Re-evaluation of Commercial Reagents for Detection of *Histoplasma capsulatum* Antigen in Urine

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Abstract (250 words)

Detection of *Histoplasma capsulatum* urine 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 showed low positive agreement (64.5%) with the MiraVista (MVista) *Histoplasma* Ag Quantitative EIA (MiraVista Diagnostics, Indianapolis, IN). Here, we have re-evaluated the IMMY GM ASR following modification of our original assay protocol and introduction of an indeterminate range. 150 prospectively collected urine samples were tested by both the IMMY and the MVista EIAs, and clinical histories were recorded for all study subjects. The IMMY GM ASR showed a positive and negative agreement of 82.3% (14/17) and 100% (121/121), respectively (12 indeterminate results excluded), and an overall percent agreement of 90% (135/150), with the MVista EIA. Of the three IMMY GM ASR negative/MVista EIA positive patients, one was tested for initial diagnostic purposes (<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 was positive in 6/12 samples which were 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 *Histoplasma* UAg.
Introduction

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

Due to the non-specific clinical presentation of histoplasmosis, alongside 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), histopathology, and antibody and antigen 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, histopathology and NAATs 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], biopsy), which may be contraindicated in patients with severe disease. Finally, the...
prolonged incubation (2-4 weeks) required for recovery of the organism in culture can delay
initiation of antifungal treatment. Serologic assays for detection of antibodies to *H. capsulatum*
are advantageous as they are performed on an easily accessible specimen source (e.g., serum)
and provide high specificity (>90%), however, results may be falsely negative in patients with
recent infection and in 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,
specifically by 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 (75-80% and >90%, respectively) (3, 11, 12).
Also, unlike other diagnostic methods, monitoring quantitative *Histoplasma* antigen values in
urine and/or serum can be used to follow treatment response and 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 the turnaround time to result
reporting and affect patient management. Finally, while an FDA-cleared assay for detection of
*Histoplasma* UAg is available, the ALPHA *Histoplasma* Antigen EIA (IMMY), 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 MVista EIA and found positive, negative and overall percent agreements of
64.5% (40/62), 99.8% (939/941) and 97.6% (979/1003), respectively. Despite the high overall agreement, the low positive agreement of the IMMY GM ASR with the MVista EIA was unacceptable. In this study, we re-evaluated the IMMY GM ASR following modification of both our original methodology and 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.
Materials and Methods

Study design. Prospective urine samples (n=150) collected from Mayo Clinic patients (n=143) between August 2013 and February 2014, and submitted for routine clinical evaluation by the MVista Histoplasma Ag Quantitative EIA, were also tested by 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 to patient clinical history. Qualitative and quantitative results were compared for both assays and medical charts were reviewed for all subjects at the time of testing and six 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 the microtiter wells which are classified as Research Use Only; for the purposes of this manuscript, the assay will be referred to as the ‘IMMY GM ASR’. Testing was performed on the Triturus automated EIA analyzer (Grifols, Miami, FL) using 100 µL of undiluted urine added directly to microtiter wells coated with a monoclonal antibody specific to GM and incubated for 55 min at 37°C. Wells were washed and 100 µL of horseradish peroxidase (HRP) conjugated anti-GM monoclonal antibody was added, incubated for 40 min at 25°C, followed by a second wash step. 100 µL of 3,3’,5,5’ tetramethylbenzidine was added next, incubated for 25 minutes at 25°C, followed by addition of 100 µL of 2N sulfuric acid (stop solution). The optical density (OD) in each well was measured at a dual excitation wavelength of 450/620 nm. A standard curve was generated with each run using eight calibrators: a 0.0 ng/mL (wash buffer) calibrator was added to the seven calibrators (individual concentrations: 0.4, 0.8, 1.6, 3.2, 6.3, 12.5 and 25 ng/mL)
recommended by the manufacturer. ODs from 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 and ≥0.50 ng/mL, positive. Following completion of testing, results for both the IMMY GM ASR and the MVista EIA 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 cut-off criteria and interpretations for the IMMY GM ASR are as follows: specimens with values falling between 0.11 ng/mL and 0.49 ng/mL were considered indeterminate, values ≤0.10 ng/mL were considered as negative, and consistent with the manufacturer’s recommendations, values ≥0.5 ng/mL were considered as positive. Specimens with results >23.0 ng/mL were considered ‘positive, above the limit of quantification’ (ALQ).

