

Evaluation of the IMMY ALPHA *Histoplasma* Antigen Enzyme Immunoassay for Diagnosis of Histoplasmosis Marked by Antigenuria[∇]

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The sensitivity of detection of antigenuria in patients positive by the MiraVista Diagnostics *Histoplasma* enzyme immunoassay (MVD EIA) was 44% with the IMMY ALPHA *Histoplasma* antigen EIA. The specificity was 84% with the IMMY EIA versus 98% with the MVD EIA. The correlation between assays for positive cases was weak ($r^2 = 0.430$).

Histoplasma antigen detection is an important laboratory parameter that is used to rapidly and noninvasively diagnose and manage patients with serious manifestations of histoplasmosis. First developed in 1986 with a radioimmunoassay (5), conversion to an enzyme immunoassay (EIA) (2) has seen two important generations of refinement that have significantly improved the assay's sensitivity and specificity (3). In 2006, a *Histoplasma* antigen detection EIA (IMMY ALPHA *Histoplasma* Antigen EIA [HAG102]; Immuno-Mycologics, Inc., Norman, OK) became available commercially. It is a sandwich EIA that uses rabbit anti-*H. capsulatum* antibodies for the capture and detector steps. Results with the IMMY EIA were reported to agree well with those at MiraVista Diagnostics (1). In that study, antigenuria was falsely negative with the IMMY EIA for 29% of specimens testing positive and falsely positive for 8% of those testing negative at MiraVista Diagnostics.

The purpose of this study was to evaluate the IMMY EIA with urine specimens from patients with presumed histoplasmosis, based on having repeatedly positive samples for *Histoplasma* antigen when tested with the MiraVista Diagnostics second-generation *Histoplasma* antigen EIA (MVD EIA) (4). Control specimens from both healthy subjects and patients in whom histoplasmosis was excluded were also tested in each assay.

Fifty urine specimens were selected from patients for whom the MVD EIA for *Histoplasma* antigen was requested on three or more occasions and results were positive, spanning the range from weak to highly positive (1.6 to 64.7 units). Fifty control urine specimens consisted of specimens from 25 healthy subjects and 25 patients for whom histoplasmosis was excluded clinically and by serology and/or culture. All specimens were stored frozen at -20°C for 2 years or less.

The IMMY ALPHA *Histoplasma* Antigen EIA (lot 001HA2) was used according to the package instructions. There are four incubations: (i) controls, standards, and patient specimens in wells of a microtiter plate coated with rabbit anti-*Histoplasma* antibodies for 60 min at 37°C ; (ii) biotinylated anti-*Histo-*

plasma detection antibody for 60 min at 37°C ; (iii) streptavidin-horseradish peroxidase for 30 min at 25°C ; and (iv) substrate for 10 min at 22 to 25°C . After incubations i, ii, and iii, the plate is washed, and after incubation with substrate, stop solution is added. The optical density is measured at a wavelength of 450 nm, with a reference wavelength of 630 nm. Results are expressed as antigen units by extrapolation using standard curve reagents with the following *Histoplasma* antigen values: 1,000, 300, 100, 30, and 2 units. Results of 2 units or higher are considered positive.

The second-generation MVD EIA for *Histoplasma* antigen has been modified to reduce reactivity with anti-rabbit antibodies (4). The biotinylated detector antibody is the F(ab')₂ fragment of rabbit anti-*Histoplasma* immunoglobulin G, which is diluted in buffer containing normal rabbit serum. Results are expressed as antigen units, determined by dividing the optical density of the test specimen by three times that of the control containing no antigen. Results of 1 unit or higher are considered positive. Specimens were thawed and their identities were masked by a technician not involved in the testing or data analysis. Reproducibility and comparison of results with the MVD EIA and the IMMY EIA were determined by linear regression.

Results with the IMMY EIA were positive for 22 of the 50 MVD EIA-positive cases (44%) (Fig. 1). False-negative IMMY EIA results occurred throughout the range of samples having positive results in the MVD EIA, including those that were strongly positive: 7 of 10 urine samples with MVD EIA results between 10.0 and 19.9 were falsely negative, and 4 of 20 with MVD EIA results that were ≥ 20 units were falsely negative. Results with the MVD EIA were positive in all 50 cases. Results for the 22 cases that were positive in both assays were compared by linear regression analysis; the correlation was weak ($r^2 = 0.4305$).

