Evaluation of the CMV-CUBE Assay for Detection of Cytomegalovirus Serologic Status in Marrow Transplant Patients and Marrow Donors

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We evaluated a new membrane dot immunobinding assay (CMV-CUBE; Difco Laboratories) for the detection of cytomegalovirus (CMV) antibody in marrow transplant patients and donors. The CMV-CUBE assay was compared with a commercially available enzyme immunoassay (EIA; CMV STAT) and a latex agglutination (LA; CMVScan) test. Serum samples were collected from 311 transplant patients and donors prior to transplantation. A total of 164 serum specimens were positive for CMV antibody by one or more of the three assays, with 153 of 164 samples (93.3%) positive by all three tests. A total of 147 serum specimens were CMV antibody negative. CMV-CUBE detected 154 of 164 (94%) of the positive samples, EIA detected 160 of 164 (97.5%), and LA detected 157 of 164 (95.7%) CMV-positive samples. Compared with EIA, CMV-CUBE had a sensitivity of 95.6% and a specificity of 99.3%. Compared with LA, CMV-CUBE had a sensitivity of 97.5% and a specificity of 99.4%. CMV-CUBE is a simple and rapid visual assay which can be used for the qualitative detection of antibody to CMV in patient serum.

Cytomegalovirus (CMV) infection remains a major cause of morbidity and mortality after allogeneic marrow transplantation, despite advances in antiviral therapy for CMV disease (8, 9). Patients who are CMV seropositive before transplantation presumably develop infection from a reactivation of the latent virus, whereas patients who are seronegative for CMV at the time of transplantation have a 40% risk of acquiring CMV infection during the first 3 months after transplantation from transfused blood components or from seropositive marrow unless they are given seronegative blood components (3, 4, 7). Thus, detection of serum antibody can help in identifying those individuals with prior CMV exposure who may develop recurrent CMV infection if they are blood donors as well as in identifying patients at risk for primary CMV infection.

The objective of this study was to evaluate a new rapid membrane dot immunobinding assay called CMV-CUBE that determines the presence of antibody to CMV in serum from potential marrow transplant recipients and marrow donors. The CMV-CUBE assay was compared with the commercially available enzyme immunoassay (EIA; CMV STAT) and the latex agglutination (LA; CMVScan) test.

MATERIALS AND METHODS

Sera. A total of 311 serum samples were collected from transplant patients and marrow donors prior to marrow transplantation at the Fred Hutchinson Cancer Research Center.

CMVScan LA procedure. The LA test was performed in accordance with the procedures described in the CMVScan package insert (BBL Microbiology Systems, Cockeysville, Md.). Serum samples were screened undiluted on a disposable card slide. A nonreactive (negative) result was indicated by a fine granular or cloudy background with no agglutination. A reactive (positive) result was indicated by distinct large clumps against a clear or slightly cloudy background. A

high- and low-positive control and a negative control provided with the kit were run with each assay (1, 2, 5, 7).

EIA procedure. The EIA was performed in a 96-well microplate format as specified by the manufacturer (CMV-STAT; Whittaker M.A. Bioproducts, Walkersville, Md.). Sera were tested at a 1:21 dilution in wells containing CMV antigen. 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 with the EIA kit (2, 5, 7).

CMV-CUBE procedure. Sera were tested by the CMV-CUBE assay (Difco Laboratories, Detroit, Mich.) as follows. All reagents and positive and negative controls were supplied with the CMV-CUBE kit. Serum samples and controls were diluted 1:5 with CMV-CUBE sample diluent, and 3 drops were applied to the surface of individual membrane cassettes. Three drops of CMV-CUBE wash reagent were then added to the membrane, after which 3 drops of CMV-CUBE conjugate were applied to the membrane and incubated for 1 min. The cassettes were washed with 5 drops of wash reagent. Three drops of CMV-CUBE chromogen reagent were then applied to the membrane, and after 2 min the reaction was terminated with 3 drops of CMV-CUBE stopping reagent. The reaction was then read. A positive result consisted of a blue spot in the center of the membrane with a white area surrounding it. A negative result (no reaction) consisted of a white membrane with negligible background staining. On the cassettes, a blue control C appearing around the perimeter of the cassette membrane indicated proper performance of the kit reagents. If this C did not appear, then the test was invalid (10).

RESULTS

A total of 164 serum samples (52.7%) were positive for antibody to CMV by one or more of the three assays, with 153 of 164 (93.3%) samples positive by all three tests. A total of 147 serum samples (47.3%) were CMV antibody negative by all three tests. CMV-CUBE detected 154 of 164 positive

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TABLE 1. Correlation of CMV-CUBE, LA, and EIA results

Test and result	No. of CMV-CUBE results	
	Positive	Negative
LA		
Positive	153	4
Negative	1	153
EIA		
Positive	154	6
Negative	1	150

samples (94%), EIA detected 160 of 164 positive samples (97.5%), and LA detected 157 of 164 positive samples (95.7%).

Compared with EIA, CMV-CUBE had a sensitivity of 96.3% and a specificity of 99.3%. Compared with the LA test, CMV-CUBE had a sensitivity of 97.5% and a specificity of 99.4%. Conversely, the EIA had a sensitivity of 99.3% and a specificity of 96.2% compared with CMV-CUBE, and the LA test had a sensitivity of 99.4% and a specificity of 97.5% compared with CMV-CUBE (Table 1).

In the three assays, a total of 11 serum samples had discrepant results. Of these, six serum samples were from patient transplant recipients and five were from marrow donors. Six serum samples were EIA positive (all low positives, with a range of predictive index of 1.02 to 2.24) but LA and CMV-CUBE negative. Three serum samples were LA positive. One serum sample was EIA and LA positive only, and one serum sample was CMV-CUBE positive only.

DISCUSSION

In marrow transplant recipients, the screening of blood products of CMV has been shown to significantly reduce CMV infection and CMV disease in seronegative recipients at risk of primary infection (4, 7, 8). An accurate and practical method of testing CMV serologic status is important in identifying CMV immune status in both potential marrow recipients and marrow donors, with primary emphasis on identifying those patients who might benefit from CMV-seronegative blood components and secondary emphasis on the screening of blood donors. Determination of CMV serologic status is also important in patients such as renal allograft recipients (6).

It is clear from this and similar studies that no single assay provides 100% sensitivity for the determination of CMV immune status in the marrow transplant population, which includes patients as well as donors. Different assays will provide discordant results with a small number of serum samples, and consequently "true" antibody status in these

patients or donors may need to be defined with additional laboratory and clinical information or both or through use of multiple serologic assays (2, 4-7).

CMV-CUBE is a simple and rapid visual assay which can be used for the qualitative detection of CMV antibody in serum. It takes approximately 10 min to run 10 to 20 samples in this assay (10). These data show that CMV-CUBE is a useful test for the determination of patient and donor serologic status regarding CMV in the marrow transplant setting, and there is excellent concordance with both the EIA and the LA test.

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