MAJOR ARTICLE

A Multicenter Evaluation of Tests for Diagnosis of Histoplasmosis

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Background. The sensitivity of the MVista *Histoplasma* antigen enzyme immunoassay (MiraVista Diagnostics) has been evaluated in disseminated histoplasmosis in patients with AIDS and in the "epidemic" form of acute pneumonia. Moreover, there has been no evaluation of the sensitivity of antigenemia detection in disseminated histoplasmosis after the implementation of methods to dissociate immune complexes and denature released antibodies. The goal of this study was to determine the sensitivity of the current antigen assay in different categories of histoplasmosis.

Methods. Urine and serum specimens obtained from 218 patients with histoplasmosis and 229 control subjects, including 30 with blastomycosis, were tested.

Results. Antigenuria was detected in 91.8% of 158 patients with disseminated histoplasmosis, 83.3% of 6 patients with acute histoplasmosis, 30.4% of 46 patients with subacute histoplasmosis, and 87.5% of 8 patients with chronic pulmonary histoplasmosis; antigenemia was present in 100% of 31 tested cases of disseminated histoplasmosis. Among patients with disseminated cases, antigenuria was detected more often and at higher concentrations in immunocompromised patients and those with severe disease. Specificity was 99.0% for patients with nonfungal infections ($n = 130$) and in healthy subjects ($n = 69$), but cross-reactivity occurred in 90% of patients with blastomycosis.

Conclusions. The sensitivity of antigen detection in disseminated histoplasmosis is higher in immunocompromised patients than in immunocompetent patients and in patients with more severe illness. The sensitivity for detection of antigenemia is similar to that for antigenuria in disseminated infection.

Antigen detection is widely used to diagnose progressive disseminated histoplasmosis (PDH). The original assay was a radioimmunoassay [1]; subsequently, an enzyme immunoassay (EIA) [2], designated the first-generation EIA, was used. A second-generation EIA was introduced in 2004 to reduce the number of false-positive results caused by human antirabbit antibodies [3], followed by a third-generation EIA in 2007, which permitted quantification [4]. Pretreatment of serum with ethylene diamine tetraacetic acid (EDTA) at 104°C increased the sensitivity for detection of antigenemia, by dissociation of antigen-antibody complexes and denaturation of dissociated antibody, preventing it from interfering with antigen detection [5].

However, the performance characteristics of the current assay have not been fully assessed. Areas of need include determination of sensitivity in patients with PDH complicating diseases other than AIDS and in

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those with pulmonary histoplasmosis [6]. In addition, the effect of EDTA pretreatment of serum, first described in antigen negative specimens from patients with AIDS [5], on sensitivity for detection of antigenemia has only been studied in acute pulmonary histoplasmosis, improving sensitivity by 38% over testing of urine samples alone [7].

A multifaceted approach is commonly used for the diagnosis of histoplasmosis, with combination of histopathological examination, culture, and antigen and antibody detection. The sensitivities of these tests have not been examined since improvements were incorporated into the MVista *Histoplasma* antigen EIA (MiraVista Diagnostics).

METHODS

Study Cohort

Specimens were obtained from patients evaluated at 8 medical centers during the period from November 2004 through December 2007 and were tested for *Histoplasma* and/or *Blastomyces* antigen at MiraVista Diagnostics. The start date was chosen because the second-generation MVista *Histoplasma* antigen assay was implemented on 8 November 2004. The results of the other diagnostic tests (culture, cytology, pathology, and antibody detection using immunodiffusion [ID] and complement fixation [CF]) were obtained by medical record review of tests performed at the originating institution or other commercial laboratories. The protocol was approved by the institutional review committees at each institution.

The criteria for diagnosis included a positive result of culture, antigen, histopathology, cytology, or *Histoplasma* antibody tests demonstrating H or M precipitin bands by ID or titers of CF antibodies of $\geq$1:8 [8]. Positive culture, cytology, or histopathology results demonstrating yeast-like structures characteristic of *Histoplasma capsulatum* were required for classification as proven disease, whereas positive antigen or antibody test results were required for classification as probable histoplasmosis, combined with compatible clinical and radiographic findings.

Cases were excluded if there was no clinical information available, if histoplasmosis occurred before November 2004 or after December 2007, if histoplasmosis was not diagnosed according to the medical record, or if the original antigen test result was positive but the specimen was not stored. There were also eligible cases with negative antigen test results when originally tested in which the specimens were not stored. To reduce bias in the evaluation of sensitivity of antigen testing, these were included and considered to be negative for antigen, even though specimens were not tested.

