity of 81% and a specificity of 95% in detecting *H. capsulatum* antigen in urine. This assay relies on simple technology that can be performed in institutions with limited resources. Use of this test will facilitate rapid diagnosis of PDH in countries where mortality is high, expediting treatment and likely reducing PDH-related mortality.

Histoplasmosis is an environmentally acquired mycosis caused by the dimorphic fungus *Histoplasma capsulatum*, which exists in its mycelial form at ambient temperatures and in its yeast form in infected tissues. Although the microorganism is found worldwide, major foci exist in the Midwestern United States and Central America, in areas where the soil is enriched with bird and bat guano. Infection occurs when the soil is disturbed and fungal conidia are inhaled. Histoplasmosis typically manifests as a benign respiratory infection and, at times, may be entirely asymptomatic. In some individuals, especially immunocompromised persons, *H. capsulatum* readily disseminates, causing significant morbidity and mortality (12).

Progressive disseminated histoplasmosis (PDH) is often fatal to persons with AIDS, especially those residing in rural, resource-poor areas of endemicity with limited access to clinical care and highly active antiretroviral therapy. Often, human immunodeficiency virus (HIV)-infected persons from rural areas do not seek treatment until secondary symptoms render them unable to work. Furthermore, PDH symptoms are nonspecific, and in the case of persons with AIDS, they may appear similar to those of other infections that require different treatment strategies (4, 8).

Laboratory methods to detect *H. capsulatum* infections in resource-limited countries, particularly in Central America, are labor-intensive, expensive, and slow, precluding a specific and timely diagnosis. Clinicians must rely on culture methods to confirm the presence of *H. capsulatum*. The slow growth of the organism can sometimes delay diagnosis and treatment by several weeks. It is not uncommon for persons with AIDS to die before a diagnosis of PDH is confirmed. A simple, rapid method to detect *H. capsulatum* infection in these regions of endemicity would dramatically decrease the time to diagnosis and treatment, leading to reduced morbidity and mortality.

Other high-efficacy antigen-capture enzyme-linked immunosorbent assays (ELISAs) to detect histoplasmosis have been reported in the literature (2, 3, 7, 26). One of these assays, in particular, is commercially available in the United States (MiraVista Diagnostics assay) (3, 26), but the reagents and methodology are proprietary and the assay is not performed outside of that laboratory. These factors, combined with the difficulty and expense of intercontinental specimen transportation, the cost of the test, and the added delay in receiving the results, severely limit the utility of this commercial assay in resource-poor settings.

Prior work in our laboratory resulted in the selection of a polyclonal antibody (PAb) reagent generation protocol for use in an *H. capsulatum* enzyme immunoassay (EIA) to detect antigenuria (16). The EIA has since been modified, resulting in increased assay sensitivity and reduction of the nonspecific background. Here we evaluate this test as a diagnostic tool for

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histoplasmosis in a prospective study of an HIV-infected population with febrile illness treated at a clinic in Guatemala.

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MATERIALS AND METHODS

Study site. The Clinica Familiar Luis Angel Garcia (CFLAG) is a nonprofit HIV/AIDS clinic housed within the Hospital General San Juan de Dios, one of Guatemala's two national hospitals, located in Guatemala City. The clinic performs more than 4,000 HIV tests per year, 17% of which give positive results. Patients at CFLAG are usually uninsured and of low socioeconomic status. They receive free HIV testing, counseling, and disease management drugs at the clinic.

Study design and specimen collection. From January 2005 through November 2007, CFLAG prospectively enrolled adult patients diagnosed with HIV who presented with at least three of six common indicators for disseminated histoplasmosis (fever, weight loss, diarrhea, hepatomegaly and/or splenomegaly, pancytopenia, and radiologic evidence [miliary pattern on chest X-ray]). Informed consent was obtained for each participant. Patients who consented to join were asked to respond to questions specific to their medical history and had baseline blood, bone marrow, and urine specimens collected on the initial clinic visit. A portion of the blood was allowed to clot, and serum was obtained. Urine and serum specimens were sent, without identifiers, to the Centers for Disease Control and Prevention (Atlanta, GA) for further study. Additionally, baseline urine specimens from patients with culture-proven nonhistoplasmosis fungal infections from prior studies were included. Urine specimens were also collected from healthy volunteers in Guatemala and the United States (CDC Blood Services Group, Atlanta, GA).

