

Clinical Significance of Low-Positive *Histoplasma* Urine Antigen Results

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***Histoplasma* urine antigen (UAg) detection is an important biomarker for histoplasmosis. The clinical significance of low-positive (<0.6 ng/ml) UAg results was evaluated in 25 patients without evidence of prior *Histoplasma* infection. UAg results from 12/25 (48%) patients were considered falsely positive, suggesting that low-positive UAg values should be interpreted cautiously.**

Early recognition of *Histoplasma capsulatum* infection is essential for successful patient outcome, particularly among immunocompromised patients who are at increased risk for severe disease, including hematogenous dissemination. Available diagnostic modalities for histoplasmosis include fungal culture, nucleic acid amplification tests (NAAT), histopathologic examination of tissue biopsy specimens, and *Histoplasma* antibody and antigen detection from body fluids. Among these methods, *Histoplasma* antigen detection from urine offers high clinical sensitivity for identification of patients with disseminated disease compared to alternative diagnostic methodologies (1–3). Additionally, *Histoplasma* antigen levels decline during disease resolution and are therefore monitored as a measure of patient response to therapy (4).

To date, the most utilized *Histoplasma* urine antigen (UAg)

assay is the *Histoplasma capsulatum* Quantitative Antigen enzyme immunoassay (EIA) performed at MiraVista Diagnostics (Indianapolis, IN) (2, 5). As of April 2012, the current quantifiable range of the MiraVista UAg EIA is 0.4 to 19 ng/ml, with samples falling within this range reported as “positive” (official notification from

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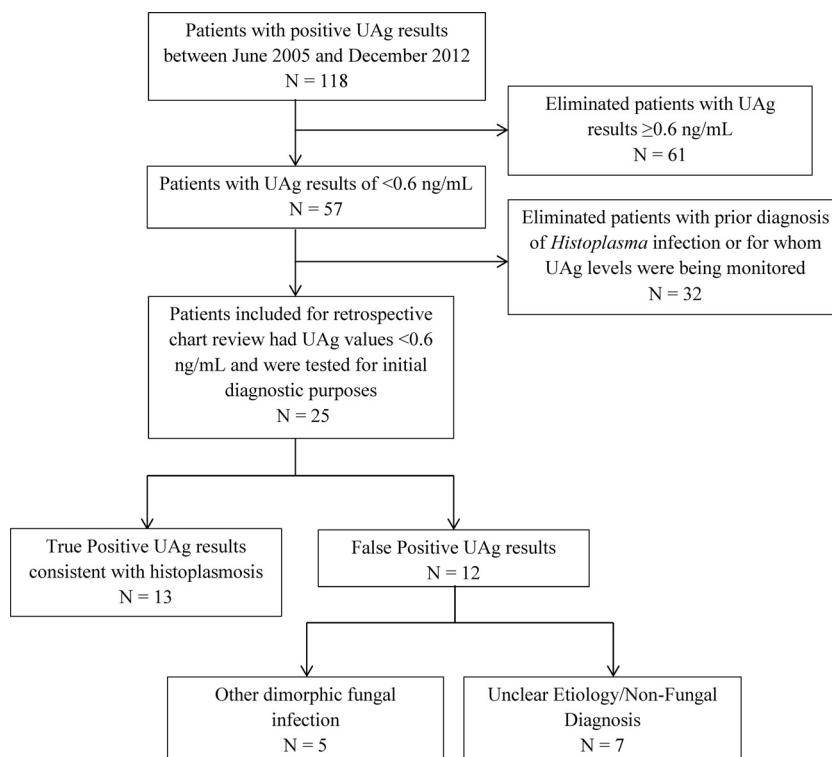


FIG 1 Selection for and evaluation of patients with low-positive (<0.6 ng/ml) *Histoplasma* UAg results by retrospective chart review.

