Reevaluation of Commercial Reagents for Detection of *Histoplasma capsulatum* Antigen in Urine

Elitza S. Theel,^a Julie A. Harring,^a Ala S. Dababneh,^b Leonard O. Rollins,^a Jeannie E. Bestrom,^a Deborah J. Jespersen^a

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,^a and Division of Infectious Diseases,^b Mayo Clinic, Rochester, Minnesota, USA

Detection of the *Histoplasma capsulatum* urinary antigen (UAg) is among the most sensitive and rapid means to diagnose histoplasmosis. Previously, we evaluated analyte-specific reagents (ASR) manufactured by IMMY (Norman, OK) for detection of *Histoplasma* galactomannan (GM) in urine using an enzyme immunoassay (EIA), and we showed low positive agreement (64.5%) with the MiraVista (MVista) *Histoplasma* antigen (Ag) quantitative EIA (MiraVista Diagnostics, Indianapolis, IN). Here we re-evaluated the IMMY GM ASR following modification of our original assay protocol and introduction of an indeterminate range. A total of 150 prospectively collected urine samples were tested with both the IMMY and MVista EIAs, and clinical histories were recorded for all study subjects. The IMMY GM ASR showed positive and negative agreements of 82.3% (14/17 samples) and 100% (121/121 samples), respectively (with exclusion of 12 indeterminate results), and overall agreement of 90% (135/150 samples) with respect to the MVista EIA. Of the three patients with negative IMMY GM ASR results and positive MVista EIA results, testing was performed for initial diagnostic purposes for one patient (<0.4 ng/ml by the MVista EIA) and UAg levels were being monitored for the remaining two patients (both <0.7 ng/ml by the MVista EIA). The MVista EIA results were positive for 6/12 samples that tested indeterminate by the IMMY GM ASR. We also show that the IMMY GM ASR can be used to serially monitor *Histoplasma* UAg levels. In conclusion, we demonstrate that, with modification, the IMMY GM ASR is a reliable rapid assay for detection of *Histoplasma* UAg.

Localized largely along the Ohio River and Mississippi River valleys, *Histoplasma capsulatum* continues to be a significant cause of morbidity and death, particularly among individuals with weakened cellular immunity and those over the age of 65 years (1, 2). Despite the high rates of exposure (60 to 90%) among residents of these regions of endemicity, otherwise healthy individuals are able to control infections with minimal disease manifestations (3, 4). However, immunocompromised patients exposed to *H. capsulatum* typically present with a nonspecific febrile illness, which can rapidly progress to pneumonia, respiratory insufficiency, disseminated disease, and death (2, 5). Histoplasmosis can be treated effectively with proper antifungal management, but timely diagnosis is essential.

Due to the nonspecific clinical presentation of histoplasmosis, as well as lengthy, costly, and potentially toxic therapeutic regimens, laboratory testing to confirm the diagnosis is critical. Many methods are available for this purpose, including culture, nucleic acid amplification testing (NAAT), histopathological examination, and antibody and antigen (Ag) detection. The individual performance characteristics of these methods vary, with their overall clinical utility being largely dependent on disease presentation (localized versus disseminated), level of patient immunosuppression, and symptom duration prior to testing. Briefly, while culture, histopathological examination, and NAAT offer high specificity, the overall sensitivity of these methods is variable, ranging from 10% to >90%, depending on the disease state, specimen source, and assay methodology (3, 6–10). Additionally, these three methods often require collection of specimens directly from the site of infection through invasive procedures (e.g., bronchoalveolar lavage [BAL] or biopsy), which may be contraindicated for patients with severe disease. Finally, the prolonged incubation (2 to 4 weeks) required for recovery of the organism in culture can delay the initiation of antifungal treatment. Serological assays for detection of antibodies to *H. capsulatum* are advantageous, as they

are performed with an easily accessible specimen source (e.g., serum) and provide high specificity (>90%); however, false-negative results may occur for patients with recent infections and those with compromised immunity (3, 11). Furthermore, low-level seropositivity following exposure or resolved infection is not uncommon and may be diagnostically confounding.