Specimen culture. Blood and bone marrow specimens were apportioned and cultured for bacteria (under anaerobic and aerobic conditions) and for fungi by the lysis centrifugation concentration method according to the manufacturer's instructions (Wampole Isolator microbial system; Inverness Medical Professional Diagnostics, Princeton, NJ). Specimen concentrates were subsequently cultured for fungi and mycobacteria by use of Mycosel (BD, Franklin Lakes, NJ), Sabouraud dextrose agar (BD), and Löwenstein-Jensen medium (BD). Only patients with positive *Histoplasma capsulatum* cultures obtained from blood, bone marrow, or other sterile sites were considered to have confirmed cases of histoplasmosis. Baseline urine samples from patients from whom other infectious agents were recovered by culture were also included for testing.

ELISA reagent preparation. (i) Immunogen. *H. capsulatum* yeast-phase cells (Thon strain) were cultured at 37°C for 72 h in Pine's liquid medium [20] and then killed with thimerosal as described by Lindsley et al. [16]. Yeast cells were harvested by centrifugation at $1,500 \times g$ and rinsed three times in 0.01 M phosphate-buffered saline, pH 7.2 (PBS). Killed yeast cells were resuspended in PBS containing 0.01% thimerosal and packed by centrifugation to a 40% (vol/vol) solution of cells. Packed yeast cells were resuspended and diluted in PBS containing 0.01% thimerosal to achieve a final solution of 0.4% (vol/vol) whole, killed *H. capsulatum* immunogen.

(ii) Rabbit immunization. Female New Zealand White rabbits ($n = 33$) weighing 2 to 4 kg were immunized intravenously with 0.5 ml of the 0.4% *H. capsulatum* immunogen on days 0, 1, 4, 8, 10, 15, 18, 23, 29, and 32 (10 injections in three separate trials), as previously described by Lindsley et al. [16], but no subsequent intramuscular injections were given. During the first trial performed at the CDC ($n = 6$ rabbits), postimmunization test bleeds (5 ml) were taken on days 14, 28, and 42 and rabbits were exsanguinated on day 49. Rabbits from both the second trial (CDC) ($n = 6$) and the third trial (Lee Laboratories, Grayson, GA) ($n = 21$) were test bled on days 14 and 28 and exsanguinated on day 42. Sera from all test bleeds and exsanguinations were collected for screening by indirect ELISA.

(iii) Screening of rabbit sera by indirect ELISA. Common cell wall antigen (C-Ag), a heterogeneous polysaccharide [11], was fractionated from histoplasmin prepared from a mycelial culture filtrate of *H. capsulatum* (ATCC 26320) by cation-exchange chromatography [21, 30]. The C-Ag contained no detectable protein, as measured by the Bradford protein assay [1] (Bio-Rad, Hercules, CA). The carbohydrate concentration of the C-Ag was determined using the phenol-sulfuric acid method [5]. The C-Ag was then diluted in PBS to a concentration of 2 µg/ml and adsorbed (100 µl/well) to Immulon 2HD flat-bottomed, 96-well microtiter plates (Thermo Fisher Scientific Inc., Waltham, MA), which were incubated at room temperature (RT) for 2 h while being shaken on a titer plate shaker (Lab-Line, Melrose Park, IL). Plates were rinsed six times in 250 µl/well PBS containing 0.05% Tween 20 (PBS-Tw) on an automatic plate washer (ELx50 AutoStrip washer; Bio-Tek Instruments, Inc., Winooski, VT). Rabbit test serum was diluted 1:100 in PBS containing 0.3% Tween 20 and 5% nonfat dry milk. The

