Latex Agglutination and Enzyme-Linked Immunosorbent Assays for Cytomegalovirus Serologic Screening of Transplant Donors and Recipients

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The effectiveness of three serologic assays (two enzyme-linked immunosorbent assays [ELISAs] and latex agglutination) for cytomegalovirus (CMV) serologic matching of donors and recipients was assessed over a 2-year period in a major organ transplant program. Sera with equivocal test results were investigated by repeat testing of serum samples and additional specimens from the individuals involved and monitoring of CMV infection in recipients. An in-house ELISA identified all CMV-infected donors as seropositive. Of 63 ELISA-positive donors, 5 were negative by latex agglutination; recipients from 3 of these donors developed primary CMV infection posttransplantation. The in-house ELISA and a commercial ELISA (Abbott enzyme immunoassay; Abbott Laboratories, North Chicago, Ill.) had a 93% concordance of results; follow-up testing indicated that the Abbott assay was sensitive but had a false-positive rate of about 11%. One recipient with a false-positive result developed symptomatic primary CMV infection after receiving a seropositive organ. Thus, performance characteristics of currently used screening assays affect recipient outcome.

Cytomegalovirus (CMV)-seropositive donors of blood and transplanted organs are an important source of CMV infection in their recipients (16). Although not all CMV-seropositive donors appear to be infective (10a), exclusion of seropositive donors is an effective means of preventing infection in seronegative recipients. Organ transplant programs have increasingly adopted CMV serologic status as one of the criteria for donor-recipient matching.

Rapid and reasonably accurate identification of CMV seroreactivity is possible through use of several current assays, such as enzyme-linked immunosorbent assays (ELISAs) or latex agglutination. However, approximately 5% of sera (from populations with a medium prevalence of CMV seroreactivity) usually show interassay discordance. Equivocal results could lead to erroneous donor-recipient serologic matching. Numerous reports (1–6, 11–15, 17–23, 25) based on laboratory comparisons of various assays are inconclusive as to the most reliable method for donor and recipient screening, although there has been recent wide acceptance of the latex agglutination assay because of its rapidity.

Determining the significance of borderline or discordant test results requires clinical correlation as well as comparisons of test kits. It has been questioned whether weakly seropositive donors can transmit CMV (25). Low or borderline CMV antibody levels detected in some donors may have resulted from blood transfusions given antemortem (e.g., after major trauma), rather than previous CMV infection in the donor, and the infectivity of these donors is uncertain (24).

During several years of prospective studies of CMV transmission in organ recipients at our transplant center (7–9, 10a), we used three assays (two ELISAs and a latex agglutination assay) for serologic classification of several hundred recipients and donors. Clinical, virologic, and serologic follow ups of recipients were obtained and used to study the results of various categories of donor-recipient CMV serologic matching. Our reference CMV serologic assay is an in-house ELISA modified from one previously described (11). For comparison, prospective cadaver donors were also screened with a commercial latex agglutination assay, whereas recipients and living related donors were also tested with a commercial ELISA (Abbott enzyme immunoassay; Abbott Laboratories, North Chicago, Ill.). Both commercial assays are widely used for these purposes. We present our experience with these assays, with emphasis on clinical and serologic follow ups pertaining to donors and recipients whose specimens gave equivocal or discordant results.

MATERIALS AND METHODS

Test sera were collected from organ (kidney, heart, and pancreas) transplant donors and recipients seen from 1985 to 1987 at Oregon Health Sciences University, as part of routine virologic surveillance. Sera were stored at −70°C until tested; initial latex agglutination assays of cadaver donor sera were performed on fresh specimens. Sera used for this study were those for which comparative testing data were available, with no further exclusion criteria applied. Information was obtained on the occurrence of CMV infection (seroconversion and virus shedding) in the recipients following grafting of organs from the donors tested.

The latex agglutination assay (CMVScan; BBL Microbiology Systems, Cockeysville, Md.) was performed on sera from prospective cadaver donors by using undiluted serum as directed by the manufacturer. The commercial ELISA (CMV total antibody enzyme immunoassay; Abbott) was performed on sera from recipients and living related donors as directed by the manufacturer, with a single serum dilution of 1:21.