Among the many available laboratory methods, detection of *H. capsulatum* antigen (Ag), specifically with the MiraVista (MVista) *Histoplasma* Ag quantitative enzyme immunoassay (EIA) (MiraVista Diagnostics, Indianapolis, IN), provides a high level of sensitivity for clinical disease in both acute and disseminated cases of infection (75 to 80% and >90%, respectively) (3, 11, 12). Also, unlike other diagnostic methods, monitoring of quantitative *Histoplasma* antigen values in urine and/or serum can be used to follow treatment responses and to monitor disease progression (13, 14). The primary limitation of the MVista assay is the requirement that all specimens be submitted to MiraVista Diagnostics for testing, which can delay result reporting and affect patient management. Finally, while an FDA-cleared assay for detection of *His*

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Address correspondence to Elitza S. Theel, theel.elitza@mayo.edu.

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toplasma urinary Ag (UAg), the Alpha *Histoplasma* antigen EIA (IMMY), is available, the performance of this assay has been reported to be poor (15).

In an effort to provide timely, clinically useful results, we previously evaluated analyte-specific reagents (ASR) produced by IMMY for detection of *Histoplasma* galactomannan (GM) by an EIA (16). We compared the results from over 1,000 urine samples tested by both the IMMY GM ASR and the MVista EIA, and we found positive, negative, and overall agreements of 64.5% (40/62 samples), 99.8% (939/941 samples), and 97.6% (979/1,003 samples), respectively. Despite the high overall agreement, the low positive agreement of the IMMY GM ASR and MVista EIA results was unacceptable. In this study, we reevaluated the IMMY GM ASR following modification of both our original methodology and the interpretive criteria. We show that, with these changes, the IMMY GM ASR, alongside other laboratory methods, is a reliable assay for detection of *Histoplasma* UAg.

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MATERIALS AND METHODS

Study design. Urine samples ($n = 150$) collected prospectively from Mayo Clinic patients ($n = 143$) between August 2013 and February 2014 and submitted for routine clinical evaluation with the MVista *Histoplasma* Ag quantitative EIA were also tested with the IMMY GM ASR in our laboratory, for detection of *Histoplasma* GM. The technologist performing the IMMY assay was blinded to both the MVista results and patient clinical history. Qualitative and quantitative results were compared for the two assays, and medical charts were reviewed for all subjects at the time of testing and 6 months thereafter. This study was approved by the Mayo Clinic Institutional Review Board.

IMMY GM ASR. The IMMY GM ASR is a quantitative, antigen capture EIA for detection of *H. capsulatum* GM. All assay components are considered ASR except for the microtiter wells, which are classified as Research Use Only; here this assay is referred to as the IMMY GM ASR. Testing was performed on the Triturus automated EIA analyzer (Grifols, Miami, FL). One hundred microliters of undiluted urine was added to microtiter wells coated with a monoclonal antibody specific to GM and incubated for 55 min at 37°C. Wells were washed, 100 μ l of horseradish peroxidase (HRP)-conjugated anti-GM monoclonal antibody was added, wells were incubated for 40 min at 25°C, and a second wash step was performed. One hundred microliters of 3,3',5,5'-tetramethylbenzidine was then added, wells were incubated for 25 min at 25°C, and 100 μ l of 2 N sulfuric acid (stop solution) was added. The optical density (OD) in each well was measured at dual excitation wavelengths of 450 and 620 nm. A standard curve was generated with each run by using eight calibrators, i.e., a 0.0-ng/ml calibrator (wash buffer) was added to the seven calibrators recommended by the manufacturer (0.4, 0.8, 1.6, 3.2, 6.3, 12.5, and 25 ng/ml). OD values for each of the eight calibrators were plotted using a linear regression curve, and quantitative patient results (in ng/ml) were calculated by mapping the sample OD value against the standard curve.

The manufacturer's interpretive criteria for this assay are as follows: <0.50 ng/ml, negative; ≥ 0.50 ng/ml, positive. Following completion of testing, the IMMY GM ASR and MVista EIA results were reviewed, as were the clinical presentations and diagnoses for all 143 patients. Based on these data, it was determined that modified interpretive criteria were needed to optimize the clinical sensitivity and specificity of the IMMY GM ASR. Specifically, the modified cutoff criteria and interpretations for the IMMY GM ASR are as follows: values falling between 0.11 ng/ml and 0.49 ng/ml were considered indeterminate, values of ≤ 0.10 ng/ml were considered negative, and values of ≥ 0.5 ng/ml were considered positive (consistent with the manufacturer's recommendations). Specimens with re-

TABLE 1 Comparison of IMMY GM ASR and MVista EIA for detection of *Histoplasma* antigen in urine using manufacturer's interpretive criteria ($n = 150$)

IMMY GM ASR result ^a	No. with MVista EIA result of:	
	Positive	Negative
Positive	14	0
Negative	9	127

^a Positive agreement, 60.9% (95% CI, 40.7 to 77.9%); negative agreement, 100% (95% CI, 96.5 to 100%); overall agreement, 94.0% (95% CI, 88.8 to 97.0%); κ , 0.72 (95% CI, 0.55 to 0.89).

sults of >23.0 ng/ml were considered positive, above the limit of quantification.

