Determination of Patient Herpes Simplex Virus Immune Status by Latex Agglutination

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A rapid latex agglutination (LA) test was compared with complement fixation and enzyme immunoassay for the determination of herpes simplex virus immune status in sera of patients. Of 173 samples, 88% gave concordant results in all three assays (115 positive and 37 negative). The LA and complement fixation tests agreed 92% of the time, and the LA agreed with the enzyme immunoassay 94% of the time. LA is a simple and rapid test which can be used for determining the herpes simplex virus immune status in sera of patients.

The detection of antibody to herpes simplex virus (HSV) can be an important aid in identifying immunocompromised patients at risk for severe HSV infections (2, 4). In marrow transplant patients, HSV is the primary viral pathogen seen in the pre-engraftment period, with viral reactivation occurring in 80% of patients who are seropositive before transplantation (3). Acyclovir is effective treatment for HSV infection and has also been shown to be effective in preventing HSV infections in immunocompromised patients when given prophylactically (5). Therefore, determination of the HSV immune status of individuals before immunosuppression can be important for the clinical management of these patients.

We evaluated a new rapid latex agglutination (LA) assay (Virogen HSV antibody test; Wampole Laboratories, Cranbury, N.J.) for determining the presence of antibody to HSV in potential marrow transplant recipients. The sensitivity, specificity, and technical features of the LA test were compared with those of the complement fixation (CF) test as used in our laboratory and with those of a commercially available enzyme immunoassay (EIA).

A total of 173 serum samples from 166 potential marrow transplant recipients and 7 normal individuals (5 laboratory personnel and 2 marrow transplant donors) were prospectively assayed for HSV immune status by the LA test, EIA, and CF test. The CF test was performed by standard microdilution methods with all sera tested in quantitative fashion starting at a dilution of 1:8 and ending at a dilution of 1:512 (1). Antibody to HSV was considered present if a CF titer of $\geq 1:8$ was obtained. Antibody was considered absent if the CF titer was $< 1:8$. The LA assay was performed as outlined in the test kit and as was previously described (P. C. De Girolami, J. Dakos, and K. Eichelberger, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1987, C-153, p. 348; A. D. Abbot, G. Ignatofsky, W. Wallen, J. T. Matthews, J. Docherty, and J. Iltis, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1987, C-154, p. 349). Included in the test kit were the latex reagent sensitized with HSV antigen, buffered diluent, and positive and negative control sera. Briefly, sera were diluted 1:4 and 1:64 in the buffered diluent, after which 25 μ l of the serum dilutions was added to different wells of a glass slide. One drop (15 μ l) of the latex reagent was then added to each of the wells and mixed. The slide was then rotated on a serologic rotator for 10 min at 100 rpm. The wells were

examined for agglutination under incandescent lighting and compared with the control wells for a positive or negative reaction. Agglutination was defined as follows. A nonreactive or negative result was indicated by a fine granular background or a cloudy background without any observable clumping. A reactive or positive result was indicated by distinct large clumps against a clear or slightly cloudy background or small but definite clumps against a cloudy background. The EIA (Herpes Stat; Whittaker M.A. Bioproducts, Walkersville, Md.) was performed as specified by the manufacturer in a 96-well microdilution plate format. Sera were tested at a single 1:21 dilution in wells containing HSV antigen. There was no antigen control well in the Herpes Stat format. After incubation with the enzyme substrate, the sample wells were read at a 550-nm wavelength on a Microplate Autoreader EL310 (BIO-TEK Instruments; provided by Whittaker M.A. Bioproducts). Samples that were antibody positive by EIA were those with absorbance values greater than or equal to the low-positive standard value provided in the EIA kit. Tests of sera giving discrepant results were repeated by all three assays.

Of 173 serum samples tested, 115 (66%) were positive for HSV antibody and 37 (21%) were negative for HSV antibody by all three assays. The LA test and EIA agreed 94% of the time (163 of 173 serum samples tested), and the LA and CF tests agreed 92% of the time (159 of 173 serum samples tested) (Table 1). Twenty-one (12%) samples tested yielded discrepant results between the three assays (Table 2). Of these 21, 14 were positive by LA, 10 were positive by EIA, and only 8 were positive by CF.

Repeat testing of these 21 serum samples in our laboratory showed no changes in the initial test results. Of the 21 discrepant samples, 14 were also sent to Wampole Laboratories for retesting by LA. Results with 12 of the 14 serum samples agreed with the original LA results obtained in our laboratory (8 of 10 positive results and 4 of 4 negative results). However, two of the three LA-positive samples found to be negative by EIA and CF were also negative by the LA test as performed at Wampole.

The LA test had a sensitivity of 97% and a specificity of 80% compared with CF and a sensitivity of 98% and a specificity of 35% compared with the EIA. Conversely, the EIA had a sensitivity of 95% and a specificity of 93% compared with LA, and the CF test had a sensitivity of 92% and a specificity of 91% compared with LA. The LA test had an apparent false-positive rate of 2%, that is, 3 of the 173

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TABLE 1. Correlation of LA, CF, and EIA results

Test and result	No. (%) of LA results	
	Positive	Negative
EIA		
Positive	122 (71)	3 (2)
Negative	7 (4)	41 (24)
CF		
Positive	119 (69)	4 (2)
Negative	10 (6)	40 (23)

serum samples were LA positive but CF and EIA negative. However, two of these three samples were also shown to be LA negative when retested by Wampole, as noted above. No sera were LA negative but positive by both CF and EIA.

The LA test is a simple and rapid test which is easy to perform without the need for expensive equipment or exten-

TABLE 2. Intertest comparison of 21 serum samples with discrepant results^a

Result	No. of samples
LA positive; CF and EIA negative	3
CF positive; LA and EIA negative	4
EIA positive; LA and CF negative	3
LA and CF positive; EIA negative	4
LA and EIA positive; CF negative	7
CF and EIA positive; LA negative	0

^a A total of 115 samples (66%) were positive by all three tests, and 37 (21%) were negative by all three tests.

sive technical expertise and with a shorter turnaround time than either the CF test or the EIA. The LA test also appeared to be the most sensitive of the three tests. Additionally, the LA test can be used for either the qualitative or quantitative determination of HSV antibody in sera of patients. This test can serve as an aid in the rapid determination of patient immune status to HSV and as an acceptable alternative to other serologic tests now in common use.

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