test serum solution was titrated twofold across the microtiter plates (100 µl/well) and incubated at RT for 5 min, with shaking. Plates were rinsed as before, followed by detection of reactive antibody, using Immunopure goat anti-rabbit immunoglobulin G (heavy plus light chains)–peroxidase (Pierce, Waltham, MA) diluted in PBS-Tw and dispensed at 100 µl/well. Plates were incubated at RT for 5 min with shaking, rinsed in PBS-Tw as described above, and then rinsed once with 250 µl/well deionized water. Enzyme substrate (TMB SureBlue peroxidase substrate system; KPL, Gaithersburg, MD) was dispensed (100 µl/well) and incubated at RT for 5 min with shaking, followed by the addition of 100 µl/well 1 M H_2SO_4 to stop the colorimetric reaction. Absorbance was read at 450 nm, using a SpectraMax 250 microplate spectrophotometer and SoftMax Pro data acquisition software (Molecular Devices, Toronto, Canada). The reactivities of individual rabbit sera from all three trials were compared on each plate to the reactivity of an unimmunized rabbit as well as to that of a positive control rabbit that had been immunized using a similar protocol in a previous trial [16]. Sera from rabbits with the highest reactivities to C-Ag were precipitated individually, using three rounds of 35% saturated ammonium sulfate as described by Hebert [10], and dialyzed twice against a 1,000× volume of PBS at 4°C for 24 h. The resulting PAb were quantitated using the Bradford protein assay [1] with a gamma globulin standard, diluted in PBS–40% glycerol, and stored at −20°C for later evaluation in an antigen-capture ELISA format.

(iv) Conjugation of PAb used as detection reagent. Exsanguination serum from an immunized rabbit showing high reactivity to C-Ag was ammonium sulfate precipitated as described above, and the resulting PAb was conjugated to horseradish peroxidase (HRPO) type VI (Sigma-Aldrich, St. Louis, MO), using the periodate method described by Wilson and Nakane [29]. The anti-*H. capsulatum* rabbit PAb–HRPO conjugate was then dialyzed in 3,000-molecular-weight-cutoff tubing (Pierce, Rockford, IL) against 4 liters of PBS at 4°C, changed three times over a 36-h period. The conjugate was stored at −20°C in 40% glycerol.

(v) Standard curve. An eight-point standard reference curve was prepared at a 10× concentration, using *H. capsulatum* C-Ag diluted in PBS. The final 10× concentrations of the C-Ag standards (ng/ml) were 5, 10, 25, 50, 100, 250, 500, and 1,000. The lower limit of C-Ag antigen detection in the ELISA when C-Ag was diluted to 1× was then 0.5 ng/ml, and the upper limit was 100 ng/ml. Unit values from 0.5 to 100 were assigned to the standards to represent ng/ml antigen detection, and a zero standard (PBS only) was included for a total of nine reference points.

Selection of rabbit PAb capture reagent by ELISA. Rabbit PAb against whole, killed *H. capsulatum* yeast cells were evaluated for reactivity against three of the C-Ag reference standards (units 5, 10, and 25), urines from patients with culture-proven cases of PDH ($n = 3$), and urines from healthy persons ($n = 3$) in an antigen-capture format. The PAb were diluted to 10 µg/ml in PBS and adsorbed to Immulon 2HB 96-well microtiter plates at RT for 2 h with shaking. Test urines were boiled for 5 min and set aside at RT. Plates were rinsed six times with PBS-Tw, and PBS (90 µl/well) was dispensed into the wells receiving C-Ag standards while the remaining PAb-coated wells received 200 µl/well of boiled urine. The 10× C-Ag reference standards were then added (10 µl/well) singly to the wells containing PBS. For each PAb tested, a well containing PBS in place of urine was used to detect the background signal, and a blank well was included in each plate. Plates were incubated at RT for 1 h with shaking and then rinsed in PBS-Tw. The previously prepared rabbit PAb-HRPO conjugate was diluted in PBS containing 3% nonfat dry milk, and 100 µl/well was dispensed into the plates and incubated at RT for 30 min to detect bound antigen, followed by rinsing in PBS-Tw as described above. Colorimetric detection was achieved using 100 µl/well TMB (KPL) enzyme substrate. After the addition of the substrate, plates were incubated at RT for 15 min, followed by the addition of 100 µl/well 1 M H_2SO_4 stop solution. Absorbance was read at 450 nm, using a SpectraMax 250 spectrophotometer. Those PAb with the highest signal/noise ratios in detecting *H. capsulatum* antigen were pooled and used as an antigen capture reagent to test study patient urines and negative controls.