The quantifiable range (0 to 23 ng/ml) for the IMMY GM ASR was established with linearity studies, by testing serial 2-fold dilutions (from 1:2 to 1:8,192) of a strongly GM-positive clinical urine sample in duplicate, according to CLSI guidelines (17). Standard linear regression analysis comparing the mean observed and expected concentrations at each dilution showed good correlation (see Fig. S1A in the supplemental material) with acceptable result differences (see Fig. S1B in the supplemental material) across the quantifiable range.

MVista *Histoplasma* Ag quantitative EIA. All 150 urine samples were evaluated with the MVista *Histoplasma* Ag quantitative EIA performed at MiraVista Diagnostics. Results are reported as not detected or positive, with quantified results provided for values between 0.4 ng/ml and 19 ng/ml. Samples with values of <0.4 ng/ml or >19 ng/ml are reported as positive, below the limit of quantification, or positive, above the limit of quantification, respectively.

Patient chart review. Medical records were reviewed, and the following information was recorded: symptoms at presentation, comorbidities, immunological status, radiological findings, exposure history, other laboratory data (including culture and serological results), antimicrobial prophylaxis, final diagnosis, and whether antifungal treatment was initiated. Additionally, patient charts were reviewed again 6 months after testing, to exclude the possibility that a case of histoplasmosis had been missed by both assays.

Statistical analysis. GraphPad software (GraphPad, La Jolla, CA) was used to determine positive agreement, negative agreement, overall agreement, κ values, and 95% confidence intervals (CIs) for qualitative comparisons of IMMY GM ASR and MVista EIA results.

RESULTS

Patients and test agreement. The 150 urine samples analyzed in this study were submitted from 143 unique patients, with a median age of 58 years (range, 12 to 89 years); 60% (86/143 patients) were male. The MVista EIA was ordered for initial diagnostic purposes for 124 patients and to monitor *Histoplasma* urinary antigen levels for an additional 19 patients. Utilization of the manufacturer-recommended cutoff criteria to interpret the IMMY GM ASR results for these 150 samples led to positive, negative, and overall agreement of 60.9%, 100%, and 94.0%, respectively, compared to the MVista EIA results (Table 1) (18). Following application of our modified interpretive criteria, we demonstrated overall agreement of 90.0% (135/150 samples) and a κ value of 0.72, indicating good strength of agreement (Table 2). Notably, using our modified criteria, 12 (8%) of the 150 urine samples yielded indeterminate results with the IMMY GM ASR. A detailed analysis of these 12 indeterminate samples, the IMMY GM ASR showed positive and negative agreements of 82.3% (14/17 samples) and 100% (121/121 samples), respectively, compared to the MVista EIA re-

TABLE 2 Comparison of IMMY GM ASR and MVista EIA for detection of *Histoplasma* antigen in urine using modified interpretive criteria ($n = 150$)

IMMY GM ASR result ^a	No. with MVista EIA result of:	
	Positive	Negative
Positive	14 ^b	0
Negative	3 ^c	121
Indeterminate ^d	6	6

^a Positive agreement, 82.3% (95% CI, 58.2 to 94.6%); negative agreement, 100% (95% CI, 96.3 to 100%); overall agreement, 90.0% (95% CI, 84.6 to 94.0%); κ , 0.72 (95% CI, 0.55 to 0.89).

^b See Table S1 in the supplemental material for detailed clinical information.

^c See Table 4 for detailed clinical information.

^d For samples with indeterminate results by the IMMY GM ASR, the results of testing with the MVista EIA were applied; these results were not included for calculation of positive and negative agreement.

sults (Table 2). The turnaround time from specimen collection to result availability was monitored for both assays. The range, average, and median times to results were 2 to 6 days, 2.7 days, and 2 days for the MVista EIA and 0.5 to 3 days, 1.07 days, and 1 day for the IMMY GM ASR, respectively.