Control specimens were obtained from 130 patients evaluated at Indiana University Health Medical Centers in whom the diagnosis of fungal infection was excluded, 30 patients with culture-proven pulmonary and/or extrapulmonary blastomycosis, and 69 healthy commercial blood donors (SeraCare Life Sciences; Milford, Massachusetts).

PDH was defined as the presence of clinical, laboratory, or imaging evidence of extrapulmonary involvement. The diagnosis of pulmonary histoplasmosis required respiratory symptoms and pulmonary radiographs and/or computerized tomography that demonstrated infiltrates and/or mediastinal lymphadenopathy, in the absence of evidence for PDH. Pulmonary cases were further classified as acute, subacute, and chronic pulmonary histoplasmosis [9], as follows: acute pulmonary histoplasmosis, symptom duration of $<$1 month and presence of diffuse or multinodular pulmonary infiltrates; subacute pulmonary histoplasmosis, symptom duration of $\geq$1 month with presence of focal infiltrates and/or mediastinal lymphadenopathy; and chronic pulmonary histoplasmosis, symptom duration of $>$3 months, presence of cavitary pulmonary infiltrates, and presence of underlying emphysema.

Histoplasmosis was classified as severe if patients required treatment in an intensive care unit, moderately severe if hospitalization was required, and mild if hospitalization was not required.

Antigen Detection

The specimens were obtained during the period 2004–2007 and stored frozen at MiraVista Diagnostics until 2010, when they were retested for this study. Only 1 sample was tested for each patient. The specimens were obtained at the time of diagnosis in 202 patients, after the diagnosis was made and treatment initiated in 12 patients, and before the time of diagnosis in 4 patients. The MVista *Histoplasma* antigen EIA [4] was modified to permit quantification below the level of 0.6 ng/mL by incorporating 0.2 ng/mL and 0.4 ng/mL calibrators and removing those of $\geq$19 ng/mL. Serum specimens were treated with 4% EDTA at 104°C before testing [5].

Statistical Analysis

Receiver operator characteristic (ROC) curve analysis was performed to determine the cutoff for positivity. The proportions of patients with positive results were compared using the χ^2 test. The student t test was used for pairwise comparisons of mean antigen levels among the different clinical syndrome groups. Multiple pairwise comparisons were adjusted using step-down Bonferroni multiple comparison procedure. Ninety-five percent confidence intervals (CIs) and proportions were calculated using the Wilson score method for small sample with asymmetrical distribution [10]. An overall significance level of $\alpha = .05$ was used for all comparisons.

RESULTS

Patients

Of 381 patients who were identified, 163 were excluded for the following reasons: histoplasmosis outside the interval of

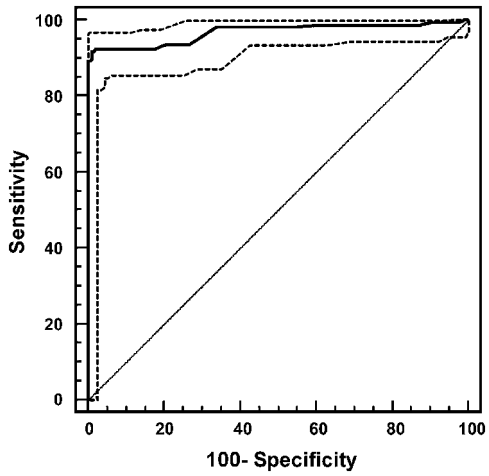

Figure 1. Receiver operating characteristics curve for determination of cutoff and assay sensitivity and specificity. Data included cases with proven histoplasmosis and controls including healthy subjects and patients without histoplasmosis or blastomycosis. The broken lines represent 95% confidence intervals.

the study, 100 patients; no laboratory documentation or inadequate information for classification, 29 patients each; and positive specimen not stored, 5 patients. Of the 218 evaluable patients, 95 were from Indiana University Health (Indianapolis, Indiana), 41 were from the Mayo Clinic (Rochester, Minnesota), 33 were from the University of Kentucky (Lexington, Kentucky), 20 were from the University of Kansas (Wichita, Kansas), 16 were from the University of Kansas Medical Center (Kansas City, Missouri), and 13 were from the Infectious Diseases Associates of Kansas City (Kansas City, Missouri).