Antigen-capture ELISA evaluation with human urine. Baseline urine specimens were used to evaluate the *H. capsulatum* antigen-capture ELISA in a blinded method in which no patient identifiers or cultural information was made available until after the completion of all testing. The antigen-capture PAb reagent (10 µg/ml) was adsorbed to plates, and test urines were boiled for 5 min and set aside to cool at RT. After 2 h, the plates were rinsed as described above, and PBS (90 µl/well) and the nine 10× standards (10 µl each) were dispensed along the top row of each assay plate. The test urines were then dispensed (200 µl) singly in the remainder of the wells in the plate. Using the antigen-capture assay format described above, all patient urines and negative controls were tested on two consecutive days, and a third time if the coefficient of variance (CV) of
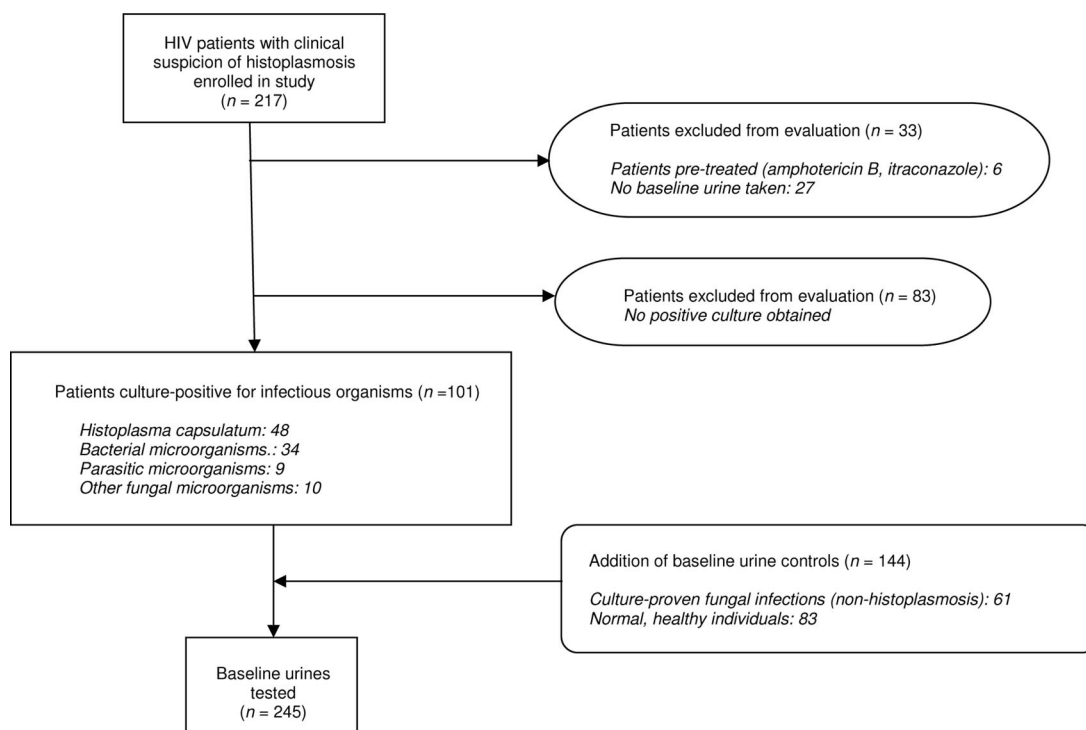
FIG. 1. Flow chart of study subjects.

the results was >15%. In addition, two reference standards from the linear portion of the curve (units 10 and 25) were included in each plate as unknown controls to ensure intraassay validity. Values for these standard controls were then compared from run to run to ensure reproducibility. Assay plates having standard control results with CVs of >15% from the mean of the previous runs were considered invalid. Additionally, a blank well and a well with PBS in place of urine were run in each plate to ensure that background noise would not affect the final test results.

Serology. Complement fixation (CF) and immunodiffusion (ID) tests were performed on baseline serum specimens from 48 patients who had positive *H. capsulatum* cultures. Patient sera were tested at the CDC against *Histoplasma* antigens prepared in-house according to the methods described by Kaufman et al. (13, 14, 17). In the CF test, patient sera were assayed against both whole yeast and histoplasmin, whereas in ID tests, sera were tested against histoplasmin alone.

Statistical analyses. A receiver operator characteristic curve was generated to evaluate the overall performance of this assay (SigmaPlot 11; Systat Software, Inc., San Jose, CA). Test reproducibility was analyzed using Microsoft Excel.