Accuracy of IMMY GM ASR. Among the 138 samples that tested positive or negative by the IMMY GM ASR, 14 were positive by both the IMMY and MVista EIAs (Table 2). Thirteen of the 14 samples were from subjects who either had a first-time diagnosis

of *H. capsulatum* infection ($n = 4$) or were being monitored for response to treatment ($n = 9$) (see Table S1 in the supplemental material). The remaining subject was diagnosed with acute respiratory distress syndrome secondary to pulmonary blastomycosis, which was confirmed by the growth of *Blastomyces dermatitidis* from BAL fluid. This finding indicates that, similar to the MVista *Histoplasma* UAg EIA, the IMMY GM ASR may lead to false-positive results for patients with blastomycosis. Importantly, during this evaluation, there were no samples that were IMMY GM ASR positive and MVista EIA negative, and none of the subjects associated with the 121 samples that were negative by both assays developed histoplasmosis in the 6 months following testing.

Three (2.2%) of the 138 samples were IMMY GM negative and MVista EIA positive (Table 4). Two of the three samples were from patients who had been diagnosed with disseminated histoplasmosis 2 or more years prior to the time of testing and had been maintained on itraconazole therapy since the time of diagnosis. In the case of the third subject, testing had been performed for initial diagnostic purposes and histoplasmosis was confirmed for this patient based on positive anti-*Histoplasma* antibody results, which were available 48 h prior to receipt of the MVista *Histoplasma* UAg report.

Indeterminate results with IMMY GM ASR. Of the 150 urine samples studied, 12 (8%) yielded indeterminate results (0.15 to 0.44 ng/ml) by the IMMY GM ASR (Table 3). Seven of the 12 samples were submitted for initial diagnostic purposes, and five

TABLE 3 Review of patients with indeterminate IMMY GM ASR results ($n = 12$)

Patient	Age (yr)/sex	Quantitative results (ng/ml) (qualitative results) ^a		Purpose of test	Patient history and significant laboratory findings
		IMMY ASR	MVista EIA		
1	27/M	0.24	ND	Initial diagnosis	Refractory T-cell-mediated autoimmune disease, receiving cyclosporine; <i>Aspergillus fumigatus</i> from BAL fluid culture; pulmonary aspergillosis Dx
2	60/F	0.28	ND	Initial diagnosis	Liver and kidney transplant in June 2013, receiving mycophenolate mofetil, tacrolimus, and prednisone; all microbiology laboratory test results Neg.; granulomatous hepatitis Dx
3	29/M	0.42	ND	Initial diagnosis	Not ICH; presented with SOB and mediastinal mass on CT scan; <i>Parvimonas</i> and <i>Actinomyces</i> spp. from BAL fluid culture; pulmonary lung abscess Dx
4	55/F	0.28	ND	Initial diagnosis	Not ICH; presented with unintentional weight loss; all microbiology laboratory test results Neg.; Dx unclear (not infectious)
5	61/F	0.44	2.29 (Pos.)	Initial diagnosis	Not ICH; blastomycosis Dx in 2010 s/p 15 mo of itraconazole; <i>Blastomyces</i> ID Pos., <i>Histoplasma</i> CF and ID Neg.; relapsed pulmonary blastomycosis Dx
6	38/M	0.35	0.67 (Pos.)	Initial diagnosis	Psoriatic arthritis; presented with FUO and 11-mm nodular lung opacity; <i>Histoplasma</i> CF Myc., 1:16; CF Yst., 1:256; M band by ID; pulmonary histoplasmosis Dx
7	35/F	0.29	ND	Initial diagnosis	Crohn's disease, receiving azathioprine; presented with abdominal pain; all microbiology test results Neg.; exacerbated Crohn's disease Dx
8	69/M	0.24	0.56 (Pos.)	Monitoring	Lymphocytic leukemia Dx in 2009; disseminated histoplasmosis Dx in April 2013; remains on itraconazole
9	50/M	0.24	ND	Monitoring	Not ICH, hypertension; pulmonary histoplasmosis Dx in June 2013; remains on itraconazole
10	44/F	0.15	0.92 (Pos.)	Monitoring	Pancreas and kidney transplant in 2001, receiving mycophenolate mofetil and tacrolimus; disseminated histoplasmosis Dx in September 2012; remains on itraconazole
11	61/F	0.22	0.49 (Pos.)	Monitoring	Crohn's disease, receiving azathioprine; disseminated histoplasmosis Dx in November 2012; remains on itraconazole
12	33/F	0.35	7.21 (Pos.)	Monitoring	Crohn's disease, receiving adalimumab and methotrexate; disseminated histoplasmosis Dx in January 2011; remains on voriconazole

^a Dx, diagnosis; BAL, bronchoalveolar lavage; ND, not detected; ICH, immunocompromised host; s/p, status post; FUO, fever of unknown origin; CT, computed tomography; SOB, shortness of breath; CF, complement fixation; ID, immunodiffusion; Myc., mycelial antigen; Yst., yeast antigen; Pos., positive; Neg., negative.

