Abstract

Introduction: Histoplasma antigen detection in urine is useful for diagnosing and monitoring treatment of systemic histoplasmosis. Immuno-Mycologics, Inc (Immy®, Norman, OK) has produced analyte specific reagents (ASRs) for a monoclonal based enzyme immunoassay for the qualitative/quantitative detection of this antigen. A literature search noted several studies where it was shown that the Immuno-Mycologics assay demonstrated high specificity, but low sensitivity when compared to MiraVista Diagnostics’ proprietary Immunoassay, which some consider the industry’s gold standard with regards to antigen detection. The aim of our study was to examine if ultrafiltration using Amicon Ultra 2 mL Centrifugal Filters would improve the detection and recovery of Histoplasma antigen.

Material and Methods: A total of 57 urine samples previously tested by MiraVista Diagnostics were used for this study. 16 of the samples had antigen levels detected by MiraVista at levels >0.4 ng/mL. The remaining 41 samples were below the established cutoff value and considered negative. The positive urine samples were tested neat with reagents from Immy to establish a baseline on a Dynex DSX-4 plate ELISA processing system. Following the analysis, these samples were concentrated according to the manufacturer’s instructions for Amicon Ultra 2 Centrifugal Filter Unit with Ultracel-3 membrane (EMD Millipore UFC200324). The concentrated samples were then tested, and samples that had the antigen concentrated to 0.4 ng/mL or greater were considered positive. The results were than compared to those reported by MiraVista.

Results: Correlation between Immy’s ASRs and MiraVista for the detection of Histoplasma antigen increased from 56.3% (9/16) positive agreement to 93.8% (15/16) positive agreement following ultrafiltration. Negative agreement remained nearly the same, but did fall slightly from 100% (41/41) to 97.6% (40/41). Overall agreement with MiraVista increased from 86.0% to 96.5% neat versus ultrafiltration, respectively. The urine sample was concentrated from a starting volume of 2.0 mL down to 150 µL, representing, mathematically, a 13-fold increase in concentration, but for unknown reasons, the amount that it was concentrated varied from sample to sample. The amount of antigen recovered after ultrafiltration ranged from 4-times to 29-times the original concentration.

Conclusion: The monoclonal enzyme immunoassay from Immy provides a unique opportunity for laboratories to test urine for Histoplasma antigen. The ability of laboratories to accurately test for Histoplasma antigen can lead to a faster turnaround time and provide the physicians with useful information to make an initial diagnosis. However, the low sensitivity of the assay could mean that patients with low levels of Histoplasma capsulatum antigen may go undetected. Our findings suggest that the concentration of the urine sample by ultrafiltration would lead to a better detection rate of the antigen using Immy’s ASRs. This method offers an accurate and sensitive method for qualitatively detecting Histoplasma antigen.

Methods and Materials

A total of 57 urine samples previously tested by MiraVista Diagnostics were used for this study. 16 of the samples had antigen levels detected by MiraVista at levels >0.4 ng/mL. The remaining 41 samples were below the established cutoff value and considered negative. The following reagents from Immy were used in this study: Histoplasma capsulatum Galactomannan Antigen (HGM000), Anti-Histoplasma Galactomannan mAb-HRP Conjugate (HGMIDAB), Anti-Histoplasma Galactomannan mAb Coated Microwells (HGMMAW1), and ELA Reagents, which contained Wash solution, TMB, and Stop solution (EIABU3).

The urine samples were first tested neat using the ASRs from Immy on a Dynex DSX-4 plate ELISA processing system. This allowed a baseline to be established before ultrafiltrating the samples and to determine the effectiveness of the ultrafiltration. The urine specimens were ultrafiltered by the following method. The urine samples were spun at 6,000 g to remove any sediment. Then 2 mL was removed and added to an Amicon Ultra-2 Centrifugal Filter unit with Ultracel-3 membrane (EMD Millipore UFC200324). It was spun for 40 minutes at 4,000 g. Next, the remaining samples were desalted by adding 1.5 mL of Millipore water and then spinning them for an additional 60 minutes at 4,000 g. The final volume of the ultrafiltered samples was 75 µL. They were diluted to 150 µL using 1x wash solution to allow for 100 µL of sample to be used and for adequate dead volume for the instrument.

The assay was performed by adding 100 µL of sample, calibrators and controls to the coated plate. It was incubated at 37°C for 1 hour. The plate was washed 3-times, and then 100 µL of conjugate was added. The plate was incubated at 25°C for 45 minutes. It was then washed 3-times and then 100 µL of TMB was added to each well. The plate was incubated for 35 minutes at 25°C. Then 100 µL of stop solution was added to each well. Each well was read at a wavelength of 450 nm with a reference wavelength of 620 nm. Samples that were concentrated to 0.4 ng/mL or greater were considered positive.

Results

Correlation between Immy’s ASRs and MiraVista for the detection of Histoplasma antigen increased from 56.3% (9/16) positive agreement (Table 1) to 93.8% (15/16) positive agreement (Table 2) following ultrafiltration. Negative agreement remained nearly the same but did fall slightly from 100% (41/41) to 97.6% (40/41) (Table 2). Overall agreement with MiraVista increased from 86.0% to 96.5% neat versus ultrafiltration, respectively.

Figure 1 and Figure 2 show a graphical representations of the data above. The red circles correspond to result disagreement between MiraVista and our assay, while the green circles show agreement between results. The larger the area of the circle, the more samples that agreed or disagreed. As can be seen when comparing figure 1 to figure 2, the overall disagreement (the red circles) decreased considerably after ultrafiltration. The results were analyzed using EP Evaluator®, release 9.

The urine samples were concentrated from a starting volume of 2.0 mL down to 150 µL, which represents mathematically a 13-fold increase in concentration, but for unknown reasons, the amount that it was concentrated varied from sample to sample. The amount of antigen recovered after ultrafiltration ranged from 4-times to 29-times the original concentration.

Conclusion

The monoclonal enzyme immunoassay from Immy provides a unique opportunity for laboratories to test urine for Histoplasma antigen. The ability of laboratories to accurately test for Histoplasma antigen can lead to a faster turnaround time and provide the physicians with useful information to make an initial diagnosis. However, the low sensitivity of the assay could mean that patients with low levels of Histoplasma capsulatum antigen may go undetected. Our findings suggest that the concentration of the urine sample by ultrafiltration would lead to a better detection rate of the antigen using Immy’s ASRs. This method offers an accurate and sensitive method for qualitatively detecting Histoplasma antigen.

References