The quantifiable range (0 to 23 ng/mL) for the IMMY GM ASR was established with linearity studies by testing serial two-fold dilutions (from 1:2 to 1:8192) of a high GM-positive clinical urine sample in duplicate per CLSI document EP06-A (17). Standard linear regression analysis comparing the mean observed and expected value concentrations at each dilution showed good correlation (Supplemental Figure 1A) with acceptable result differences (Supplemental Figure 1B) across the quantifiable range.

**MVista Histoplasma Ag Quantitative EIA:** All 150 urine samples were evaluated by the MVista Histoplasma Ag Quantitative EIA performed at MiraVista Diagnostics. Results are reported as ‘not detected’ or ‘positive’ with a quantified result provided for values between 0.4
ng/mL and 19 ng/mL. Samples with values <0.4 ng/mL or >19 ng/mL are reported as ‘positive, below the limit of quantification’ or ‘positive, above the limit of quantification’.

**Patient Chart Review:** Medical records were reviewed and the following information was recorded: Symptoms at presentation, comorbidities, immunologic status, radiologic findings, exposure history, other laboratory data (culture, serology, etc.), antimicrobial prophylaxis, final diagnosis and whether antifungal treatment was initiated. Additionally, patient charts were re-reviewed six months after testing to exclude the possibility that a case of histoplasmosis had been missed by both assays.

**Statistical Analysis:** GraphPad software (http://www.graphpad.com/quickcalcs/, La Jolla, CA) was used to determine positive agreement, negative agreement, overall agreement, kappa values and 95% confidence intervals (CI) for qualitative result comparison between the IMMY GM ASR and the MVista EIA.
Results

The 150 urine samples analyzed in this study were submitted from 143 unique patients with a median age of 58 years (range 12-89 years) and 60% (86/143) were male. The MVista EIA was ordered for initial diagnostic purposes in 124 patients and to monitor *Histoplasma* urine antigen levels in an additional 19 patients. Utilization of the manufacturer recommended cut-off criteria to interpret the IMMY GM ASR results from these 150 samples led to a positive, negative and overall percent agreement of 60.9%, 100% and 94.0%, respectively, when compared to the MVista EIA (Table 1) (18). Following application of our modified interpretive criteria, we demonstrated an overall percent agreement of 90.0% (135/150) and a kappa value of 0.72, indicating a ‘good’ strength of agreement (Table 2). Notably, using our modified criteria, 12 of the 150 (8%) urine samples resulted as indeterminate by the IMMY GM ASR. A detailed analysis of these 12 samples is provided below and in Table 3. Excluding the 12 indeterminate samples, the IMMY GM ASR showed a positive and negative agreement of 82.3% (14/17) and 100% (121/121), respectively, when compared to the MVista EIA (Table 2). The turnaround time from specimen collection to result availability was monitored for both assays. The range, average and median time to results was 2-6 days, 2.7 days and 2 days for the MVista EIA and 0.5-3 days, 1.07 days and 1 day for the IMMY GM ASR, respectively.

**Accuracy of the IMMY GM ASR.** Among the 138 samples resulted as positive or negative by the IMMY GM ASR, 14 were positive by both the IMMY and MVista EIAs (Table 2). Thirteen of the 14 were from subjects with either a first time diagnosis of *H. capsulatum* infection (n=4) or were being monitored for response to treatment (n=9) (Supplemental Table 1). The remaining subject was diagnosed with acute respiratory distress syndrome secondary
to pulmonary blastomycosis, confirmed by growth of *Blastomyces dermatitidis* from BAL fluid.

This indicates that similar to the MVista *Histoplasma* UAg EIA, the IMMY GM ASR may lead to false positive results in patients with blastomycosis. Importantly, during this evaluation, there were no samples that were IMMY GM ASR positive/MVista EIA negative, and none of the subjects associated with the 121 samples negative by both assays developed histoplasmosis in the six months following testing.