TABLE 4 Review of patients with discordant IMMY GM ASR and MVista EIA results ($n = 3$)

Patient	Age (yr)/sex	Quantitative results (ng/ml) (qualitative results) ^a		Purpose of test	Patient history
		IMMY ASR	MVista EIA		
1	52/M	0 (Neg.)	<0.4 (Pos., BLoQ)	Monitoring	Disseminated histoplasmosis Dx in February 2012, receiving prednisone and azathioprine for treatment of frontal orbital inflammatory pseudotumor at time of Dx; remains on itraconazole
2	28/F	0 (Neg.)	0.64 (Pos.)	Monitoring	Disseminated histoplasmosis Dx in December 2010, receiving adalimumab and azathioprine for Crohn's disease at time of Dx; remains on itraconazole
3	68/M	0 (Neg.)	<0.4 (Pos., BLoQ)	Initial diagnosis	AML s/p allogeneic SCTx in December 2012; pulmonary histoplasmosis Dx in October 2013 by serology (CF Yst., 1:64; H and M bands by ID) ^b

^a Dx, diagnosis; Pos., positive; Neg., negative; AML, acute myeloid leukemia; s/p, status post; SCTx, stem cell transplant; CF, complement fixation; Yst., yeast antigen; ID, immunodiffusion; BLoQ, below the limit of quantification.

^b Serological results were finalized 24 h following serum collection, and MVista EIA results were finalized 72 h following urine collection; the two specimens were collected on the same day.

were collected to monitor UAg levels in patients previously diagnosed with histoplasmosis. Two of the seven patients evaluated due to clinical concerns regarding primary *H. capsulatum* infection tested positive by the MVista EIA. One patient (patient 5 in Table 3) was diagnosed with relapsed pulmonary blastomycosis based on positive *B. dermatitidis* immunodiffusion results, and the second patient (patient 6 in Table 3) was diagnosed with pulmonary histoplasmosis based on positive *Histoplasma* serological results, which were available 24 h prior to receipt of the MVista *Histoplasma* UAg report. Among the five indeterminate samples from patients for whom *Histoplasma* UAg levels were being monitored, four were positive by the MVista EIA.

Serial patient testing using IMMY GM ASR. Of the 143 subjects included in our study, four were serially tested during the study period (Table 5). While quantitative values determined by the IMMY GM ASR were consistently lower than those determined by the MVista EIA, the two assays showed similar trending of *Histoplasma* UAg levels over time for all four subjects.

DISCUSSION

We have reevaluated the IMMY GM ASR for detection of *Histoplasma* GM in urine following modification of our previously published protocol and interpretive criteria (16). Specifically, we added another calibrator to those provided by the manufacturer, and we established an indeterminate result range. With these modifications, we show that the IMMY GM ASR has overall agree-

ment of 90.0% (135/150 samples) with respect to the MVista EIA and, following exclusion of samples with indeterminate results, positive and negative agreements of 82.3% (14/17 samples) and 100% (121/121 samples), respectively. This is a significant improvement in positive agreement over that observed using the manufacturer-recommended criteria (60.9%) (Table 1) and reported in our original evaluation (64.5%) of the IMMY GM ASR (16). Also, in our previous study we identified an optimal cutoff value of ≥ 0.15 ng/ml for positive results using receiver operating characteristic (ROC) analysis; application of this criterion to the current data set resulted in seven false-positive IMMY GM ASR results in patients without histoplasmosis, discrediting this cutoff value as clinically appropriate (data not shown) (16). Finally, we show that the time from specimen collection to acquisition of results was improved from an average of 2.7 days with the MVista EIA to an average of 1.07 days with the IMMY GM ASR performed in our laboratory.