RESULTS

Study population and specimens tested. Patient selection is described in Fig. 1. Over the 2-year study period, a total of 217 patients were enrolled. Of these, 33 were excluded from evaluation because no baseline urine was available or they were receiving antifungal therapy at the time of the clinic visit and 83 were excluded because no infection was demonstrated by culture. Of the 101 remaining patients, 48 were diagnosed with culture-proven PDH and 53 were diagnosed with other infectious diseases. Bacterial infections were diagnosed in 34 patients (32 *Mycobacterium* and 2 *Shigella* infections), parasitic infections were diagnosed in 9 patients (5 *Toxoplasma*, 3 *Cryptosporidium*, and 1 *Cyclospora* infection), and other nonhistoplasmosis fungal infections were diagnosed in 10 patients (7 *Cryptococcus* and 3 *Coccidioides* infections). A total of 144 baseline urine specimens that were not a part of the Guate-

malan study cohort were also available for testing. These were comprised of samples from 61 patients with culture-proven fungal infections (20 *Aspergillus*, 12 *Candida*, 25 *Paracoccidioides*, and 4 other molds) and 83 specimens from healthy individuals (50 from Guatemala and 33 from the United States; CDC Blood Services Group).

Study population demographics. The demographics of the 48 patients with PDH were similar to those of the 53 patients diagnosed with other infectious diseases (Table 1). Patients with PDH were predominantly male (77%) and had a median age of 32 years (range, 19 to 70 years), compared with 75% male and a median age of 35 (range, 19 to 67 years) for patients with other infections. The median CD4 count for patients diagnosed with PDH (24/μl; range, 1 to 193/μl) was lower than that for patients with other infections (33/μl; range, 1 to 328 per μl). A larger number of patients with PDH died during the study period (40%; 19/48 patients) compared with patients with other diseases (26%; 14/53 patients). Four patients with PDH died prior to confirmation of diagnosis.

TABLE 1. Characteristics of HIV-infected patients with culture-proven infections

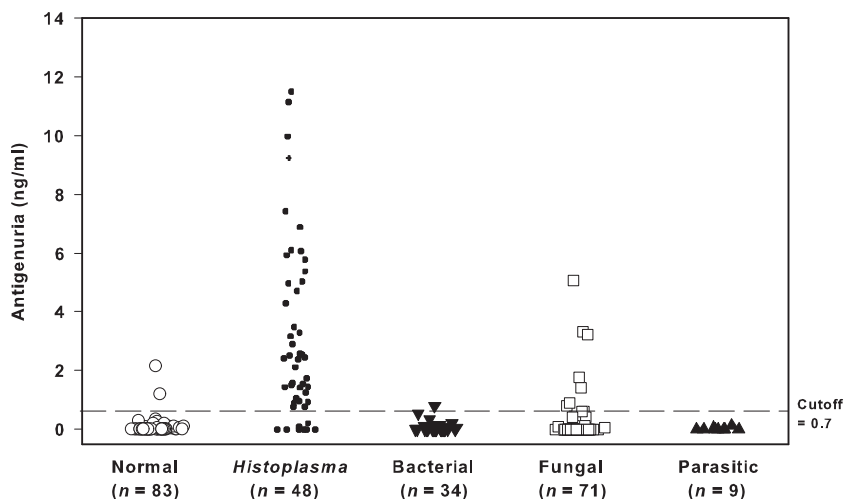| Study group (n)	No. (%) of males	Median age (yr) (range)	Median CD4 count (/μl) (range)	No. (%) of deaths
Patients with histoplasmosis (48)	37 (77)	32 (19–70)	24 (1–193)	19 (40)
Patients with other infections (53)	40 (75)	35 (19–70)	33 (1–328)	14 (26)

FIG. 2. Reactivities of urine specimens from healthy (normal) and infected patients in the *H. capsulatum* antigen-capture ELISA by disease status.