All sera were tested with an in-house ELISA which we have used extensively over the past several years. With standard 96-well plates (Immulon-2; Dynatech Laboratories, Inc., Alexandria, Va.), alternate paired columns of wells

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TABLE 1. Comparison of Abbott and in-house ELISAs

<table>
<thead>
<tr>
<th>In-house ELISA result</th>
<th>No. of Abbott ELISA results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>116</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
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were coated with 50 μl of glycine-extracted CMV strain AD169 antigen or uninfected-cell control antigen per well, respectively. Antigens were prepared and diluted for use as previously described (10). Coated plates were tightly covered and stored from overnight to 10 days at 4°C, washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (Twee 20-PBS), and emptied, and protein-binding sites remaining on the plates were blocked by addition of 0.2 ml of PBS per well containing 10% calf or horse serum. After 2 h, the plates were emptied and the ELISA was conducted by using procedures similar to those previously described (11).

Wells were loaded with 50 μl of test serum per well at a single 1:1,000 dilution (in PBS–10% calf serum). Each specimen was loaded into two wells coated with CMV antigen and two wells coated with uninfected-cell antigen. Duplicate loading of specimens was used to control for uniformity of plate coating and loading, and testing of each specimen against an uninfected-cell control antigen was used to control for nonspecific reactivity. Known positive and negative serum pools at the same dilution were included with each run. After 2 h at 37°C, plates were washed three times with Tween 20-PBS, and peroxidase-conjugated goat anti-human immunoglobulin G antibody (1:2,500 dilution, 50 μl per well; Tago, Burlingame, Calif.) was added. After another 2 h at 37°C, the plates were washed five times with Tween 20-PBS and five times with distilled water. Wells were then loaded with 0.1 ml of a substrate solution [0.5 mg of 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., St. Louis, Mo.) per ml, 0.03% hydrogen peroxide in 50 mM citrate buffer (pH 4.5)] per well. After 15 min, the reaction was stopped with 0.1 ml of a 1% aqueous solution of electrophoresis grade sodium dodecyl sulfate per well. The A₉₀₀ was read on an automated ELISA reader. Specific absorbance was calculated by averaging the absorbance in the two CMV antigen wells and subtracting from this the average absorbance in the two uninfected-cell antigen wells. A specific absorbance of greater than 0.1 was considered positive in this assay, provided that the positive control pool (consisting of sera known to be strongly reactive) gave a specific absorbance of greater than 1.0 (usually about 1.5) and the negative control pool gave an absorbance of less than 0.05 (usually about 0.01).

RESULTS

ELISA screening of recipients and living related donors. Results of testing 284 serum specimens with the two ELISAs are shown in Table 1. The sera were from 238 individuals, about 95% of whom were recipients; the rest were from living related donors. With the defined cutoff absorbance value for each ELISA, concordance of results was observed in 264 (93%) cases. Absorbance values in the Abbott ELISA averaged 1.060 for all positive specimens and ranged up to 1.808. In our ELISA, the corresponding values were 1.106 and 1.922, respectively.

Discordant results were observed with 20 serum specimens. We attempted to resolve these discrepancies by using several criteria. The first was reproducibility on repeat testing of the same specimen. Secondly, we checked the consistency of the result with tests of other sera from the same individual by using both ELISAs. Finally, for those individuals whose serologic status was changing (i.e., primary infection), we sought corroborative evidence of active infection, such as CMV shedding in urine or blood.

Table 2 shows the results of examining discordant results. Of the 16 serum specimens initially positive by the Abbott ELISA (absorbance ranging up to 0.233) but negative by our ELISA, 10 were negative on retesting of the same sera by the Abbott kit. The negative retesting result was found to be consistent with results for subsequent sera from these individuals and with clinical and microbiologic data (absence of CMV shedding or illness). Four specimens gave a repeatedly low positive result by the Abbott kit, but additional sera from the same individuals were negative by both ELISAs. The negative results were likewise consistent with clinical and microbiologic data. One individual showing a low positive result by the Abbott kit and a negative result by our ELISA was in the process of seroconverting (and had just begun shedding CMV in urine). Conversely, two of the four sera specimens positive by our ELISA (absorbances of 0.205 and 1.230) and negative by the Abbott ELISA were also from individuals in the process of seroconverting. Of the remaining two serum specimens positive in our ELISA and negative in the Abbott ELISA, one was negative on retesting with our ELISA (controls on the initial assay showed high nonspecific absorbance) and the other was repeatedly positive by our ELISA, but a later specimen was negative. Finally, one case of discordance could not be resolved with additional sera or clinical data. The serum was positive by the Abbott ELISA and negative by the other two assays.