A number of findings in our study deserve to be highlighted. First, the IMMY GM ASR did not yield false-positive results, compared to the MVista EIA, during this evaluation, and none of the subjects who tested negative by both assays developed histoplasmosis in the 6 months following initial testing. Three subjects did have discordant results, however, with negative IMMY GM ASR results and positive MVista EIA results. The first of these patients was tested for initial diagnostic purposes and, based on the MVista

TABLE 5 Comparison of serial IMMY GM ASR and MVista EIA results for four patients

Serial test	Quantitative results (ng/ml) (qualitative results) ^a							
	Patient 1 ^b		Patient 2 ^c		Patient 3 ^d		Patient 4 ^e	
	IMMY ASR	MVista EIA	IMMY ASR	MVista EIA	IMMY ASR	MVista EIA	IMMY ASR	MVista EIA
1	0.24 (Ind.)	0.56 (Pos.)	9.66 (Pos.)	15.94 (Pos.)	1.45 (Pos.)	7.49 (Pos.)	0.6 (Pos.)	1.31 (Pos.)
2	0 (Neg.)	ND	11.1 (Pos.)	14.03 (Pos.)	1.70 (Pos.)	10.2 (Pos.)	0.6 (Pos.)	1.35 (Pos.)
3	0 (Neg.)	ND	4.51 (Pos.)	7.48 (Pos.)	—	—	—	—

^a Ind., indeterminate; Pos., positive; Neg., negative; ND, not detected; —, testing not performed.

^b Patient 1 was a 69-year-old man who was diagnosed with disseminated histoplasmosis in April 2013 and remained on itraconazole therapy; 3 months elapsed between serial tests 1 and 2, and 2 months elapsed between serial tests 2 and 3.

^c Patient 2 was a 62-year-old man who was diagnosed with disseminated histoplasmosis in October 2012 and remained on itraconazole therapy; 2 months elapsed between serial tests 1 and 2, and 2.5 months elapsed between serial tests 2 and 3.

^d Patient 3 was a 22-year-old man who was diagnosed with disseminated histoplasmosis in December 2011 and remained on voriconazole therapy; 3 months elapsed between serial tests 1 and 2.

^e Patient 4 was a 65-year-old man who was diagnosed with disseminated histoplasmosis in January 2013 and remained on itraconazole therapy; 2.5 months elapsed between serial tests 1 and 2.

EIA result of positive, below the limit of quantification, and positive *Histoplasma* serological results, the IMMY GM ASR result should be considered falsely negative. This subject was diagnosed with pulmonary histoplasmosis based on the presence of anti-*Histoplasma* antibodies, which were reported 48 h prior to the MVista EIA result. This case underscores the importance of using multiple available laboratory methods to aid in the diagnosis of *H. capsulatum* infection, regardless of which *Histoplasma* UAg assay is used.

The remaining two subjects with discordant results had been diagnosed with histoplasmosis over 2 years prior to testing in the current study, were receiving suppressive antifungal therapy, and were being monitored for *Histoplasma* UAg levels. In both cases, the MVista EIA provided low-level positive results, suggesting that the IMMY GM ASR results were falsely negative. In the 6 months following this study, serial testing with the MVista EIA revealed that these two subjects tested negative or persistently tested positive, below the limit of quantification, for *Histoplasma* UAg (data not shown). Similar to the first discordant case, it can be argued that the IMMY GM ASR is less sensitive than the MVista EIA and that patients monitored with the IMMY GM ASR begin testing negative for *Histoplasma* UAg sooner than if monitoring is performed with the MVista EIA. The question that arises, however, regards the clinical significance of persistent low-level positive *Histoplasma* UAg results for patients who have completed a full course of antifungal treatment and remain asymptomatic. Of note, the most recent Infectious Diseases Society of America guidelines on *H. capsulatum* infections indicate that persistent low-level *Histoplasma* antigenuria may not be reason enough to prolong antifungal treatment (5). Additionally, a study of immunoreconstituted AIDS patients (CD4⁺ T cell counts of >150 cells/mm³) with histoplasmosis who were treated for at least 12 months found no relapse of disease in the 2 years following discontinuation of antifungal suppressive therapy, despite low-level *Histoplasma* antigenuria in ~20% of patients (19). While these guidelines and studies suggest that persistent *Histoplasma* antigenuria may not be indicative of ongoing infection, further studies to better define the kinetics of *Histoplasma* UAg clearance from urine and to clarify the clinical significance of prolonged low-level antigenuria in relation to clinical disease are needed.

