Comparison of Radioimmunoassay and Enzyme-Linked Immunoassay Methods for Detection of \textit{Histoplasma capsulatum} var. \textit{capsulatum} Antigen

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Histoplasmosis is diagnosed in a variety of ways. Culture is the most definitive method of diagnosis, but it may be falsely negative for up to 15% of patients (1, 8), often requires invasive procedures, and may take up to 4 weeks until results become available. Measuring patient antibody response to \textit{Histoplasma capsulatum} can be confusing since background positivity rates in areas where the disease is endemic can be as high as 5% (6). False-positive results occur for patients with other fungal infections, and false-negative results may occur during the first month of illness. Also, immunosuppressed patients may not develop antibody in response to \textit{H. capsulatum}, resulting in positive serologies only about 60% of the time.

A radioimmunoassay (RIA) for the detection of an \textit{H. capsulatum} antigen is an established method for the diagnosis of histoplasmosis and monitoring the response to treatment (7). Antigen detection was the most common method of initial diagnosis for 80% of patients with disseminated histoplasmosis in Indianapolis in an outbreak between 1988 and 1993 (5), providing results within 1 working day. Antigen can be detected in the urine of 92% and the serum of 82% of patients with disseminated disease (8). For patients with disseminated cases of disease with meningeal or pulmonary involvement, antigen can also be detected in the cerebrospinal fluid of 40% and bronchoalveolar lavage fluid of 70% of patients, respectively (7). Antigen clears with treatment and increases with relapse (2), so antigen levels provide assistance in monitoring the effectiveness of drug therapy (3).

An enzyme-linked immunosorbent assay (ELISA) has several advantages over an RIA. An ELISA does not expose laboratory workers to radioactivity and does not require licensing by the Nuclear Regulatory Commission. Anti-Histoplasma antibody is more conserved with an ELISA system because of the long-term stability of the conjugate (years) compared to that of the radioactive \textsuperscript{125}I label, which has a half-life of 60 days. Also, an ELISA is easier than an RIA to adapt into a kit form, and adaptation allowing interpretation of the results by visual inspection would make ELISA practical for widespread use of this test.

An ELISA method previously described by our laboratory was inferior to the RIA (9). Ten percent of the RIA-positive patients were false negative by ELISA, and 3 of 41 controls were false positive by ELISA, whereas 1 of 41 controls were false positive by RIA. In the current study, a new ELISA system that uses a biotin-conjugated antibody detected with streptavidinhorseradish peroxidase was compared to the established RIA by performing both tests concurrently with stored specimens.

\textbf{MATERIALS AND METHODS}

\textbf{Patients with histoplasmosis.} Urine was collected from histoplasmosis patients from 1988 to 1992 during the Indianapolis outbreak and were stored at 4°C. Previous RIA results for these patients were reviewed, and specimens were chosen to provide a range of results from negative to high positive. Urine specimens from 45 non-AIDS patients (16 with disseminated histoplasmosis, 22 with pulmonary histoplasmosis, 5 with cavitary histoplasmosis, and 2 with periodontal disease) and 41 AIDS patients (40 with disseminated histoplasmosis and 1 with pulmonary histoplasmosis) were used to compare both methods of antigen detection.

\textbf{Control patients.} Control specimens were urine specimens from the following individuals: 20 healthy laboratory personnel, 28 patients with fungal infections other than \textit{H. capsulatum} (12 with Candida, 3 with Aspergillus, 2 with Blastomyces dermatitidis, 1 with Panococcioides brasiliensis, 1 with Coccioides immitis, 3 with Cryptococcus neoformans, 2 with Pneumocystis carinii, and 4 with miscellaneous fungal infections), 24 patients with urinary tract infections (12 with Escherichia coli, 5 with Klebsiella pneumoniae, 1 with Proteus mirabilis, 4 with Staphylococcus spp, 1 staphylococcus group D, and 1 with Citrobacter freundii infections), and 24 patients with nonfungal pneumonia (20 with Strepococcus pneumoniae, 2 with Haemophilus influenzae, 1 with Mycobacterium tuberculosis, and 1 with Nocardia sp. infections).

