NOTES

Variability in Commercial Histoplasma Complement Fixation Antigens

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Using Immuno-Mycologics (IMMY; Norman, Okla.) histoplasmal yeast (HY) and mycelial (HM) antibody complement fixation test antigens, we retested 1,386 samples that were initially tested with Meridian Diagnostics, Inc. (Cincinnati, Ohio), antigens. Histoplasmal antibody was identified (≥1:16) in 20% of HY and 5% of HM samples reported to have titers of <1:8 with Meridian reagents. IMMY titers were at least fourfold higher than Meridian titers in 39% of HY and 54% of HM samples that initially had titers of ≥1:8 with Meridian antigens. Because 30 of 58 (52%) samples from confirmed cases of histoplasmosis yielded negative results with Meridian antigens and positive results upon retesting with IMMY antigens, we concluded that the Meridian antigens had less reactivity with human histoplasmal antibody.

Serologic testing is the most convenient approach for diagnosing histoplasmosis. The complement fixation (CF) test for histoplasmal (histo) antibodies (Abs), which was performed in a standard microtitration system (2) with yeast and mycelial antigens, provides excellent specificity and is more sensitive than the immunodiffusion test (3). CF test titers of ≥1:32 or rising titers with yeast antigens, mycelial antigens, or both are strong presumptive evidence of histoplasmosis; titers of 1:8 or 1:16 are generally considered suggestive of histoplasmosis but are less readily interpreted (1).

Because we obtained negative (<1:8) histo Ab CF test results in five serum specimens from two patients with confirmed histoplasmosis, we evaluated the reactivity of our CF test antigens. In a small pilot study, we tested the Meridian antigens (Meridian Diagnostics, Inc., Cincinnati, Ohio) that we were using in our CF test, antigens supplied by the Centers for Disease Control (CDC), and antigens purchased from Immuno-Mycologics (IMMY; Norman, Okla.) in parallel against 13 human serum specimens with known histo Ab profiles. The Meridian antigens yielded negative results for all 11 serum specimens that contained yeast Abs and for 7 of 9 serum specimens that contained mycelial Abs; all of these samples had comparable titers of ≥1:8 with CDC and IMMY yeast antigens, mycelial antigens, or both. The Meridian antigens were subsequently recalled by the manufacturer. Because the IMMY antigens consistently produced titers that were within 1 doubling dilution of those produced by the CDC reference antigens, we selected the IMMY antigens for use in our test system.

To evaluate how the differences in the reactivities of histo antigen preparations affected histo CF test titers of clinical samples tested previously in our laboratory, we used the IMMY antigens to retest 1,386 clinical samples (1,373 serum specimens, 11 cerebrospinal fluid specimens, 1 pleural fluid specimen, and 1 paracentosis fluid specimen) originally tested by using Meridian antigens and 161 clinical samples (all sera) originally tested by using antigens from American MicroScan (AMS; West Sacramento, Calif.). Although parallel testing with the three antigens would have provided the most meaningful data, this could not be done because the Meridian and AMS antigens were not available.

In repeat CF tests with IMMY antigens, titers of ≥1:16 were obtained in 243 of 1,231 (20%) samples that initially had titers of <1:8 with Meridian yeast antigens and in 14 of 135 (10%) samples that initially had titers of <1:8 with AMS yeast antigens (Table 1). There was a significant difference (P < 0.001) between IMMY and Meridian yeast antigen results and between IMMY and AMS yeast antigen results.

With IMMY mycelial antigen, CF test titers of ≥1:16 were obtained in 63 of 1,272 (5%) samples that initially had titers of <1:8 with Meridian mycelial antigens and in 2 of 159 (1%) samples that initially had titers of <1:8 with AMS mycelial antigens (Table 1). There was no significant difference between IMMY and AMS mycelial antigen titers (not significant at 0.05), although there was a significant difference (P < 0.001) between IMMY and Meridian mycelial antigen results.

By assigning a number to each titer to represent the number of doubling dilutions (1 = 1:8, 2 = 1:16, 3 = 1:32, 4 = 1:64, 5 = 1:128, 6 = 1:256, 7 = 1:512), we were able to calculate the magnitude of the differences in titers obtained with various antigens (Table 2). The IMMY titers were higher by an average of 3.3 doubling dilutions in 32 of 83 (39%) samples initially tested by using Meridian yeast antigen and by an average of 3.0 doubling dilutions in 20 of 37 (54%) samples originally tested by using Meridian mycelial antigen. The IMMY result was higher by an average of 2 doubling dilutions in 4 of 24 (17%) samples that initially had titers of ≥1:8 with AMS yeast antigen. No samples initially had titers of ≥1:8 with AMS mycelial antigen. The IMMY titers were lower by 1 doubling dilution in 8% of samples initially tested with Meridian yeast or mycelial antigens and in 17% of samples initially tested with AMS yeast antigen. The IMMY titers were generally lower than the initial titer by more than 1 doubling dilution.