Second, during our study period, four patients who had been previously diagnosed with histoplasmosis were serially tested, at intervals of 2 to 3 months, with both the IMMY GM ASR and the MVista EIA. Despite the consistently lower quantitative UAg levels provided by the IMMY GM ASR, the overall trends of results with the two assays were similar. Serial testing results for one patient (patient 3 in Table 5) stand out, however, as an increase in quantitative values from the MVista EIA was not mirrored by the IMMY GM ASR results. After the conclusion of our study, *Histoplasma* UAg testing of this patient showed fluctuating values with the MVista EIA (6.72 and 10.31 ng/ml at 6 and 7 months poststudy, respectively) but a consistent IMMY GM ASR value (1.8 ng/ml at 9 months poststudy). The clinical significance of the fluctuating MVista EIA values was not readily apparent, as the patient remained asymptomatic with antifungal therapy. The overall disparity in quantitative antigen levels between the two assays is likely due to the different detection antibodies and target antigens used; the IMMY GM ASR employs a monoclonal antibody to *Histoplasma* galactomannan, whereas the MVista EIA utilizes a polyclonal antibody to a *Histoplasma* polysaccharide antigen (15, 20).

Collectively, however, these findings support application of the IMMY GM ASR as a means to monitor *Histoplasma* UAg levels and disease progression in patients receiving antifungal therapy. Future studies confirming these findings by following a larger cohort of patients receiving therapy for histoplasmosis over a longer period of time will be pursued.

The final aspect deserving discussion is the inclusion of an indeterminate range for the IMMY GM ASR. Among the 12 indeterminate specimens in this study, 6 (50%) were positive by the MVista EIA, and four of those were from patients with a prior diagnosis of *H. capsulatum* infection. These four patients had been diagnosed with disseminated histoplasmosis 3 or more years prior to the current study, and three of the four had low MVista EIA quantitative values. One of the six positive patients, however, was tested for initial diagnostic purposes and would have been missed by the IMMY GM ASR assay if an indeterminate range had not been applied. Collectively, these data confirm the need for an indeterminate range for the IMMY GM ASR, with samples falling within these limits requiring either further testing with an alternative assay or repeat testing with a fresh urine sample.

In summary, we demonstrate that, following modification of our original protocol and interpretive criteria, the IMMY GM ASR can be used as a reliable and timely (when implemented on site) assay for detection of *Histoplasma* UAg. Along with routine diagnostic testing, including culture, serological testing, and NAAT, the IMMY GM ASR can be used both to diagnose histoplasmosis and to monitor UAg levels as markers of disease progression. While introduction of an indeterminate range may be viewed as a limitation, use of a single cutoff value would have led to six additional false-negative results (compared to the MVista EIA), one of which would have occurred for a patient with a first-time diagnosis of *H. capsulatum* infection. Excluding indeterminate results, we achieved 100% negative agreement (121/121 samples) and 82.3% positive agreement (14/17 samples) with the MVista EIA. Among the three subjects with negative IMMY GM ASR results and positive MVista EIA results, the IMMY result should be considered falsely negative for only one patient. For the remaining two patients with discordant results, the clinical significance of persistent low-level antigenuria, as detected by the MVista EIA, was not entirely clear; therefore, inaccuracy of the IMMY GM ASR results in these cases cannot be determined with certainty.

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REFERENCES

- Baddley JW, Winthrop KL, Patkar NM, Delzell E, Beukelman T, Xie F, Chen L, Curtis JR. 2011. Geographic distribution of endemic fungal infections among older persons, United States. *Emerg Infect Dis* 17:1664–1669. <http://dx.doi.org/10.3201/eid1709.101987>.
- Kauffman CA. 2008. Diagnosis of histoplasmosis in immunosuppressed patients. *Curr Opin Infect Dis* 21:421–425. <http://dx.doi.org/10.1097/QCO.0b013e328306eb8d>.
- Wheat LJ. 2006. Improvements in diagnosis of histoplasmosis. *Expert Opin Biol Ther* 6:1207–1221. <http://dx.doi.org/10.1517/14712598.6.11.1207>.
- Kauffman CA. 2009. Histoplasmosis. *Clin Chest Med* 30:217–225. <http://dx.doi.org/10.1016/j.ccm.2009.02.002>.
- Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Loyd