Three of the 138 samples (2.2%) were IMMY GM negative and MVista EIA positive (Table 4). Two of the three were from patients diagnosed with disseminated histoplasmosis two 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 in this patient based on positive anti- *Histoplasma* antibody results, available 48 hours prior to receipt of the MVista *Histoplasma* UAg report.

**Indeterminate Results by the IMMY GM ASR.** Of the 150 urine samples studied, 12 (8%) yielded indeterminate results by the IMMY GM ASR (0.15 to 0.44 ng/mL, Table 3). Seven of the 12 samples were submitted for initial diagnostic purposes and five were collected to monitor UAg levels in patients previously diagnosed with histoplasmosis. Two of the seven patients evaluated due to clinical concern for primary *H. capsulatum* infection were positive by the MVista EIA. One (Patient #5, Table 3) was diagnosed with relapsed pulmonary blastomycosis based on a positive *B. dermatitidis* immunodiffusion result, and the second (Patient #6, Table 3) was diagnosed with pulmonary histoplasmosis based on positive *Histoplasma* serology results, available 24 hours 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 the IMMY GM ASR. Of the 143 subjects included in our study, four were serially tested during the study period (Table 5). While quantitative values by the IMMY GM ASR were consistently lower than those reported by the MVista EIA, the two assays showed similar trending of Histoplasma UAg levels overtime for all four subjects.
Discussion

We have re-evaluated the IMMY GM ASR for detection of *Histoplasma* GM in urine following modification of our previously published protocol and interpretive criteria (16).

Specifically, we included an additional calibrator to those provided by the manufacturer and established an indeterminate result range. With these modifications we show that compared to the MVista EIA, the IMMY GM ASR has an overall percent agreement of 90.0% (135/150), and following exclusion of samples with indeterminate results, a positive and negative agreement of 82.3% (14/17) and 100% (121/121), 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 had identified an optimal cut-off value of ≥0.15 ng/mL for positive results using ROC analysis; application of this criterion to the current data set resulted in seven falsely positive IMMY GM ASR results in patients without histoplasmosis, discrediting this cut-off 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 by the MVista EIA to an average of 1.07 days using the IMMY GM ASR when performed in our laboratory.

A number of findings in our study deserve to be highlighted. First, when compared to the MVista EIA, the IMMY GM ASR did not yield false positive results during this evaluation and none of the subjects who tested negative by both assays developed histoplasmosis in the six months following initial testing. Three subjects did however have discordant, IMMY GM ASR negative and MVista EIA positive results. The first of these patients was tested for initial
diagnostic purposes, and based on the ‘positive, below limit of quantification’ MVista EIA result and positive *Histoplasma* serologies, the IMMY GM ASR result should be considered as falsely negative. This subject was, however, diagnosed with pulmonary histoplasmosis based on the presence of anti-*Histoplasma* antibodies, which were reported 48 hours 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 two 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 a low positive result, suggesting that the IMMY GM ASR was falsely negative. In the six months following this study, serial testing by the MVista EIA revealed that these two subjects became negative or were persistently ‘positive, below 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 by the IMMY GM ASR become negative for *Histoplasma* UAg sooner than if monitoring is performed by the MVista EIA. The question that arises however is to the clinical significance of a persistently low positive *Histoplasma* UAg result in a patient who has completed a full course of antifungal treatment and remains asymptomatic. Of note, the most recent Infectious Diseases Society of America guidelines on *H. capsulatum* infections indicate that persist low-level *Histoplasma* antigenuria may not be reason enough to prolong antifungal treatment (5). Additionally, a study in immune reconstituted AIDS patients (CD4+ T cell count...
>150 cells/mm$^3$) with histoplasmosis who were treated for at least 12 months, found no relapse of disease in the two 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.

