NOTES

Evaluation of a New Latex Agglutination Method for Detection of Antibody to Herpes Simplex Virus

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A new latex agglutination (LA) test (Wampole Laboratories, Cranbury, N.J.) for detection of antibody to herpes simplex virus was compared with a reference complement fixation (CF) method in a premarket evaluation. Of 102 serum samples tested, 19 were LA negative and CF negative, 79 were LA positive and CF positive, and 4 were LA positive and CF negative. An enzyme immunoassay (EIA) (M. A. Bioproducts, Walkersville, Md.) performed on the four LA-positive and CF-negative serum samples agreed with LA in all cases. Most LA titers were two to four doubling dilutions higher than CF titers. We conclude that this new LA test is a rapid, sensitive, and simple method for documentation of past infection with herpes simplex virus.

The serologic assays most commonly employed for diagnosis of human infection with herpes simplex virus (HSV) include complement fixation (CF), indirect immunofluorescence, and enzyme immunoassay (EIA) (5). Other methods such as radioimmunoassay, indirect hemagglutination, and neutralization have also been applied (3, 5). The value of serologic methods for detection of antibody to HSV is unquestioned in the setting of seroepidemiologic investigation (1). However, their usefulness for the clinical diagnosis and management of patients with herpetic infections is limited. In most cases of recurrent herpetic infection, serologic testing contributes little or nothing to the understanding of the disease or the patient’s management (2). However, in selected cases, such as fever of unknown origin or other atypical clinical presentations particularly in immunosuppressed patients, serology may prove useful (5). Other indications for serologic testing for HSV are suspected neonatal herpes and documentation of past infection in a patient who is a candidate for organ transplantation or is to receive immunosuppressive drugs (5, 7). Serologic testing for diagnosis of herpetic encephalitis may be useful as a confirmatory test but does not usually provide the answer in a timely fashion (4).

While simple, rapid methods have been in use for some time for detection of antibody to other commonly encountered viruses (e.g., latex agglutination [LA] for rubella and cytomegalovirus), most available methods for detection of antibody to HSV either are technically demanding (e.g., CF, neutralization) or require special instrumentation (e.g., indirect immunofluorescence, EIA, radioimmunoassay). This study evaluated a premarket formulation of a new LA kit for detection of antibody to HSV.

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A total of 102 serum samples (82 single and 10 paired samples) collected from 92 adult patients for detection of antibody to HSV were tested with a reference CF method and with a premarket formulation of an LA kit for detection of antibody to HSV (Wampole Laboratories, Cranbury, N.J.). All testing was performed in a double-blind fashion. Serum samples yielding qualitatively discrepant or borderline results (borderline-positive CF titers of 4; borderline-negative LA titers of 2) were tested with an EIA (M. A. Bioproducts, Walkersville, Md.).

For CF testing we used the Laboratory Branch Complement Fixation Method from the Bureau of Laboratories, Centers for Disease Control, Atlanta, Ga. (6). Antigen for the CF test was obtained from Hillcrest Biologicals, Cypress, Calif. Serum samples with titers of ≥4 were considered positive by CF.

The LA kit included latex reagent sensitized with HSV antigen, a buffered diluent, positive and negative controls, and reusable glass slides. According to the manufacturer, the LA kit detects immunoglobulins G and M to both HSV type 1 (HSV-1) and HSV-2, but does not provide type-specific antibody determination. Serum dilutions were prepared in microtiter plates, and 25-μl samples were placed on glass slides and mixed with 15 μl of latex reagent with the aid of disposable stirrers. The slides were placed under humidifying covers, rotated on a mechanical rotator for 10 min at 100 rpm, and then immediately observed for agglutination under incandescent light projected obliquely. Agglutination was defined as distinct large clumps against a clear or slightly cloudy background or small but definite clumps against a cloudy background. Positive and negative controls were tested with each run. All serum samples were titrated to endpoint. Specimens with titers of ≥4 were considered positive by LA.

Testing by EIA was performed and interpreted according to the manufacturer’s instructions. Serum samples with values of ≥1 were considered positive by EIA. Positive and negative controls were tested with each patient run.

Of 102 serum samples tested, 23 (22.5%) were negative for antibody to HSV by CF, and 79 (77.5%) had CF titers ranging from 4 to 256. Of 10 patients with paired sera, 1 showed a fourfold increase in CF titers (16 to 64).

The LA method yielded 19 (19%) negative and 83 (81%)...
positive results, with LA titers ranging from 4 to 4,096. The patient with a fourfold increase in CF titers (16 to 64) showed a corresponding eightfold increase in LA titers (64 to 512). Of the 19 specimens considered LA negative, 18 showed no agglutination at all and 1 showed weak agglutination at a 1:2 dilution only.

Nineteen (19%) serum samples were negative for antibody to HSV with both LA and CF, and 79 (77%) were positive with both methods. A prozone effect was observed with LA in two specimens (2.5% of positive sera) with LA titers of 256 and 1,024, which failed to agglutinate at a 1:4 dilution but showed strong agglutination first detected at 1:8. Four samples (4%) yielded qualitatively discrepant results; all four were positive by LA and negative by CF. Predictive values of positive and negative results for the LA compared with the reference CF were 95 and 100%, respectively. EIA performed on the four LA-positive, CF-negative samples, however, agreed with the LA results in all four cases. Of the four CF-negative, LA-positive, EIA-positive samples, three had low LA titers (4 or 8) and borderline-low positive EIA values (1.0 or 1.1), and one had an LA titer of 256 and a correspondingly high positive EIA value (7.8). The latter specimen was obtained from a patient who presented clinically with acute herpetic gingivostomatitis documented by isolation of HSV from the oral lesions at the time of serum collection. It is possible that the LA method, similarly to other techniques such as neutralization, may be able to detect a recent HSV infection sooner than CF, thereby explaining the discrepancy observed between LA and CF results in this case.

EIA testing was also performed on seven serum samples which were positive by LA and had borderline-positive CF titers of 4. Low levels of antibody to HSV were detected by EIA in six of the seven samples. There was a single borderline-negative specimen which showed weak agglutination by LA at a 1:2 dilution and was negative also by CF and EIA.

When LA titers were compared with CF titers for the 79 LA-positive, CF-positive specimens, no linear correlation was found. However, for 74 samples (94% of all specimens positive with both CF and LA) the LA titers were higher than the CF titers. Most specimens (85%) had LA titers two to four doubling dilutions higher than CF titers (Fig. 1).

As all serum samples which were positive by CF at titers of ≥4 or by EIA had LA titers of ≥4, we recommend diluting the serum 1:4 for LA screening. In view of the prozone effect observed in 2.5% of the positive serum samples, it is advisable to add a second serum dilution for routine screening to insure detection of all sera with high antibody titers. Given the distribution of the LA titers and their general correlation to CF titers, routine screening of 1:4 and 1:64 serum dilutions seems appropriate and would provide semiquantitative results which should suffice for most patients. If a more precise semiquantitative were desired, a third dilution (1:256) could be added. Titration to endpoint is also easily performed with the LA method by testing doubling serum dilutions prepared in microdilution plates. This more expensive test format should be reserved for selected patients in whom determination of precise antibody titers to HSV is deemed clinically useful.

We conclude that this new LA kit for HSV serology offers a sensitive, simple, and rapid alternative method for detection of immunoglobulin G to HSV, without the need for expensive equipment or special technical expertise.

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**LITERATURE CITED**