\textbf{ELISA.} The ELISA method has been modified from the procedure which was described previously (9). Immulon 2 microtiter wells were coated with 100 μl of...
stopped by the addition of 100 μl of 0.1 M citrate buffer (pH 4.0), and 100 μl of substrate (tetramethylbenzidine; Cappel) was dissolved in a citrate buffer (0.1 M HCl (pH 8.0) (0.1 M Tris) and washed as described above. Peroxidase streptavidin-horseradish peroxidase in 0.1 M Tris–5% bovine serum albumin to antigen adhering to the solid-phase antibody was measured by adding 100 μl of streptavidin-horseradish peroxidase in 0.1 M Tris–5% bovine serum albumin to each well. The plate was incubated and washed as described above. Peroxidase substrate (tetramethylbenzidine; Cappel) was dissolved in a citrate buffer (0.1 M HCl (pH 8.0) (0.1 M Tris) and washed as described above. Finally, Histoplasma antigen adhering to the solid-phase antibody was measured by adding 100 μl of streptavidin-horseradish peroxidase in 0.1 M Tris–5% bovine serum albumin to each well. The plate was incubated and washed as described above. Peroxidase substrate (tetramethylbenzidine; Cappel) was dissolved in a citrate buffer (0.1 M HCl (pH 8.0) (0.1 M Tris) and washed as described above. Finally, Histoplasma antigen adhering to the solid-phase antibody was measured by adding 100 μl of streptavidin-horseradish peroxidase in 0.1 M Tris–5% bovine serum albumin to each well.

RESULTS

Sensitivity and specificity. We tested 86 urine specimens from patients with histoplasmosis and 96 control specimens. Histoplasma antigen was detected by both EIA and RIA in 61 of the 86 specimens (71%) from histoplasmosis patients and 1 of the 96 (1%) control specimens (Fig. 1). Both systems detected antigen in a specimen from a control patient with paracoccidioidomycosis, indicating the presence of a cross-reacting antigen. Both the RIA and the EIA detected Histoplasma antigen in 50 of 56 (89%) patients with disseminated histoplasmosis. Among patients with AIDS and disseminated disease, antigen was detected in 38 of 40 (95%) patients, while among patients with disseminated histoplasmosis without AIDS, antigen was detected in 12 of 16 (75%) patients. Among patients with nondisseminated cases of infection, antigen was detectable in 11 of 30 (37%) patients (Table 1). Among those patients with nondisseminated histoplasmosis, antigen was detected in 10 of 23 (43%) patients with acute pulmonary disease, 1 of 5 (20%) patients with cavitary histoplasmosis, and 0 of 2 patients with pericardial disease.

Correlation of EIA and RIA. The results of the RIA and EIA were compared by linear regression analysis. There was a good correlation between the two methods, with a correlation coefficient of 0.974 and a P value of <0.0001. The slope was 0.915, and the intercept was 0.013 (Fig. 2). The RIA system had a greater range of results, with the highest result being 27.0 units. In contrast, for the same urine sample the result was 20.1 units by the EIA (Fig. 1).

Reproducibility. The reproducibility was established by testing specimens from 34 patients with histoplasmosis and 10 control specimens in two consecutive EIAs within a period of 1 week. There was an excellent correlation by linear regression analysis, with a correlation coefficient of 0.995 and a P value of <0.0001. The slope was 0.990, and the intercept was 0.0001. The slope was 0.951, and the intercept was 0.013 (Fig. 2). The RIA system had a greater range of results, with the highest result being 27.0 units. In contrast, for the same urine sample the result was 20.1 units by the EIA (Fig. 1).