Secondly, during our study period, four patients previously diagnosed with histoplasmosis were serially tested two to three months apart by both the IMMY GM ASR and the MVista EIA. Despite the consistently lower UAg quantitative values provided by the IMMY GM ASR, overall trending of results between the two assays was similar. Serial testing for one patient (Patient #3, Table 5) stands out however, as an increase in quantitative values by the MVista EIA was not mirrored by the IMMY GM ASR. Following the conclusion of our study, subsequent *Histoplasma* UAg testing of this patient showed fluctuating values by the MVista EIA (6.72 and 10.31 ng/mL at 6 and 7 months post-study), yet a consistent IMMY GM ASR value (1.8 ng/ml at 9 months post-study). The clinical significance of the varying MVista EIA values was not readily apparent as the patient remained asymptomatic on antifungal therapy. The overall disparity in quantitative antigen levels between the two assays is likely due to the differing 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 on 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 in patients with a prior diagnosis of *H. capsulatum* infection. These four patients had been diagnosed with disseminated histoplasmosis two 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 had an indeterminate range not been applied. Collectively, these data support the need for an indeterminate range of the IMMY GM ASR, with samples falling within these limits requiring either further testing by an alternative assay or repeat testing on 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, when implemented on-site, timely assay for detection of *Histoplasma* UAg. Alongside routine diagnostic testing including culture, serology and NAAT, the IMMY GM ASR can be used to both diagnose histoplasmosis and to monitor UAg levels as a marker of disease progression. While introduction of an indeterminate range may be viewed as a limitation, use of a single cut-off value would have led to an additional six false negative results (compared to the MVista EIA), one of which would have occurred in a patient with a first time diagnosis of *H. capsulatum* infection. Excluding indeterminate results, we achieved 100% (121/121) negative agreement.
and 82.3% (14/17) positive agreement with the MVista EIA. Among the three IMMY GM ASR negative/MVista EIA positive subjects, the IMMY result should be considered falsely negative for only one patient. For the remaining two discordant patients, the clinical significance of persistently low-level antigenuria as detected by the MVista EIA was not entirely clear and therefore consideration of the IMMY GM ASR results as inaccurate in these cases cannot be determined with certainty.

Acknowledgements

We would like to thank all technologists in the Central Clinical Laboratory and the Infectious Diseases Serology Laboratory who helped aliquot and prepare urine samples for this study.
**Supplemental Figure 1 Legend**

**A. Linear regression.** Standard linear regression showed strong correlation (solid line; $r = 0.991$) between the expected and mean observed values for the IMMY GM ASR.

**B. Difference plot analysis.** Plot of the differences between the corresponding expected and mean observed values versus the expected value showed an overall mean difference of 1.15 ng/mL, with all but one difference falling within a ±1.96 SD range of -5.85 ng/mL to 8.15 ng/mL.

These data verified a quantification range of 0.00 to 23.00 ng/mL for IMMY GM ASR. ULQ, upper limit of quantification.


Table 1. Comparison of the IMMY GM ASR and MVista EIA for Detection of *Histoplasma* Antigen in Urine using the Manufacturer’s Interpretive Criteria (n=150)

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Positive Agreement</th>
<th>Negative Agreement</th>
<th>Overall Agreement</th>
<th>( \kappa )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMMY GM ASR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>0</td>
<td>60.9% (CI: 40.7-77.9%)</td>
<td>100% (CI: 96.5-100%)</td>
<td>94.0% (CI: 88.8-97.0%)</td>
<td>0.72 (CI: 0.55-0.89)</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>127</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, 95% Confidence Interval; \( \kappa \), kappa coefficient
Table 2. Comparison of the IMMY GM ASR and MVista EIA for Detection of *Histoplasma* Antigen in Urine using Modified Interpretive Criteria (n=150)

<table>
<thead>
<tr>
<th>IMMY GM ASR</th>
<th>MVista EIA</th>
<th>Positive Agreement</th>
<th>Negative Agreement</th>
<th>Overall Agreement</th>
<th>( \kappa )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>14(^a)</td>
<td>0</td>
<td>82.3% (CI: 58.2-94.6%)</td>
<td>100% (CI: 96.3-100%)</td>
<td>90.0% (CI: 84.6-94.0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>3(^a)</td>
<td>121</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indeterminate(^a)</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, 95% Confidence Interval; \( \kappa \), kappa coefficient

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

\(^b\) Refer to Supplemental Table 1 for detailed clinical information.