One hundred fifty-eight cases were classified as disseminated infection and 60 were classified as pulmonary infection, including 6 with acute, 46 with subacute, and 8 with chronic pulmonary manifestations. One hundred fifty-seven patients were immunocompromised, as follows: AIDS, 57 patients; receipt of solid-organ transplants, 26 patients; treatment with tumor necrosis factor antagonists, 24 patients; treatment with other immunosuppressive medications for hematologic or inflammatory conditions, 34 patients; other immunodeficiency states, 16 patients (including lymphoma in 5); primary immunodeficiencies, 9 patients; and gastric bypass and prematurity, 1 patient each.

ROC Analysis

Urine specimens obtained from 111 patients with proven PDH and 199 control subjects, including 69 healthy subjects and 130 patients without systemic fungal infection, were tested (Figure 1). The sensitivity was 91.0%, the specificity was 99.0%, and the area under the curve (AUC) was 0.967. The ROC analysis was repeated using 158 cases of proven and probable PDH, yielding a sensitivity of 91.8%, a specificity of 99.0%, and an AUC of 0.968.

Disseminated Histoplasmosis

Antigenuria was detected in 91.8% of patients (95% CI, 86.3%–95.1%), including 94.6% of those with AIDS, 93.1% with other immunocompromising conditions, and 73.3% of immunocompetent patients ($P = .837$) (Table 1). Culture results were positive in 74.2%, pathology test results were positive in 76.3%, and results of tests for anti-*Histoplasma* antibodies were positive in 75% of cases for which the test was performed. Antigen levels were higher in immunocompromised than immunocompetent patients (mean level, 11.78 and 6.92 ng/mL, respectively; $P = .023$). Additionally, antigen levels were higher in patients with AIDS (mean, 13.8 ng/mL) than in those with other types of immunocompromise (mean, 10.48 ng/mL) or in immunocompetent patients (mean, 6.92 ng/mL; $P < .05$). Antigen levels were higher in patients with positive blood culture results than in those with negative culture results (mean, 14.72 and 8.06 ng/mL, respectively; $P = .049$). Antigenuria was detected in 100 (90.1%) of 111 patients with proven cases, compared with 25 (100%) of 25 with probable cases for whom pathology test and/or culture results were negative ($P= .862$). Culture results were positive in 91.6%, pathology test results were positive in 85.3%, and antibody test results were positive in 73.6% of proven cases (Table 2).

Table 1. Comparison of Diagnostic Tests in All Cases

	Disseminated cases ($n = 158$)				Pulmonary cases ($n = 60$)		
All tests	AIDS ($n = 56$)	OIC ($n = 87$)	NIC ($n = 15$)	All ($n = 158$)	Acute cases ($n = 6$)	Subacute cases ($n = 46$)	Chronic cases ($n = 8$)
Culture	34/48 (70.8)	57/75 (76.0)	7/9 (77.8)	98/132 (74.2)	0/3 (0)	14/26 (53.8)	4/6 (66.7)
Pathology	18/25 (72.0)	32/43 (74.4)	8/8 (100)	58/76 (76.3)	0/2 (0)	8/19 (42.1)	3/4 (75.0)
Antigen	53/56 (94.6) [13.80–7.67][a]	81/87 (93.1) [10.48–7.62]	11/15 (73.3) [6.92– 7.65]	145/158 (91.8) [11.32–7.88]	5/6 (83.3) [2.41–2.26]	14/46 (30.4) [0.53–1.23]	7/8 (87.5) [0.93–0.83]
Antibody	15/19 (78.9)	37/53 (71.2)	8/9 (88.9)	60/80 (75.0)	4/6 (66.7)	39/41 (95.1)	5/6 (83.3)

NOTE. Data are no. of patients with positive test results / no. of patients tested (%). NIC, nonimmunocompromised; OIC, other causes of immunocompromise.

[a] Mean antigen concentration, standard deviation in ng/mL. Among the OIC group, antibody tests were positive in 2 (18.2%) of 11 patients who had undergone organ transplantation, 12 (85.7%) of 14 who were receiving tumor necrosis factor antagonists, and 20 (62.5%) of 32 with other causes for immunocompromise.

