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Evaluation of a Latex Particle Agglutination Assay for the Detection of Cytomegalovirus Antibody in Patient Serum

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The Virogen CMV Antibody Test is a simple and rapid latex agglutination assay for the detection of cytomegalovirus antibody in human serum and plasma. Evaluation of this assay with respect to enzyme immunoassay yielded a sensitivity of 98% with a specificity of 100%. In comparison to CMVScan, the Virogen CMV Antibody Test had a sensitivity of 98.4% and a specificity of 100%.

The majority of cytomegalovirus (CMV) infections are asymptomatic, but there is a risk of serious complications when CMV infections occur congenitally or in immunocompromised individuals (5–9). Detection of serum antibody can help in identifying those individuals with prior CMV exposure and may aid in confirming acute CMV infection (P. Klevjer-Anderson, Clin. Microbiol. Newsl. 9:36–38, 1987). Moreover, absence of CMV antibody by serological testing can identify those individuals at risk for CMV infection. This is extremely important in immunocompromised patients who may be receiving blood products and, based on CMV seronegativity, should be given seronegative CMV blood products (3, 4).

CMV antibodies in patient sera can be measured by a variety of serological immunoassays. These include complement fixation, solid-phase radioimmunoassay, latex agglutination assay (LA), indirect hemagglutination assay, indirect fluorescent-antibody test, anti-complement fluorescent-antibody test, neutralization, and enzyme immunoassay (EIA) (1, 3, 4, 6). We report here the use of a simple and rapid LA for the detection of CMV antibody in human serum or plasma.

A total of 427 serum specimens from 303 potential marrow transplant recipients and 124 random samples from adult blood donors were used in the evaluation of the Virogen CMV Antibody Test (Wampole Laboratories, Cranbury, N.J.), an LA. All 427 serum specimens were also assayed with CMVScan LA (BBL Microbiology Systems, Cockeysville, Md.), and 360 of the samples were available for testing by EIA (CMV Stat; Whittaker M.A. Bioproducts, Walkersville, Md.). The Virogen test was performed per the package insert as follows. Sera were screened undiluted by mixing 0.025 ml of serum sample with one drop of Virogen CMV latex on a black disposable card slide. The card slide was rotated at 100 rpm for 10 min, and the agglutination patterns were immediately examined under an obliquely projected light and compared with the negative control wells for a positive or negative reaction. In the paired-serum study, 0.025 ml of serum was mixed with 0.025 ml of sample diluent (provided in Virogen kit) and serial twofold dilutions of each serum sample were performed on the card slide to a 1:1,024 dilution. All dilutions were tested to determine the endpoint titer by mixing 0.025 ml of serum dilution with one drop of Virogen CMV latex.

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Agglutination was defined as follows. A nonreactive or negative result was indicated by a fine granular background or a cloudy background with absence of agglutination. 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 CMVScan test was performed in accordance with the procedures described in the package insert and as previously described (1, 2). Serum samples were screened undiluted, and endpoint titrations were performed by serially diluting samples on the disposable card slide.

In a 96-well microdilution format, the EIA was performed as specified by the manufacturer. Sera were tested at a 1:21 dilution in wells containing CMV antigen. There was no antigen control well in the CMV 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 value obtained with the low-positive standard provided in the EIA kit.

Serum and plasma samples containing sodium heparin, EDTA, or sodium citrate anticoagulants were drawn from each of eight donors. All samples were screened by Virogen for CMV antibody as described above.

Of 427 serum samples tested by both the Virogen CMV Antibody Test and CMVScan, 243 (57%) were positive for CMV antibody and 180 (42%) were negative for CMV antibody by both assays. Four serum samples (1.0%) were considered CMV antibody positive by CMVScan but nonreactive by the Virogen LA. The two LAs agreed 99% of the time (423 of 427 serum samples tested). Only 360 of the above-mentioned serum samples were available for further testing with the EIA. Of these 360, 195 (54%) were positive for CMV antibody and 180 (45%) were negative for CMV antibody by both assays. The same four serum samples mentioned above were positive for CMV antibody by the EIA but again nonreactive with the Virogen test. The Virogen test and the EIA agreed 98.8% of the time (356 of 360 serum samples tested; Table 1).