A total of 58 specimens (55 serum specimens and 3 cerebrospinal fluid specimens) with IMMY results that were significantly different from Meridian results (Tables 1 and 2)

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were collected from 41 patients whose histoplasmosis was confirmed in our laboratory by culture or by positive hist urine antigen radioimmunoassay (4) test results. Thirty (52%) of the samples had negative CF test results with Meridian yeast and mycelial antigens but had titers of 1:8 with IMMY yeast antigen, mycelial antigen, or both; 28 (48%) of the samples had titers of 1:8 with Meridian yeast, mycelial antigen, or both, but had IMMY titers that were fourfold or more higher than the initial Meridian result. An additional 45 serum specimens from 35 patients, whose histoplasmosis disease status was unknown, produced bands in histo immunodiffusion tests; 21 of these specimens had negative CF test titers with the Meridian antigen but had titers of 1:8 with IMMY antigens.

We were unable to evaluate the significance of the differences in titer obtained with IMMY and AMS antigens because none of the serum specimens which had results that were significantly different between the two antigens were from patients whose histoplasmosis was confirmed in our laboratory by culture or by a positive urine antigen radioimmunoassay result. Six serum specimens that had bands in immunodiffusion tests had CF test titers of 1:8 with both AMS and IMMY antigens.

Although some differences in serologic test results can be expected in the retesting of stored serum specimens, the large differences between CF test results with IMMY antigens and Meridian antigens cannot be attributed solely to storage changes. When we used IMMY antigens to retest sera that were collected 24 to 28 months earlier, tested with antigens from AMS, and stored under conditions identical to those used for the Meridian samples, we found less of a difference between the IMMY and AMS results than we found between the IMMY and Meridian results.

The clinical significance of the lower reactivities of the Meridian antigens can only be estimated. We found that 52% of serum specimens from patients with confirmed histoplasmosis were identified as antibody negative with Meridian antigens but had titers of 1:8 with IMMY antigens. Because serologic testing is convenient, sensitive, and specific in the diagnosis of histoplasmosis, such falsely negative results may needlessly complicate the diagnostic process. Lower titers, such as those produced in many cases with the Meridian antigens, may be sufficient to suggest histoplasmosis, but they may not provide the evidence needed to confirm the diagnosis. Physicians working in hospitals or areas where histo isolation or histo urine antigen tests are not available may not have sufficient evidence to confirm the diagnosis of histoplasmosis if only low titers are reported.

The lower reactivities of the Meridian antigens were not detected by clinical laboratory personnel for a prolonged period because the Meridian-histo CF test-positive control serum (rabbit) consistently produced expected values in the CF test. Such hyperimmune sera, which is produced by immunization of animals with the prepared antigen, may react with their homologous antigens to consistently produce acceptable and expected CF test titers. Until the antigens are tested against human sera known to contain histo Abs, the capacity of the antigen to detect human histo Ab is neither measured nor monitored.

In order to avoid problems such as those described in this report, it is essential for clinical laboratories to follow a comprehensive quality assurance program. We recommend the testing of each new lot of histo antigen against a panel of human sera known to contain histo yeast and mycelial Abs. Also, old and new antigen products should be tested in parallel when a change in manufacturer or in the manufacturing process of a reagent is made.

Other serologic test antigens that are marketed with positive control sera from animals are potential candidates for similar difficulties. It is essential that known human control sera be obtained and tested periodically. Manufacturers should be encouraged to test their antigens against known positive and negative human sera and to market human control sera with their antigens, if it is possible to do so.

We thank Shirley Collins and Sandra English for assisting with histo Ab CF testing, Judy Stacey for preparing the manuscript, and CDC for providing reference histo antigens and antisera.

### REFERENCES


### TABLE 1. Histo Ab CF test titers with IMMY antigens in samples that yielded titers of <1:8 with Meridian or AMS antigens

<table>
<thead>
<tr>
<th>Antigen used to determine initial CF test result</th>
<th>No. of samples</th>
<th>No. of samples with the following IMMY antigen CF test result:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meridian yeast</td>
<td>1,231</td>
<td>1:8 1:16 1:32 1:64 1:128 1:256 ≥1:512</td>
</tr>
<tr>
<td>AMS yeast</td>
<td>135</td>
<td>88 33 11 2 1 0 0 0</td>
</tr>
<tr>
<td>Meridian mycelial</td>
<td>1,272</td>
<td>159 149 8 1 1 0 0 0</td>
</tr>
</tbody>
</table>

* CF test result with a titer of <1:8.

*b Results for 77 samples initially tested with Meridian antigens and for 2 samples initially tested with AMS antigens were excluded because of anti-complementary activity.

* For titers of 1:16 to ≥1:512, values are numbers of specimens that gave results that were significantly (fourfold or more) different from the original <1:8 result.

### TABLE 2. Histo Ab CF test titers with IMMY antigens in samples that yielded titers of 1:8 with Meridian or AMS antigens

<table>
<thead>
<tr>
<th>Antigen used to determine initial CF test result</th>
<th>No. of samples</th>
<th>No. of samples with IMMY antigen CF test result by the following no. of doubling dilutions:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meridian yeast</td>
<td>83</td>
<td>51 15 10 2 0 2 3</td>
</tr>
<tr>
<td>AMS yeast</td>
<td>24</td>
<td>20 4 0 0 0 0 0</td>
</tr>
<tr>
<td>Meridian mycelial</td>
<td>37</td>
<td>17 8 7 2 3 0 0</td>
</tr>
<tr>
<td>AMS mycelial</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* For doubling dilutions of 2 to 7, values are numbers of specimens that gave results that were significantly (fourfold or more) different from the original result.

* None of the samples had a titer of ≥1:8 in the initial CF test.