Table 2. Comparison of Diagnostic Tests in Proven Cases

	Disseminated cases (n = 111)				Pulmonary cases (n = 23)[a]	
Proven tests	AIDS (n = 38)	OIC (n = 62)	NIC (n = 11)	All (n = 111)	Subacute (n = 17)	Chronic (n = 5)
Culture	34/38 (89.5)	57/61 (93.4)	7/8 (87.5)	98/107 (91.6)	14/17 (82.4)	4/4 (100)
Pathology	18/23 (78.3)	32/38 (84.2)	8/8 (100)	58/68 (85.3)	8/12 (66.7)	3/4 (75.0)
Antigen	35/38 (92.1) [14.46–7.34][b]	58/62 (93.5) [11.90–7.44]	7/11 (63.6) [5.11–7.74]	100/111 (90.1) [12.10–7.82]	7/18 (38.9) [0.62–1.23]	4/5 (80.0) [0.63–0.54]
Antibody	9/13 (69.2)	16/19 (84.2)	6/7 (85.7)	41/56 (73.2)	12/13 (92.3)	3/3 (100)

NOTE. Data are no. of patients with positive test results / no. of patients tested (%). NIC, nonimmunocompromised; OIC, other causes of immunocompromise.

[a] None of the acute pulmonary cases were proven.

[b] Mean antigen concentration, standard deviation in ng/mL. Among the OIC group, antibody test results were positive in 2 (20%) of 10 patients who had undergone organ transplantation, 7 (87.5%) of 8 who were receiving tumor necrosis factor antagonists, and 14 (66.7%) of 21 with other causes for immunocompromise.

Illness was severe in 23.4% of patients, moderately severe in 46.8% of patients, and mild in 29.7% of patients with PDH (Table 3), and the severe cases included 8 (14.3%) of 56 patients with AIDS, 27 (31.0%) of 87 with other immunocompromising conditions, and 3 (20%) of 15 without immunocompromising conditions. Antigenuria was detected in 100% of patients with severe cases, 95.9% of those with moderately severe cases, and 78.7% of those with mild cases (P = .706). Culture results were positive in 27 (75.0%) of 36 patients with severe cases versus 71 (74.0%) of 96 patients with nonsevere cases (P = .918), and pathology test results were positive in 31 (81.6%) of 38 patients with severe cases versus 39 (79.9%) of 49 patients with nonsevere cases (P = .932).

Antigen levels were higher in patients with disseminated cases than in those with pulmonary cases (mean, 11.32 vs 0.77 ng/mL; P < .001) (Figure 2). Concentrations were slightly higher in patients with proven versus probable cases (mean, 12.10 vs 9.47 ng/mL; P = .055) (Table 3).

Table 3. Analysis of the Effect of Selected Parameters on Prevalence and Magnitude of Antigenuria in All Patients With Progressive Disseminated Histoplasmosis

Parameter	No. of positive results / total (%)	P	Mean ng/mL ± SD	P
Classification				
Probable	45/47 (95.7)	.906	9.47 ± 7.80	.055
Proven	100/111 (90.1)		12.10 ± 7.82	
Immune status				
AIDS	53/56 (94.6)	.837	13.80 ± 7.67	.003
OIC	81/87 (93.1)		10.48 ± 7.62	
NIC	11/15 (73.3)		6.92 ± 7.65	
Severity				
Severe (ICU)	37/37 (100)	.706	16.08 ± 5.66	<.001
Moderately severe (hospitalized)	71/74 (95.9)		11.09 ± 7.86	
Mild (nonhospitalized)	37/47 (78.7)		7.94 ± 7.66	

NOTE. ICU, intensive care unit; NIC, nonimmunocompromised; OIC, other causes of immunocompromise; SD, standard deviation.

Mean antigen concentrations were 16.08 ng/mL in patients with severe cases, 11.09 ng/mL in those with moderately severe cases, and 7.94 ng/mL in those with mild cases, P <.001 (Table 3). Concentrations ≥19 ng/mL were present in 27 (73.0%) of 37 patients with severe cases, 29 (39.2%) of 74 with moderately severe cases, and 8 (17.0%) of 47 with mild cases (P = .004). The positive predictive value (PPV) for severe disease in patients with antigen levels ≥19 ng/mL was 42.2%, and the positive likelihood ratio was 3.11; and the negative predictive value (NPV) for severe disease was 92.3% and the negative likelihood ratio 0.35 in those with antigen levels <19 ng/mL. The PPV for severe or moderately severe disease for antigen levels ≥19 ng/mL was 87.5%, and the positive likelihood ratio was 2.75; the NPV was 35.9%, and the negative likelihood ratio was 0.70.