TABLE 1 Clinical and laboratory findings for patients with falsely positive *Histoplasma* UAg values of <0.6 ng/ml^a

Patient no.	Age (yr)/sex	Presenting symptom(s)	Comorbidity(ies)/immune status	Radiologic findings at presentation	Other microbiology findings	Final diagnosis	Antifungals started?
1	46/F	Fever; new DVT/PICC	Sacral OM 3 months prior	N/A	<i>Enterobacter cloacae</i> BSI	<i>E. cloacae</i> BSI	No
2	67/M	Weight loss, dysphagia	Coronary/peripheral artery disease	Hepatosplenomegaly	<i>H. capsulatum</i> yeast CF (1:16)	Sarcoidosis	No
3	73/M	Incidental cavitory lesion	B-cell lymphoma on chemotherapy	Left lung cavitory lesion	N/A	Unclear etiology	Empirical fluconazole
4	27/F	Fever, cough	None	Bilateral lung infiltrates	N/A	Community-acquired pneumonia	No
5	70/F	Respiratory failure	Lung carcinoma on chemotherapy	Bilateral lung infiltrates	<i>Blastomyces</i> UAg positive (2.19 ng/ml)	Disseminated blastomycosis	Itraconazole
6	81/M	Pancytopenia, noncaseating granuloma in BM	Coronary artery disease	Splenomegaly	<i>H. capsulatum</i> yeast CF (1:16); <i>H. capsulatum</i> ID; M band <i>Coccidioides</i> CF (1:2)	Granulomatous disease, secondary to sarcoidosis	No
7	74/F	Persistent pulmonary infiltrates	Hypertension	Bilateral lung infiltrates	<i>Coccidioides</i> IgG ID positive and CF (1:8)	<i>Coccidioidomycosis</i>	Fluconazole
8	74/M	Elevated ESR	RA on methotrexate	Nodular lung infiltrates	<i>Blastomyces</i> recovered from BAL fluid	Pulmonary blastomycosis	Itraconazole
9	37/M	Lymphadenopathy	Unclear	N/A	<i>Coccidioides</i> spherule identified from lymph node biopsy specimen	<i>Coccidioides</i> lymphadenopathy	Fluconazole
10	55/M	Fever, fatigue	Stem cell transplant on prednisone	Bilateral ground-glass infiltrates	<i>Pseudomonas</i> isolated from sputum	Unclear etiology, initiated on ciprofloxacin	No
11	66/F	Incidental CT finding	Remote esophageal cancer	Upper lobe nodules	N/A	Unclear etiology	No
12	11/F	Fever, dry cough	None	Cavitory infiltrate	<i>Blastomyces</i> EIA and UAg positive	Pulmonary blastomycosis	Itraconazole

^a Abbreviations: M, male; F, female; RA, rheumatoid arthritis; BM, bone marrow; DVT/PICC, deep vein thrombosis/peripherally inserted central catheter; ID, immunodiffusion; CF, complement fixation; OM, osteomyelitis; BSI, bloodstream infection; WL, weight loss; BAL, bronchoalveolar lavage; ESR, erythrocyte sedimentation rate; HA, headache; CT, computed tomography; N/A, not applicable.

MiraVista Diagnostics). Values of <0.4 ng/ml or >19 ng/ml are considered positive and “below” or “above” the limit of quantification (LoQ), respectively. Finally, MiraVista also reports a result of “none detected” which is interpreted as “negative,” though a quantitative antigen value is not provided. The quantifiable range for this assay has undergone a number of iterations over the past decade. The most recent previous range was defined as 0.6 to 39 ng/ml, and, similarly to the current assay, values of <0.6 ng/ml were considered “positive, below the LoQ.”

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In a previous study conducted by our group, we compared a commercially available *Histoplasma* UAg EIA (Immuno Mycologics, Inc. [IMMY], Norton, OK) to the MiraVista assay and encountered multiple patients whose results were classified as “positive, below the LoQ” by the MiraVista EIA and yet were negative

by the comparator assay (6). Interestingly, this subset of patients either never developed histoplasmosis or had been on antifungal therapy for 1 year or longer without active symptoms. Based on these observations, we questioned the clinical significance of low-positive MiraVista *Histoplasma* UAg results, defined as values of <0.6 ng/ml, specifically among patients without a history of histoplasmosis.