\(^c\) Refer to Table 4 for detailed clinical information.
<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age/Sex</th>
<th>IMMY ASR (ng/mL)</th>
<th>MVista EIA (ng/mL)</th>
<th>Purpose for Test?</th>
<th>Patient History and Significant Laboratory Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27/M</td>
<td>0.24</td>
<td>Not Det.</td>
<td>Initial Diagnosis</td>
<td>Refractory T-cell mediated autoimmune disease on cyclosporine; Aspergillus fumigatus from BAL Cx; Dx. with pulmonary aspergillosis</td>
</tr>
<tr>
<td>2</td>
<td>60/F</td>
<td>0.28</td>
<td>Not Det.</td>
<td>Initial Diagnosis</td>
<td>Liver and kidney Tx in June 2013 on mycophenolate mofetil, tacrolimus and prednisone; All microbiology laboratory testing negative; Dx. with granulomatous hepatitis</td>
</tr>
<tr>
<td>3</td>
<td>29/M</td>
<td>0.42</td>
<td>Not Det.</td>
<td>Initial Diagnosis</td>
<td>Not ICH; Presented with SOB and mediastinal mass on CT scan; Parvimonas species, Actinomyces species from BAL Cx; Dx. with pulmonary lung abscess</td>
</tr>
<tr>
<td>4</td>
<td>55/F</td>
<td>0.28</td>
<td>Not Det.</td>
<td>Initial Diagnosis</td>
<td>Not ICH; Presented with unintentional weight loss; All microbiology laboratory testing negative; Dx. unclear – not infectious</td>
</tr>
<tr>
<td>5</td>
<td>61/F</td>
<td>0.44</td>
<td>2.29; Pos.</td>
<td>Initial Diagnosis</td>
<td>Not ICH; Blastomycosis Dx. in 2010 s/p 15 months of itraconazole; Blastomyces ID pos., Histoplasma CF and ID neg.; Dx. with relapsed pulmonary blastomycosis</td>
</tr>
<tr>
<td>6</td>
<td>38/M</td>
<td>0.35</td>
<td>0.67; Pos.</td>
<td>Initial Diagnosis</td>
<td>Psoriatic arthritis; Presented with FUO and 11 mm nodular lung opacity; Histoplasma CF Myc. 1:16, CF Yst. 1:256, ID M-Band; Dx. with pulmonary histoplasmosis</td>
</tr>
<tr>
<td>7</td>
<td>35/F</td>
<td>0.29</td>
<td>Not Det.</td>
<td>Initial Diagnosis</td>
<td>Crohn’s Disease on azathioprine; Presented with abdominal pain; All microbiology testing negative; Dx. with exacerbated Crohn’s disease</td>
</tr>
<tr>
<td>8</td>
<td>69/M</td>
<td>0.24</td>
<td>0.56; Pos.</td>
<td>Monitoring</td>
<td>Lymphocytic leukemia Dx. 2009; Disseminated histoplasmosis Dx. in April 2013; Remains on itraconazole</td>
</tr>
<tr>
<td>9</td>
<td>50/M</td>
<td>0.24</td>
<td>Not Det.</td>
<td>Monitoring</td>
<td>Not ICH, hypertension; Pulmonary histoplasmosis Dx. in June 2013 Remains on itraconazole</td>
</tr>
<tr>
<td>10</td>
<td>44/F</td>
<td>0.15</td>
<td>0.92; Pos.</td>
<td>Monitoring</td>
<td>Pancreas and kidney Tx in 2001 on mycophenolate mofetil and tacrolimus; Disseminated histoplasmosis Dx. in September 2012; Remains on itraconazole</td>
</tr>
<tr>
<td>11</td>
<td>61/F</td>
<td>0.22</td>
<td>0.49; Pos.</td>
<td>Monitoring</td>
<td>Crohn’s Disease on azathioprine; Disseminated histoplasmosis Dx. Nov 2012; Remains on itraconazole</td>
</tr>
<tr>
<td>12</td>
<td>33/F</td>
<td>0.35</td>
<td>7.21; Pos.</td>
<td>Monitoring</td>
<td>Crohn’s Disease on adalimumab and methotrexate; Disseminated histoplasmosis Dx in Jan. 2011; Remains on voriconazole</td>
</tr>
</tbody>
</table>

Abbreviations: Dx., Diagnosis; BAL, Bronchoalveolar lavage; Cx., Culture; Det., Detected; Pt., Patient; ICH, Immunocompromised host; FUO, Fever of Unknown Origin; CF, Complement Fixation; ID, Immunodiffusion; Myc., Mycelial Antigen; Yst., Yeast Antigen;
Table 4. Chart Review of Patients with Discordant Results Between the IMMY GM ASR and MVista EIA (n=3)