Selection of rabbit PAb. In the first trial, rabbits ($n = 6$) were exsanguinated on day 49. Five of these rabbits (83%) showed hyperimmunity greater than or equal to that of the positive control rabbit immunized previously (16). When sera from blood samples drawn on day 42 were compared by ELISA to the exsanguination sera (day 49), rabbits showed greater reactivity to C-Ag at day 42. This was in keeping with previous immunization data (16), and consequently, all rabbits in later trials were exsanguinated on day 42. In the second immunization trial, all rabbits ($n = 6$) showed greater hyperimmunity than the positive control rabbit. In the third immunization trial, 66% (14/21 animals) of rabbits showed hyperimmunity greater than or equal to that of the positive control rabbit. Overall, 76% of rabbits immunized yielded serum hyperimmune to C-Ag.

Antigen-capture ELISA efficacy. ELISA results are shown in Fig. 2. The *H. capsulatum* antigen-capture ELISA had a sensitivity of 81% (39/48 cases; 95% confidence interval [95% CI], 67 to 91%) in detecting culture-proven cases of histoplasmosis and an overall specificity of 95% (187/197 cases; 95% CI, 91 to 98%), with all negative controls combined. Healthy controls from the United States ($n = 33$) and Guatemala ($n = 50$) showed a specificity of 98%, with one patient from each group showing cross-reactivity. Healthy controls were grouped together during analyses because there were no appreciable differences in their reactivities, despite the geographic disparity of these cohorts. The specificity for all clinically confirmed cases of bacterial disease ($n = 34$) was 97%. Urines from patients with nonhistoplasmosis fungal diseases displayed the highest level of cross-reactivity, with an overall specificity of 90% (Fig. 3). Of the
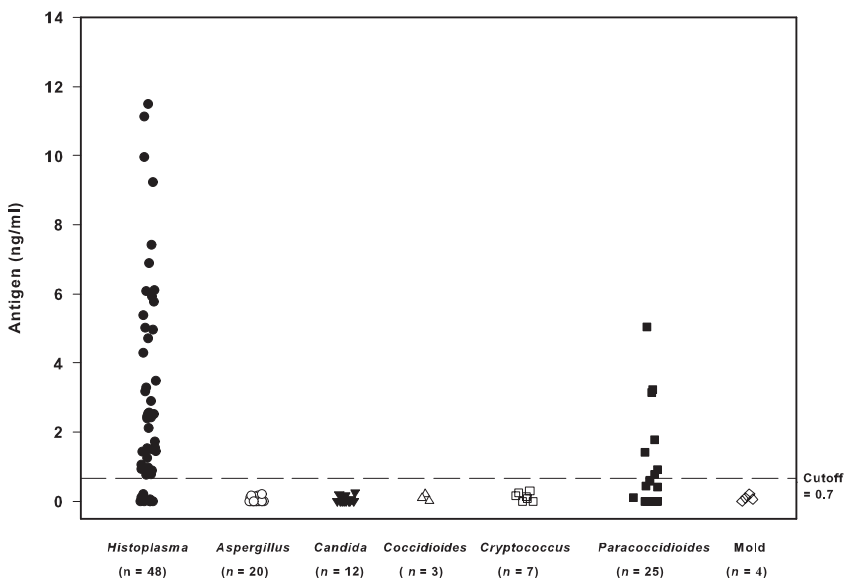

FIG. 3. Reactivities of urines from patients with culture-proven fungal infections ($n = 119$) in the *H. capsulatum* antigen-capture ELISA.
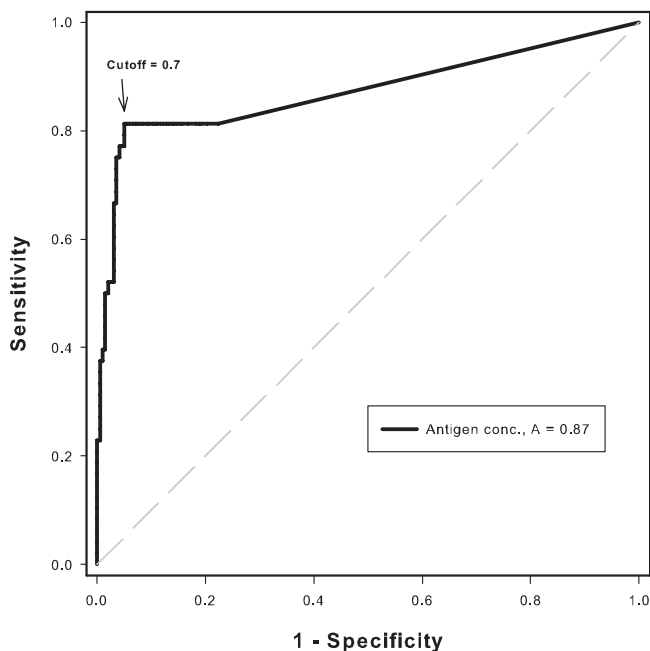
FIG. 4. Receiver operator characteristic curve of *H. capsulatum* antigen-capture ELISA efficacy, using results for human urine.