Among the 31 patients with disseminated cases for whom both urine and serum samples were tested, antigenemia was present

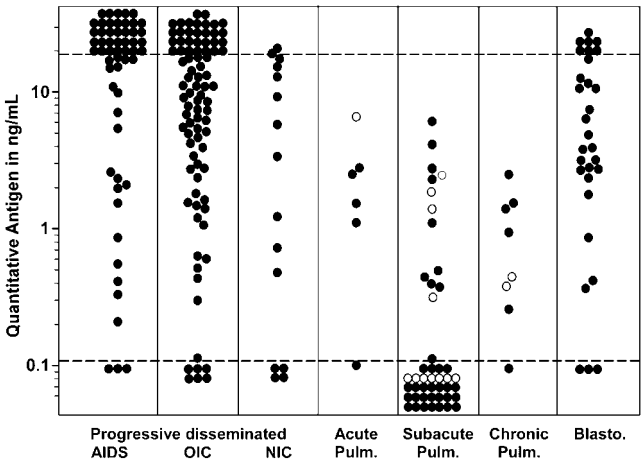

Figure 2. Results for cases of proven and probable histoplasmosis and proven blastomycosis. Antigen concentration (in ng/mL) is shown on the vertical axis. The broken horizontal line represents the cutoff value for positivity. Among the pulmonary categories, closed circles represent cases in nonimmunocompromised subjects, and open circles represent cases in immunocompromised subjects.

in all, including 1 in whom antigenuria was absent, in whom the antigen level was 0.16 ng/mL. Antigenemia was detected in 22 (71.0%) of 31 patients when originally tested without EDTA-heat pretreatment, compared with 31 (100%) of 31 patients with pretreatment ($P = .470$). Mean antigenuria (14.84 ng/mL) was higher than mean antigenemia (11.09 ng/mL), but the P value (.056) was not quite significant.

Pulmonary Histoplasmosis

Antigenuria was detected in 5 (83.3%) of 6 patients with acute cases, 14 (30.4%) of 46 with subacute cases, and 7 (87.5%) of 8 with chronic pulmonary cases, at higher concentrations in those with acute versus subacute cases (mean, 2.41 vs 0.53 ng/mL, respectively; $P < .05$) (Table 1). Serum samples were not available to test for antigenemia. Serological test results were positive for 4 (66.7%) of 6 patients with acute cases, 39 (95.1%) of 41 with subacute cases, and 5 (83.3%) of 6 with chronic pulmonary cases. Pathology test results were negative in 2 patients with acute pulmonary cases who were evaluated, but they were positive in 8 (42.1%) of 19 patients with subacute cases and 3 (75%) of 4 with chronic cases. Culture results were positive in 14 (53.8%) of 26 patients with subacute cases and 4 (66.7%) of 6 with chronic pulmonary cases but negative in all 3 patients with acute cases who were tested. Among proven cases only, antigen was detected in 7 (38.9%) of 18 patients with subacute cases and 4 (80%) of 5 with chronic cases ($P = .678$) at similar concentrations (mean, 0.62 vs 0.63 ng/mL, respectively; $P = .985$) (Table 2). No acute cases were proven.

Among immunocompromised patients with pulmonary histoplasmosis, antigen test results were positive in 7 (50%) of 14, pathology test results were positive in 5 (71.4%) of 7, and serological test results were positive in 9 (90%) of 10 (Table 4). The type of pulmonary involvement was acute in 1 case, chronic in 3 cases, and subacute in 10 cases. Among the non-immunocompromised patients with pulmonary cases, antigen test results were positive in 19 (41.3%) of 46, pathology test results were positive in 6 (33.3%) of 18, and serological test results were positive in 38 (90.5%) of 42 ($P = .031$). Mean antigen concentration was 0.91 ng/mL in immunocompromised patients and 0.73 ng/mL in nonimmunocompromised patients ($P = .806$).

Specificity of Antigen Detection

Cross-reactions occurred in 27 (90%) of 30 patients with proven pulmonary and/or extrapulmonary blastomycosis (95% CI, 74.4%–96.5%). Concentrations were higher in patients with proven PDH (mean, 12.10 ng/mL; range, 0.21 to ≥19.0 ng/mL) than in those with proven blastomycosis (mean, 7.78 ng/mL; range, 0.36 to ≥19.0 ng/mL; $P = .007$). Culture results were positive in 27 (96.4%) of 28 patients with blastomycosis, and pathology test results were positive in 21 (84%) of 25 patients with blastomycosis.