The IMMY EIA was positive for 8 of 50 controls (16%) without histoplasmosis. The false-positive results ranged from 2.0 to 2.9 units. When the cutoff was set at 3.0 units, the specificity increased to 100% but the sensitivity decreased to 34% (17 of 50). Based on a prevalence of 10%, the positive predictive value was 23.4% and the negative predictive value was 93.1%. Results with the MVD EIA were positive for 1 of the 50 controls (2%). Predictive values for the MVD EIA were

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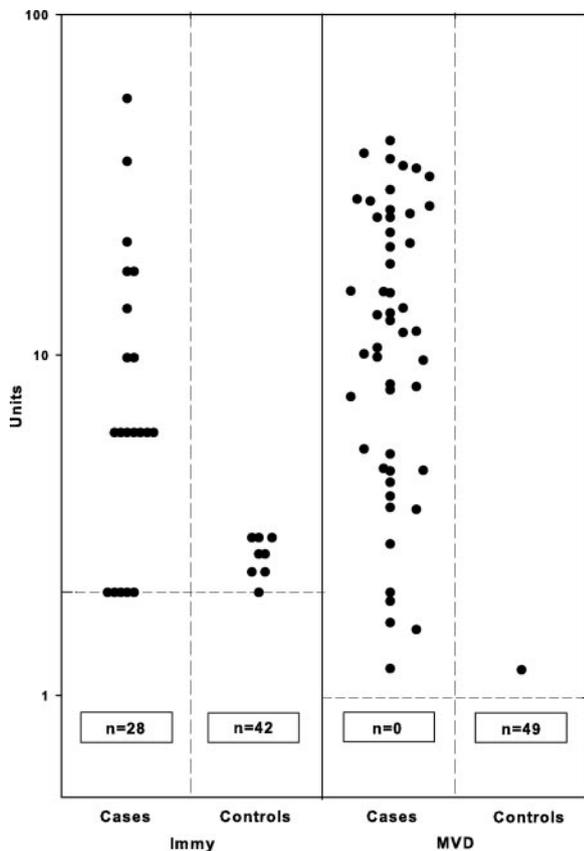


FIG. 1. Comparison of results with the MVD EIA and IMMY EIA from histoplasmosis patients and controls. The dashed horizontal lines represent the cutoff for positivity, at 2 antigen units for the IMMY EIA and 1 antigen unit for the MVD EIA. The numbers in the boxes below the dashed lines are the numbers of patients or controls with negative results.

not determined, because the cases were selected based on positive results in that assay.

Upon repeat testing with the IMMY EIA, 26 of the 50 histoplasmosis cases (52%) and 22 of the 50 controls (44%) were positive. The four additional histoplasmosis cases that were positive with the repeat IMMY EIA were weakly positive, ranging from 2.0 to 4.1 units. Results for these four specimens

in the MVD EIA were 1.2, 3.4, 14.0, and 20.1. The discrepant results for the 14 controls that were falsely positive upon repeat testing ranged from 2.0 to 13.3 units (median, 2.9 units).

The reasons for the lower sensitivity with the IMMY EIA in this study (44%) compared to that found by Cloud et al. (1) (71%) are unknown. Different case specimens were tested, however. The lower sensitivity was not explained by low-level antigenuria, as falsely negative results occurred for 11 of 30 specimens (37%) with MVD EIA levels above 10 units.

The specificity for healthy subjects and individuals without histoplasmosis was 84% with the IMMY EIA, compared to 98% with the MVD EIA. In the study of Cloud et al., the specificity was 92% (1). Cloud et al. tested specimens that were negative for *Histoplasma* antigen at MiraVista Diagnostics, while we tested specimens from healthy subjects and patients in which histoplasmosis was excluded based on clinical evaluation and negative laboratory tests for histoplasmosis.

These findings show that the two assays differ in the ability to detect *Histoplasma* antigen in urine. Strongly positive specimens from histoplasmosis patients were frequently negative with the IMMY EIA, reducing the reliability of this test for diagnosis of serious histoplasmosis. Accordingly, the guidelines published for interpreting findings with the MVD EIA (3) should not be applied to tests using the IMMY EIA.

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