25 urines from patients with *Paracoccidioides brasiliensis*, 7 displayed a positive result in this assay. None of the 64 patients with other fungal diseases demonstrated any cross-reaction in the antigen-capture ELISA.

The ideal cutoff for this assay was determined to be 0.7 U on the standard curve, the equivalent of 0.7 ng/μl of C-Ag, by receiver operator characteristic curve analysis (Fig. 4). The area under the curve was 0.87 (95% CI, 0.80 to 0.95; standard error, 0.037; $P = <0.0001$), and positive and negative likelihood ratios were 16.1 and 0.2, respectively. The antigen-capture ELISA was highly reproducible, resulting in a low inter-assay variability; CVs for the two standard controls averaged 8% after 15 consecutive assays (data not shown).

Serology. The sensitivity of the CF test with baseline specimens from patients with culture-proven PDH was 48% (23 positive of 48 total specimens), using the standard diagnostic criterion of a serum titer of ≥1:32 against either whole *H. capsulatum* yeast or histoplasmin. An additional six patients (29 of 48 patients) had serum CF titers of ≥1:16, increasing the sensitivity to 60%. The sensitivity of the ID test using histoplasmin was 40% (19/48 patients) for this AIDS patient population, and all positive sera recognized the M antigen, while none formed a precipitin with the H antigen (11).

DISCUSSION

The ability to make a definitive diagnosis of histoplasmosis in resource-limited settings is challenging, especially for patients with AIDS, who are at high risk for disseminated disease and may have other concurrent illnesses producing similar symptoms. Fungal culture requires an invasive procedure where material is obtained from bone marrow, and microorganisms may take weeks to grow, long after treatment decisions need to be made. In our study, we evaluated a rapid and

simple non-culture-based assay to detect *H. capsulatum* in urines from a patient population with AIDS in Guatemala, a country where histoplasmosis is highly endemic. The *H. capsulatum* antigen-capture assay demonstrated a high sensitivity (81%) when tested against urines from patients who were proven to have PDH by culture, the "gold standard" method of diagnosis (9, 19). Specimens from patients with other diseases and from healthy persons showed few cross-reactions in this assay (Fig. 2), with the exception of those infected with *Paracoccidioides brasiliensis* (n = 25) (Fig. 3). Although 28% of those sera displayed false positivity, assay cross-reactivity between these two etiologic agents has been described previously in the literature, with values as high as 90% (18, 25). Because of this cross-reaction, differential diagnosis based on patient symptoms and classical serological methods will be necessary to distinguish these diseases in patients from areas where both diseases are endemic.

A total of 9 of the 48 patients (19%) with PDH were missed by the ELISA, yet all had been given various medicines as prophylaxis (trimethoprim-sulfamethoxazole, dapsone, and fluconazole). Although we excluded the results for patients who had prior treatment with drugs specific for histoplasmosis (amphotericin B and itraconazole) (Fig. 1), this patient population was severely immunocompromised, often taking many different medicines. It is difficult to pinpoint any single drug as capable of interfering with patient antigenuria in this assay, yet we do not rule out the possibility that certain drugs or drug combinations may have adversely affected our ability to detect disease in these cases (27). Since *H. capsulatum* antigen levels fluctuate over time (28), it is possible that the amount of antigen present in baseline urines from false-negative PDH patients was below the detection level. A recent study by MiraVista Diagnostics, using an EIA for histoplasmosis, has shown that concentrating false-negative urine specimens by ultrafiltration improves the sensitivity by 74% (6). Furthermore, it is possible that a specimen collected at a later time might demonstrate a positive result.