To address this issue, we identified all Mayo Clinic patients who tested positive by the MiraVista UAg EIA between June 2005 and December 2012 ($n = 118$; Fig. 1). From this subset, those with UAg results of ≥ 0.6 ng/ml ($n = 61$) and those with a prior diagnosis of histoplasmosis for whom UAg levels were being monitored ($n = 32$) were excluded from analysis. The medical charts for the remaining 25 patients were retrospectively reviewed to determine the clinical impact of a low-positive UAg result. Specifically, patient presentation at the time of testing, immune status, radiologic findings, exposure history, other microbiologic labora-

tory data, and any antifungal treatment were recorded. For this review, a confirmed case of histoplasmosis was defined by isolation of *H. capsulatum* in culture, a positive NAAT result (7), identification of fungal organisms consistent with *H. capsulatum* by histopathology, and/or strongly suggestive serologic evidence, defined as the presence of at least an H-band as demonstrated by immunodiffusion (ID) and complement fixation (CF) titers of $\geq 1:32$ for the yeast and/or mycelial *Histoplasma* antigens. Patients who did not meet the above criteria and yet had positive *Histoplasma* serologies (e.g., M-band only by ID and CF titers between $\geq 1:8$ and $< 1:32$) and for whom an alternative diagnosis was not identified were classified as representing possible histoplasmosis cases. All other patients were classified as negative for histoplasmosis. This study was approved by the Mayo Clinic Institutional Review Board.

Among the 25 patients with initial low-positive *Histoplasma* UAg values, 12 were considered to have a confirmed case of histoplasmosis on the basis of identification by recovery of the organism in culture ($n = 4$) from various sources (i.e., tissue, bronchoalveolar lavage [BAL] fluid, or bone marrow), by positive histopathology ($n = 1$, lung biopsy specimen), by a positive NAAT result ($n = 1$, bone marrow), or by the patient's serologic profile ($n = 6$). An additional patient was considered to have possible pulmonary histoplasmosis as determined on the basis of the presence of an M-band by ID and a yeast CF titer of 1:16; due to the lack of an alternative diagnosis, this patient was treated with itraconazole. Collectively, the low-positive *Histoplasma* UAg values for these 13/25 (52%) patients were considered true-positive results and led to the prompt diagnosis of *H. capsulatum* infection and initiation of antifungal therapy.

Among the remaining 12 (48%) patients, the low-positive UAg values were considered falsely positive, as the patients were ultimately diagnosed with an alternative disease ($n = 9$) or never progressed to histoplasmosis ($n = 3$) (Table 1). Of these 12 patients, 3 were diagnosed with disseminated or pulmonary blastomycosis based on recovery of *Blastomyces dermatitidis* from BAL fluid or a strongly positive *B. dermatitidis* UAg test result (MiraVista Diagnostics). *Coccidioides immitis*-*Coccidioides posadasii* infections were identified in an additional two patients based on identification of a spherule from a lymph node biopsy specimen and a suggestive serologic profile. Cross-reactivity of the *Histoplasma* UAg assay with these dimorphic fungi and other fungal agents, including *Penicillium*, *Paracoccidioides*, and, rarely, *Aspergillus* species, has been described previously and remains a noted limitation of the MiraVista assay (5). However, despite the lack of analytical specificity among these five low-positive *Histoplasma* UAg results, they remain of clinical value in this patient group as the treatments of *Histoplasma* and *Blastomyces* infections are similar and the risk for *Coccidioides* infection can be determined based on prior travel history (e.g., travel to the southwest United States). The consideration of the possibility on an alternative fungal infection in at-risk patients with low-positive *Histoplasma* UAg values

is recommended, and further testing by alternative methods (e.g., serology, culture) would be beneficial in such cases.

The remaining 7/12 patients either received an alternative diagnosis ($n = 4$) or remained without a defined etiology for their symptoms ($n = 3$). Notably, one patient was started on empirical fluconazole treatment despite the absence of additional supportive data for a fungal infection, and though two patients had positive *Histoplasma* serologic findings, both were ultimately diagnosed with sarcoidosis and antifungal treatment was withheld. Importantly, neither of these patients developed histoplasmosis or infection with an alternative agent known to cross-react with the UAg assay in the year following the initial low-positive *Histoplasma* UAg result.

The major limitation of this retrospective study was the small number of patients with low-positive *Histoplasma* UAg levels ($n = 25$). However, this remains the first review of the clinical utility of low-positive UAg values and adds previously undocumented data to the ever-growing understanding of this assay.

In conclusion, our evaluation suggests that while the *Histoplasma* UAg assay remains an invaluable tool for the diagnosis of histoplasmosis, low-positive *Histoplasma* UAg results should be interpreted cautiously and correlated with additional laboratory findings (e.g., serology, culture, repeat *Histoplasma* UAg testing) to confirm the diagnosis.

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