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age/Sex</th>
<th>IMMY ASR (ng/mL)</th>
<th>MVista EIA (ng/mL)</th>
<th>Purpose for Test</th>
<th>Patient History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52/M</td>
<td>0; Neg. &lt;0.4</td>
<td>Pos., BLoQ</td>
<td>Monitoring</td>
<td>Disseminated histoplasmosis Dx. in Feb. 2012, on prednisone and azathioprine for treatment of frontal orbital inflammatory pseudotumor at time of Dx; Remains on itraconazole</td>
</tr>
<tr>
<td>2</td>
<td>28/F</td>
<td>0; Neg. 0.64</td>
<td>Pos.</td>
<td>Monitoring</td>
<td>Disseminated histoplasmosis Dx. in Dec. 2010, on adalimumab and azathioprine for Crohn’s disease at time of Dx; Remains on itraconazole</td>
</tr>
<tr>
<td>3</td>
<td>68/M</td>
<td>0; Neg. &lt;0.4</td>
<td>Pos., BLoQ</td>
<td>Initial Diagnosis</td>
<td>AML s/p allogenic SCTx in Dec. 2012; Pulmonary histoplasmosis Dx. in Oct. 2013 by serology (CF Yst 1:64; H and M bands by ID)</td>
</tr>
</tbody>
</table>

Abbreviations: Dx., Diagnosis; ICH, Immunocompromised Host; Pos., Positive; AML, Acute Myeloid Leukemia; s/p, status post; SCTx, Stem Cell Transplant; CF, Complement Fixation; Yst., Yeast Antigen; ID, Immunodiffusion.

*a Serology results finalized 24 hours following serum collection; MVista EIA results finalized 72 hours following urine collection. Both specimens were collected on the same day.*
Table 5. Comparison of Serial Test Results Between the IMMY GM ASR and MVista EIAs for Four Patients

<table>
<thead>
<tr>
<th></th>
<th>Patient 1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Patient 2&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Patient 3&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Patient 4&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/Sex</td>
<td>69/M</td>
<td>62/M</td>
<td>22/M</td>
<td>65/M</td>
</tr>
<tr>
<td>Pt. Hx.</td>
<td>Disseminated histoplasmosis Dx. in April, 2013; Remains on Itraconazole</td>
<td>Disseminated histoplasmosis Dx. in Oct. 2012; Remains on Itraconazole</td>
<td>Disseminated histoplasmosis Dx. in Dec. 2011; Remains on Voriconazole</td>
<td>Disseminated histoplasmosis Dx. in Jan. 2013; Remains on Itraconazole</td>
</tr>
<tr>
<td>Assay&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IMMY ASR</td>
<td>MVista</td>
<td>IMMY ASR</td>
<td>MVista</td>
</tr>
<tr>
<td>Serial Test #1</td>
<td>0.24; Ind.</td>
<td>0.56; Pos.</td>
<td>9.66; Pos.</td>
<td>15.94; Pos.</td>
</tr>
<tr>
<td>Serial Test #2</td>
<td>0; Neg.</td>
<td>Not Det.</td>
<td>11.1; Pos.</td>
<td>14.03; Pos.</td>
</tr>
<tr>
<td>Serial Test #3</td>
<td>0; Neg.</td>
<td>Not Det.</td>
<td>4.51; Pos.</td>
<td>7.48; Pos.</td>
</tr>
</tbody>
</table>

Abbreviations: Pt., Patient; Hx., History; Dx., Diagnosed; Ind., Indeterminate.; Det., Detected; Dash (-) indicates testing not performed.

<sup>a</sup> All quantitative values are in ng/mL for both the IMMY GM and MVista EIAs.
<sup>b</sup> 3 months elapsed between serial test #1 and #2 and 2 months elapsed between serial tests #2 and #3.
<sup>c</sup> 2 months elapsed between serial test #1 and #2 and 2.5 months elapsed between serial tests #2 and #3.
<sup>d</sup> 3 months elapsed between serial test #1 and #2.
<sup>e</sup> 2.5 months elapsed between serial test #1 and #2.