- JE, Kauffman CA. 2007. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 45:807–825. <http://dx.doi.org/10.1086/521259>.
6. Babady NE, Buckwalter SP, Hall L, Le Febvre KM, Binnicker MJ, Wengenack NL. 2011. Detection of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from culture isolates and clinical specimens by use of real-time PCR. *J Clin Microbiol* 49:3204–3208. <http://dx.doi.org/10.1128/JCM.00673-11>.
 7. Kauffman CA. 2007. Histoplasmosis: a clinical and laboratory update. *Clin Microbiol Rev* 20:115–132. <http://dx.doi.org/10.1128/CMR.00027-06>.
 8. Maubon D, Simon S, Aznar C. 2007. Histoplasmosis diagnosis using a polymerase chain reaction method: application on human samples in French Guiana, South America. *Diagn Microbiol Infect Dis* 58:441–444. <http://dx.doi.org/10.1016/j.diagmicrobio.2007.03.008>.
 9. Munoz B, Martinez MA, Palma G, Ramirez A, Frias MG, Reyes MR, Taylor ML, Higuera AL, Corcho A, Manjarrez ME. 2010. Molecular characterization of *Histoplasma capsulatum* isolated from an outbreak in treasure hunters. *BMC Infect Dis* 10:264. <http://dx.doi.org/10.1186/1471-2334-10-264>.
 10. Scheel CM, Zhou Y, Theodoro RC, Abrams B, Balajee SA, Litvintseva AP. 2014. Development of a loop-mediated isothermal amplification method for detection of *Histoplasma capsulatum* DNA in clinical samples. *J Clin Microbiol* 52:483–488. <http://dx.doi.org/10.1128/JCM.02739-13>.
 11. Hage CA, Ribes JA, Wengenack NL, Baddour LM, Assi M, McKinsey DS, Hammoud K, Alapat D, Babady NE, Parker M, Fuller D, Noor A, Davis TE, Rodgers M, Connolly PA, El Haddad B, Wheat LJ. 2011. A multicenter evaluation of tests for diagnosis of histoplasmosis. *Clin Infect Dis* 53:448–454. <http://dx.doi.org/10.1093/cid/cir435>.
 12. Wheat LJ. 2009. Approach to the diagnosis of the endemic mycoses. *Clin Chest Med* 30:379–389. <http://dx.doi.org/10.1016/j.ccm.2009.02.011>.
 13. Hage CA, Kirsch EJ, Stump TE, Kauffman CA, Goldman M, Connolly P, Johnson PC, Wheat LJ, Baddley JW. 2011. *Histoplasma* antigen clearance during treatment of histoplasmosis in patients with AIDS determined by a quantitative antigen enzyme immunoassay. *Clin Vaccine Immunol* 18:661–666. <http://dx.doi.org/10.1128/CVI.00389-10>.
 14. Wheat LJ, Connolly-Stringfield P, Blair R, Connolly K, Garringer T, Katz BP, Gupta M. 1992. Effect of successful treatment with amphotericin B on *Histoplasma capsulatum* variety *capsulatum* polysaccharide antigen levels in patients with AIDS and histoplasmosis. *Am J Med* 92:153–160. [http://dx.doi.org/10.1016/0002-9343\(92\)90106-L](http://dx.doi.org/10.1016/0002-9343(92)90106-L).
 15. Zhang X, Gibson B, Jr, Daly TM. 2013. Evaluation of commercially available reagents for diagnosis of histoplasmosis infection in immunocompromised patients. *J Clin Microbiol* 51:4095–4101. <http://dx.doi.org/10.1128/JCM.02298-13>.
 16. Theel ES, Jespersen DJ, Harring J, Mandrekar J, Binnicker MJ. 2013. Evaluation of an enzyme immunoassay for detection of *Histoplasma capsulatum* antigen from urine specimens. *J Clin Microbiol* 51:3555–3559. <http://dx.doi.org/10.1128/JCM.01868-13>.
 17. Clinical and Laboratory Standards Institute. 2003. Evaluation of the linearity of quantitative measurement procedures: a statistical approach; approved guideline. CLSI document EP06-A. Clinical and Laboratory Standards Institute, Wayne, PA.
 18. Fleiss JL, Levin B, Paik MC. 2003. Statistical methods for rates and proportions, 3rd ed. Wiley-Interscience, Hoboken, NJ.
 19. Goldman M, Zackin R, Fichtenbaum CJ, Skiest DJ, Koletar SL, Hafner R, Wheat LJ, Nyangweso PM, Yiannoutsos CT, Schnitzlein-Bick CT, Owens S, Aberg JA. 2004. Safety of discontinuation of maintenance therapy for disseminated histoplasmosis after immunologic response to antiretroviral therapy. *Clin Infect Dis* 38:1485–1489. <http://dx.doi.org/10.1086/420749>.
 20. Connolly PA, Durkin MM, Lemonte AM, Hackett EJ, Wheat LJ. 2007. Detection of *Histoplasma* antigen by a quantitative enzyme immunoassay. *Clin Vaccine Immunol* 14:1587–1591. <http://dx.doi.org/10.1128/CVI.00071-07>.