Variation in Complement Fixation Test Results with Three 
*Histoplasma capsulatum* Yeast Phase Antigens

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Antigens derived from three different yeast strains of *Histoplasma capsulatum* (GW and VC used by the Veterans Administration Serology Reference Laboratory and the Centers for Disease Control reference antigen, A811) were compared with each other to determine their equivalency. Results from 200 sera, tested concurrently by the Serology Reference Laboratory and the University of Kentucky Mycoserology Laboratory, indicated that the VC yeast antigen gave better correlation to the A811 antigen than did the GW antigen.

The complement fixation (CF) test has historically been the test of choice for the serodiagnosis of histoplasmosis. In the early years of histoplasmosis serology, laboratories independently developed their own strains of antigens for use in CF, hemagglutination, precipitin, and gel diffusion testing (1). Various mycelial and yeast phase antigen preparations were used, and it was subsequently noted that the CF titers obtained by a given laboratory were influenced by the strain of *Histoplasma capsulatum* used for antigen preparation (2, 4–6). These early studies indicated the necessity of using both a mycelial and yeast form antigen to increase the sensitivity of CF testing, as well as the need for standardization of reagents and test procedure (1).

The A811 yeast form antigen used by the Centers for Disease Control for over 20 years is generally accepted as the *H. capsulatum* reference antigen for CF testing (3). Although the Veterans Administration Serology Reference Laboratory (SRL) has used the GW and VC yeast form antigens of *H. capsulatum* for over 30 years, there has never been a published report on the comparability of these antigens to the Centers for Disease Control reference strain, A811. Therefore, we decided to determine the equivalency of these antigens by comparing results obtained using the standardized Laboratory Branch Complement Fixation (LBCF) micromethod (8). Interlaboratory reproducibility was determined by performing the LBCF test in two different laboratories.

Two hundred patient sera from the serum banks of the SRL and the University of Kentucky Mycoserology Laboratory (UKL) were used for the study. The sera were aliquotted, coded, and concurrently tested in each laboratory. CF tests for antibodies to the GW, VC, and A811 yeast form antigens of *H. capsulatum* were performed in each laboratory by the LBCF micromethod. Each laboratory used the same lot numbers of reagents, including antigens (GW, lot 681; VC, lot 381; A811, lot H90E3; American Microscan, Inc.), complement (lot 14133; Cordis Laboratories), and hemolysin (lot 17; Colorado Serum Co.). The only difference in methodology was that the SRL used an automated diluter (Titer Tek Medimixer; Flow Laboratories, Inc.), whereas the UKL made all serum dilutions manually.

A total of 200 sera, consisting of 84 high-titered sera (≥32), 47 low-titered sera (8 to 16), and 69 negative sera (<8) were coded by the SRL and sent to the CF testing sections of the SRL and UKL for evaluation. Sera were tested concurrently in each laboratory. After all sera were tested, the code was broken, and the results were compared.

Of 200 sera tested, 14 were anticomplementary, allowing the comparison of results from 186 sera. Specimens tested in the SRL demonstrated 66.7% complete agreement and 97.3% agreement at ±1 log₂ for the GW and VC antigens, 54.3% complete agreement and 90.3% agreement at ±1 log₂ for the GW and A811 antigens, and 60.9% complete agreement and 93.6% agreement at ±1 log₂ for the VC and A811 antigens. In like manner, results obtained in the UKL for the same specimens were as follows: 73.6% complete agreement and 93.6% agreement at ±1 log₂ for the GW and VC yeast form antigens; 58.6% complete agreement and 86.1% agreement at ±1 log₂ for the GW and A811 antigens; and 62.5% complete agreement and 89.4% agreement at ±1 log₂ for the VC and A811 antigens. Figures 1, 2, and 3 not only depict the above data in graphic form but also show the interlaboratory reproducibility achieved for the GW and VC, GW and A811,
and VC and A811 yeast form antigen comparisons, respectively.

Although the numerical results indicate the best correlation was between the GW and VC antigens (97.3% agreement at ±1 log₂ in the SRL), it can readily be seen that the VC and A811 antigens have a better overall correlation. The curves obtained in both the UKL and SRL for VC:A811 (Fig. 3) are almost superimposable and are symmetric with regard to the dilutional increments. On the other hand, the GW:VC (Fig. 1) and GW:A811 (Fig. 2) curves are skewed to the right, indicating that the GW antigen has a tendency to be one dilutional increment more sensitive than either the VC or A811 yeast form antigens. The yeast form antigens used in this study were prepared in the same manner; however, the antigen suspensions were not tested for their relative concentrations of protein and carbohydrates.

In an attempt to determine whether the variation seen among the different antigens was inherent in the test procedure or reagents used or both, the reproducibility of the CF tests with the same sera and two different lots of the A811 yeast form antigen (obtained from American Microscan and the Centers for Disease Control) was determined. Results were similar to those noted for the GW, VC, and A811 comparisons in that agreement at ±1 log₂ for the two lots of A811 was 96.9%.

Tradition has held that serologic tests should be reproducible within 1 log₂ (7). The antigen comparisons indicate close correlation with the above statement. However, as reflected in the skewing of Fig. 1 and 2, the observed variation may not always be around the mode, thus invalidating the above concept. Wood and Durham (9) have extended the acceptable serologic definition of reproducibility stated above by calculating the estimated reproducibility or probability that multiple tests on the same serum will remain within 1 log₂. Applying their formula to our data resulted in a lower estimated correlation than the correlation that was actually observed. For example, the estimated reproducibility for the VC:A811 comparison in the SRL was 85%, whereas the actual correlation at ±1 log₂ was 93%. This correlation held true for all of the sera comparisons in both the SRL and UKL.

Comparisons of the various yeast form antigens in the SRL indicated approximately 4% higher correlation at ±1 log₂ than the same sera tested in the UKL. This was most likely due to the fact that the SRL used automated diluters, whereas the UKL made all dilutions manually.

The overall results indicate that the three *H. capsulatum* yeast phase antigens studied (GW, VC, A811) give fairly comparable results and that these antigens could be used interchangeably, if desired.

**LITERATURE CITED**