Table 4. Findings in Pulmonary Histoplasmosis

Finding	Immunocompromised patients ($n = 14$)	Nonimmunocompromised patients ($n = 46$)	P
Culture	7/11 (63.6)	13/24 (54.2)	.44
Pathology	5/7 (71.4)	6/18 (33.3)	.10
Antigen	7/14 (50) [0.91–1.78][a]	19/46 (41.3) [0.73–1.30]	.39 .676[b]
Antibody	9/10 (90.0)	38/42 (90.5)	.806

NOTE. Data are no. of patients with positive results / no. tested (%).

[a] Mean antigen concentration, standard deviation in ng/mL.

[b] P value for comparison of mean antigen level in ng/mL between the 2 groups.

Antibody Detection

Among PDH cases, antibodies were detected in 8 (88.9%) of 9 nonimmunocompromised patients and 52 (72.2%) of 72 immunocompromised patients ($P = .889$), including 85.7% of those receiving tumor necrosis factor–inhibitor therapy, 78.9% of those with AIDS, and 18.2% of those who had undergone solid-organ transplantation (Table 1). H and/or M precipitin bands were detected by ID in 34 (63.2%) of 54 immunocompromised patients versus 6 (85.7%) of 7 non-immunocompromised patients ($P = .831$). CF titers ≥1:8 were detected in 45 (70.3%) of 64 immunocompromised patients versus 7 (77.8%) of 9 nonimmunocompromised patients ($P = .932$). Elevated antibody levels were present in 41 (73.2%) of 56 patients with proven cases versus 7 (58.3%) of 12 patients with probable cases ($P = .854$).

Elevated anti-*Histoplasma* antibody levels were detected in 39 (95.1%) of 41 patients with subacute histoplasmosis, 4 (66.7%) of 6 with acute histoplasmosis, and 7 (83.3%) of 8 with chronic pulmonary histoplasmosis (Table 1), as well as in 9 (90%) of 10 immunocompromised patients and 38 (90.5%) of 42 non-immunocompromised patients ($P = .806$) (Table 4).

Among 101 patients in whom both CF and ID were performed, results of CF alone were positive in 19 (18.8%), results of ID alone were positive in 8 (7.9%), results of both were positive in 57 (56.4%), and results of neither were positive in 17 (16.8%). Overall, of 110 patients in whom the ID was performed, M precipitin bands alone were present in 43 (39.1%), M and H precipitin bands were present in 28 (25.4%), and neither was present in 39 (35.4%). Among patients in whom CF was performed, results were positive in 52 (71.2%) of 73 patients with PDH, compared with 40 (80%) of 50 patients with pulmonary histoplasmosis ($P = .782$). The median CF titer was 1:32 in both groups.

DISCUSSION

To our knowledge, this is the largest multicenter analysis of the sensitivity of several diagnostic tests for histoplasmosis. Among

patients with proven and probable cases of PDH, antigenuria was detected in 92%, including 95% of those with AIDS, 93% of those with other immunocompromising conditions, and 73% of patients not known to be immunocompromised. Cross-reactive antigen was detected in 90% of patients with blastomycosis. The sensitivity was 76% for histopathology and 74% for culture. Among patients with PDH for whom serum and urine samples were tested, antigenuria was detected in 97% and antigenemia in 100%; 27% had results that were negative without EDTA-heat pretreatment. Assuming that histopathologic examination provided a more rapid result than antigen testing, it would have been the basis for diagnosis in 52% of proven cases; antigen, antibody, and culture tests would have formed the basis for 43%, 3%, and 2%, respectively. Although analysis of proven cases provides the greatest assurance that antigen test results were not falsely positive, it does not reflect clinical practice, where invasive procedures to obtain specimens for pathology and culture tests often are omitted if the clinical features are consistent with histoplasmosis and the results of tests for antigen are positive. Histopathological tests would have provided the initial diagnosis for 37% of proven and probable cases; antigen, antibody, and culture tests would have provided the initial diagnosis in 59%, 3%, and 1%, respectively.

Antigenuria correlated with the severity of PDH. Concentrations of ≥19 ng/mL occurred in 73% of severe cases, 39% of moderately severe cases, and 17% of mild cases. The likelihood of hospitalization was 2.75 and of intensive care unit care was 3.11 if the antigen level was ≥19 ng/mL.