Serology results indicated that ID sensitivity for PDH patients was low (40%). The sensitivity of the CF test with a cutoff of ≥1:32 was also low (48%) but increased to 60% when we considered patients with titers of ≥1:16. Although a titer of ≥1:16 is below the conventional cutoff, it may be indicative of disease in immunocompromised patients. The median CD4 cell count of these patients was 24/μl (range, 1 to 193/μl) (Table 1), and consequently, the lack of an appropriate immune response exhibited by many of these patients is not surprising. Interestingly, five of the nine patient sera that were falsely negative by the antigen-capture ELISA were positive by the CF test (four had titers of ≥1:32 and one had a titer of ≥16), three of which were positive by ID. When antigen detection and serology results are combined in this study, sensitivity is increased to 92% (44/48 patients). Previous studies have described similar serology results for this patient population (15, 23), indicating that using a combination of diagnostic methods is the most effective strategy in detecting PDH. The caveat in relying on antibody detection to diagnose PDH is that antibodies against *H. capsulatum* are known to persist for several years and may be indicative of previous exposure (12), not active disease. Furthermore, the CF test in particular is not well suited to small, low-volume laboratories with re-

source limitations because the necessary reagents have a very limited shelf life.

In this study, we have shown that the generation of our PAb reagent is highly reproducible, with an average yield of 76% hyperimmune sera from immunized rabbits in three separate trials. The average PAb yields from the first two immunization trials at the CDC were actually much higher (11/12 rabbits; 92%) than that from the third trial, performed at the commercial laboratory, yet the reasons for this are unclear since the same antigen preparation and immunization procedures were used for all three trials. Rabbits exsanguinated on day 42 of the immunization protocol described here showed peak reactivity against C-Ag and yielded between 40 and 70 ml of hyperimmune sera, theoretically containing enough PAb to test ~30,000 patient specimens in triplicate. In initially developing this assay, we compared the efficacy of an immunoglobulin M monoclonal antibody against C-Ag as a detection reagent to that of our current PAb because we wanted to have a continuous, easily renewable source of antibody reagent for our assay (22). We found that the monoclonal antibody detection reagent lacked specificity (data not shown), and consequently, we used the PAb as both the capture and detection reagent.

Since large quantities of urine from culture-proven PDH patients were not available, we used a C-Ag standard reference curve to quantify the relative *H. capsulatum* antigen concentration values and to determine the run-to-run reproducibility of the assay. This *H. capsulatum*-derived carbohydrate antigen is easily prepared in large quantities and remains stable at 4°C for at least 1 year (data not shown). The standard units are expressed in values of ng/µl C-Ag, but it is possible that our PAb, which was prepared against whole *H. capsulatum* yeast cells, is capable of detecting other protein or glycoprotein antigens. Further evaluation of the PAb reagent will be necessary to determine which antigens, besides the C-Ag, can be detected in physiological specimens.

We have not evaluated this antigen-capture ELISA for the ability to detect primary acute histoplasmosis in HIV-negative individuals. Additionally, we would like to evaluate this assay more completely using serum specimens. Early in the study, we performed a pilot experiment aimed at detecting antigen in serum specimens ($n = 51$), but the volume of patient serum required was prohibitive (1.5 ml). The preliminary results were encouraging (93% sensitivity and 92% specificity) (data not shown), and further evaluation of this assay with sera appears to be warranted.

According to the United States Agency for International Development, Guatemala is experiencing an extensive HIV/AIDS epidemic, with nearly 1% of the total population infected as of 2007 (24). There are no incidence figures for PDH in that country, and thus we cannot provide reliable predictive values for our assay. However, it is important that culture-proven PDH patients ($n = 48$) outnumbered patients with mycobacterial infections ($n = 32$) in this cohort, suggesting that histoplasmosis is a prominent opportunistic infection in this patient population. In the future, the *H. capsulatum* antigen-capture assay may be helpful in ascertaining the burden of PDH in Guatemala.

In summary, the high efficacy shown by the *H. capsulatum* antigen ELISA demonstrates its usefulness in detecting PDH in patients with AIDS in Guatemala. The assay can be per-

formed in less than a day, greatly reducing the diagnostic delay imposed by the slow growth of the organism in culture. This ELISA relies on uncomplicated technology and is simple to perform, and thus it would be well suited for use in clinical laboratories in countries where histoplasmosis is endemic.

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