DISCUSSION

The EIA is comparable in sensitivity and specificity to the RIA for the detection of Histoplasma antigen. Both systems

![FIG. 1. Sensitivity and specificity of EIA versus RIA for detection of Histoplasma antigen. (A) EIA data; (B) RIA data. Results are expressed in units which are described in the Materials and Methods section. The broken line at 1 unit separates the positive results from the negative results. The numbers in the boxes below the broken line represent the number of patients with a test result below 1.0 unit.](http://jcm.asm.org/)

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. of subjects positive/total (%)</th>
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<tr>
<td></td>
<td>RIA</td>
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<tr>
<td>Patients with histoplasmosis</td>
<td></td>
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<tr>
<td>Disseminated, AIDS</td>
<td>38/40 (95)</td>
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<tr>
<td>Disseminated, non-AIDS</td>
<td>12/16 (75)</td>
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<tr>
<td>Nondisseminated</td>
<td>11/30 (37)</td>
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<td>Controls</td>
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detected antigen in 89% of patients with disseminated cases of infection and 37% of patients with nondisseminated cases of infection. Antigen was detected in 95% of the patients with disseminated cases of infection and AIDS, whereas it was detected in 75% of those with disseminated cases of infection but without AIDS. All specimens which were positive in the RIA were positive in the EIA. Likewise, all specimens which tested negative in the RIA tested negative in the EIA. The correlation coefficient of 0.974 indicates a strong correlation between the results obtained with the two systems. The RIA had a wider range of reactivity, with RIA units of between 0.4 and 27.0. The EIA had a range of units of from 0.6 to 20.1. These differences may be attributed to the kinetics of the detection systems used in the RIA and EIA methods, resulting in the RIA having a slightly higher ceiling for detection under the conditions chosen for these experiments.

The EIA is as specific as the RIA for the detection of Histoplasma antigen. There was one false-positive result by both assays. The urine specimen with a false-positive result was from a patient with paracoccidioidomycosis. Blastomyces dermatitidis, Paracoccidioides brasiliensis, and Penicillium marneffei share cross-reacting antigens which react in the antigen assay (7).

The reproducibility of the EIA was excellent. Forty-four specimens were tested in two consecutive EIAs within a period of 1 week. The reproducibility of the results was determined by linear regression analysis, which showed an excellent correlation between assays, with a correlation coefficient of 0.995 and a P value of <0.0001. All specimens had the same result in both assays, either negative or weak, moderate, or high positive.

This EIA with a biotin-conjugated antibody appears to be as sensitive and specific as the RIA. Although the new EIA with a biotin-conjugated antibody was not directly compared to the alkaline phosphatase and peroxidase EIA methods published earlier (9), the new EIA appears better in a comparison of each method to the RIA. In the previous report on EIAs (9), the RIA was compared to two different EIA systems, one that used a horseradish peroxidase-conjugated antibody and another one that used an alkaline phosphatase-conjugated antibody. In that evaluation, 10% of the RIA-positive specimens were false negative by EIA. Also, problems with high background required that the specimens be heated to 56°C. The EIA with alkaline phosphatase-conjugated antibody was slightly less specific than the RIA, with false-positive results for 3 of 41 control specimens, compared to false-positive results for 1 of 41 specimens by RIA. Both systems, when compared previously, detected antigen in a patient with disseminated blastomycosis. The false-positive results detected by the EIA with alkaline phosphatase-conjugated antibody alone were for a patient with candidemia and a patient without a fungal infection. The EIA with horseradish peroxidase-conjugated antibody was less sensitive than the RIA for patients with nondisseminated disease, detecting nondisseminated histoplasmosis in 16% of patients, whereas RIA detected nondisseminated histoplasmosis in 48% of patients. In conclusion, we have developed a EIA system that is comparable to the established RIA for Histoplasma antigen detection. This EIA is a reasonable alternative to the established RIA and offers a potential for wider availability of Histoplasma antigen testing and development as a kit.

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REFERENCES