Pulmonary histoplasmosis accounted for 28% of cases, of which 56.5% exhibited antigenuria. The antigen concentration was <4 ng/mL in 95% of patients with pulmonary cases; higher levels raise concern about disseminated disease. Antigenuria was detected in 50% of immunocompromised patients and 41% of nonimmunocompromised patients with pulmonary histoplasmosis, whereas the results of tests for antibodies were positive in 90% of immunocompromised and 91% of nonimmunocompromised patients.

Antigenuria was present in 5 (83%) of 6 patients with acute cases. In an earlier report of 29 cases of acute pulmonary histoplasmosis, antigenuria was detected in 45%, antigenemia in 79%, and either in 83% [7]. Results of the current and prior study indicate that antigen can be detected in ~80% of patients with acute cases, especially if both urine and serum samples are tested. Antigenuria was the sole laboratory basis for diagnosis in 2 acute cases in this report, which could have been caused by either histoplasmosis or blastomycosis, considering the cross-reactivity between the 2 antigens.

Antigenuria was detected in 30% of patients with subacute pulmonary cases ($n = 46$), providing a reasonably accurate assessment of sensitivity (95% CI, 19–45). Whether also testing for antigenemia would improve the sensitivity, as noted in acute cases, is unknown. The diagnosis of subacute pulmonary histoplasmosis is usually based on detection of antibodies to *H. capsulatum;* the results were positive in 95% of cases in this report. Detection of antigen would strengthen the certainty of the diagnosis. The sensitivity of 88% for chronic pulmonary histoplasmosis was based on data for only 7 cases. Among 7 more recent cases diagnosed at the Indiana University Medical Center, antigenuria was detected in only 2. Thus, the sensitivity for diagnosis of chronic pulmonary histoplasmosis requires additional investigation.

Antibody production was slightly impaired in immunocompromised patients with PDH because of the poor response in solid-organ transplant recipients. Furthermore, the sensitivity in solid-organ transplant recipients in this study (18%) was lower than in a previous report (100%) [11]. A potential cause for the lower sensitivity in the current study is the change in the immunosuppressive regimens, which largely consisted of corticosteroids and azathioprine in the prior report [11] and calcineurin inhibitors and mycophenolate mofetil in this study—drugs that impair antibody production [12, 13].

Antibodies were detected more often in patients with subacute (95%) than acute (67%) manifestations, similar to results of an earlier study (64%) [7]. Antibody production occurs slowly after acute infection, requiring up to 3 months [14]. Although antibodies were detected more often in pulmonary cases (91%) than in disseminated cases (75%), median CF titers were 1:32 in both groups. H precipitin bands were slightly more common in disseminated cases (28.6%) than pulmonary cases (20.4%), but not enough so as to differentiate the 2 manifestations.

The highest sensitivity for antibody testing was achieved by combining CF and ID testing; the results of one or both were positive in 82% of cases. ID is easy to perform and often is used as a screening test, with performance of CF only if ID results are positive. Twenty percent of the current cases would have been missed using that approach.

Cross-reactive antigens were detected in 90% of patients with blastomycosis [4, 15]. Although the antigen concentration was higher in patients with histoplasmosis, there was considerable overlap, preventing differentiation by antigen concentration. Although the inability to distinguish histoplasmosis and blastomycosis is a limitation of antigen detection, treatment is similar. Cross-reactions also occur in ~80% patients with paracoccidioidomycosis or penicilliosis marneffei, 60% of those with coccidioidomycosis [2, 4], and nearly 10% of those with aspergillosis [16].

Molecular methods have been reported for diagnosis of histoplasmosis, with variable accuracy [17, 18]. Molecular methods are offered by several commercial laboratories but were not used in any of the cases in this report. Their role remains uncertain for diagnosis of histoplasmosis.

In conclusion, this study supports a broad approach, using antigen testing of urine and serum samples, antibody testing,

culture, and pathology for diagnosis of disseminated or pulmonary histoplasmosis. The concentration of antigenuria in PDH correlates with the severity of disease. Importantly, the sensitivity of antigen testing is not 100%, even if both urine and serum samples are tested, and negative results do not exclude histoplasmosis. Repeated testing is advised for patients with progressive illness if the initial test results are negative.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed in the Acknowledgments section.

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