The Virogen test was further evaluated for its capability to detect a diagnostically significant increase (greater than fourfold) in the CMV antibody titer in five sets of acute- and convalescent-phase paired sera. The Virogen and CMVScan results were in agreement for indicating a fourfold-or-greater

TABLE 1. Results of the Virogen LA, CMVScan, and EIA for detection of CMV antibody from human serum

Test and result	No. of Virogen LA results	
	Positive	Negative
CMVScan LA		
Positive	243	4
Negative	0	180
EIA		
Positive	195	4
Negative	0	161

increase in antibody titer (Table 2) with a normal serologic variation of plus or minus one twofold dilution.

Undiluted serum, as well as plasma specimens containing three commonly used anticoagulants (sodium heparin, EDTA, or sodium citrate), were tested with the Virogen LA. Eight donor samples (five CMV antibody positive and three CMV antibody negative) were tested. The results were unaffected by the presence of the three anticoagulants.

The Virogen CMV Antibody Test, compared with the CMVScan test, had a sensitivity of 98.4% and a specificity of 100%. With respect to the EIA, the Virogen test had a sensitivity of 98% with a specificity of 100%. The CMVScan had a sensitivity of 100% and a specificity of 98% compared with the Virogen test, and the EIA had a sensitivity of 100% and a specificity of 97% compared with the Virogen test. The Virogen test had an apparent false-negative rate of approximately 1%; that is, 4 of the 427 sera tested were both CMVScan and EIA positive but Virogen negative. Overall, these results do not demonstrate a substantial difference in performance of the three assays. Repeat testing of these four samples with the LA test and EIA did not change the initial test results. These four serum samples were obtained from marrow transplant patients prior to transplantation. Subsequent serum samples from these four patients following transplantation were shown to be CMV antibody positive by the Virogen test. Why these sera did not initially react with the Virogen test is unclear. The most likely reason for these results was that the CMV antibody titer increased to a level sufficient for detection. Although less likely, it is possible the samples were from seronegative individuals who seroconverted following marrow transplantation. Marrow transplant patients are subjected to many blood products as well as to various chemical reagents; thus, we cannot rule out the possibility of something in the serum interfering with the agglutination reaction.

TABLE 2. Clinical evaluation of Virogen CMV LA compared with CMVScan LA: paired serum study

Serum pair	Antibody titer	
	Virogen	CMVScan
1		
Acute phase	–	–
Convalescent phase	512	512
2		
Acute phase	+ (neat)	+ (neat)
Convalescent phase	16	64
3		
Acute phase	+ (neat)	2
Convalescent phase	128	64
4		
Acute phase	–	–
Convalescent phase	8	32
5		
Acute phase	–	–
Convalescent phase	16	16

The Virogen CMV Antibody Test is a rapid and sensitive test which can be used for the qualitative and quantitative detection of CMV antibody in human serum or plasma. The assay performs very well when compared with current commercially available CMV antibody EIAs and LAs. It does not require extensive hands-on incubation or wash times to achieve sensitivity similar to that of EIA, nor does it require any special handling of plasma samples containing heparin, EDTA, or citrate anticoagulants. With CMVScan use, plasma containing citrate anticoagulant must first be diluted 1:2. Plasma is not recommended for use with the CMV Stat EIA. We noted that with some of the seronegative samples the Virogen assay displayed a less grainy (smoother) nonreactivity than CMVScan. This difference was the most prominent distinction noted between the two LAs, since overall performance was comparable.

It is clear from this and similar studies (2, 4, 6; Klevjer-Anderson, Clin. Microbiol. Newsl.) that no standard assay is available for the determination of CMV antibody. Different assays will provide discordant results with a small number of specimens, and consequently, true antibody status must be defined (in these instances) with additional clinical information. Results of this study indicate that the Virogen Test can serve as an aid in the determination of CMV immune status and the diagnosis of recent CMV infection.

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