Given the above resolution of discordant data, we estimated that the Abbott ELISA has a false-positive rate of at least 14/132 (11%). Assay specificity and concordance are improved if a positive cutoff absorbance value of 0.170 is used for the Abbott assay, which is about 0.05 U higher than the manufacturer specification. Under these conditions, concordance was improved to 276/284 (97%). The 16 specimens positive by the Abbott ELISA and negative by ours were reduced to 3, and the 4 specimens positive by our assay and negative by the Abbott assay were increased by only 1 (however, this specimen was from a patient known to be seropositive). False-positive results caused potential problems for recipients. The pretransplant serum of one recipient tested positive by the Abbott ELISA (absorbance, 0.166). Because the latex agglutination assay of the same specimen was also
positive, a seropositive organ was transplanted. Several weeks later, a typical primary CMV syndrome with fever, leukopenia, and seroconversion occurred in the recipient. The results of our own ELISA, not available at the time of transplant, indicated that the pretransplant serum was negative, as did repeat testing of the same specimen with the Abbott ELISA. An additional serum specimen obtained shortly after transplant was also negative by the Abbott ELISA.

Comparison of latex agglutination and ELISA for donor screening. Results of testing 108 cadaver donor serum specimens are shown in Table 3. Concordance of results was observed in 101 (94%) cases. No donor who was seronegative by both assays was found to transmit CMV to recipients. Discordant results were observed with seven donors, and these persisted on repeat testing. We attempted to resolve these differences by running the Abbott ELISA on the same specimens and by obtaining clinical follow-up data on the recipients of organs from these donors.

Follow ups of sera with discordant results by latex assay and ELISA revealed three cases in which the serum was latex assay negative and ELISA positive and the donor transmitted CMV, one case in which the serum was latex assay negative and ELISA positive and the donor did not transmit CMV, one case in which the serum was latex assay negative and ELISA positive and the recipient was seropositive, one case in which the serum was latex assay positive and ELISA negative and the donor did not transmit CMV, and one case in which the serum was latex assay positive and ELISA negative and the recipient was seropositive. Five donors were seronegative by latex agglutination but positive by our ELISA (absorbance values, 0.122 to 0.396). Four CMV-seronegative recipients received organs from four of these donors, and three of the recipients later developed primary CMV infection with seroconversion, thus strongly implicating the donors as the source of their CMV infection. All three infective donors were also seropositive by the Abbott ELISA (absorbance values, 0.258 to 0.366) but repeatedly negative on latex agglutination (including different test kits). Of the two donors who were seropositive by latex agglutination but negative by our ELISA, one was also negative by the Abbott ELISA and three of that donor’s organs were transplanted into three different CMV-seronegative recipients. All three recipients remained seronegative posttransplant; this suggests that the seronegative classification was correct. The remaining latex-positive donor was borderline negative in our ELISA and weakly positive in the Abbott ELISA; infectivity could not be assessed, because the recipient was CMV seropositive and no CMV shedding was noted posttransplant. On the basis of the above-described findings, we estimate that the latex agglutination assay has a false-negative rate of at least 3/63 (5%).

DISCUSSION

Accurate serologic classification of organ and blood donors and recipients is necessary for prevention of primary CMV infection in recipients. Previous comparative studies of commercial assays (1–6, 11–15, 17–23, 25) have reported various levels of accuracy, depending on the reference assay used. In a transplant program, a more important measure of accuracy is the ability of the assay to predict clinical consequences. The data presented here indicate that, although two widely used commercial assays were largely satisfactory, there remained a number of specimens with equivocal results that could result in unintended donor-recipient matches and adverse clinical consequences. Avoidance of these outcomes depends on understanding the performance characteristics of the individual assays, along with cross-checking of results by alternate assays and serial specimens when necessary.

We found the Abbott ELISA to have adequate sensitivity in detecting CMV-seropositive specimens at the expense of false-positive results. False positivity with the Abbott assay has been reported previously in laboratory comparisons (15, 18). Experience with our in-house ELISA suggests that the false-positive results obtained with the Abbott kit most likely stem from the lack of negative (uninfected-cell antigen) controls for each test serum. Our assay encounters sera with highly nontypical reactions to cell control antigen at about the same frequency as that at which false-positive results are encountered with the Abbott ELISA. False positivity with the Abbott kit can be reduced considerably, with slight loss of sensitivity, by increasing the absorbance value chosen as the positive cutoff. Our current practice in using the Abbott kit is to repeat all results ranging between the manufacturer-specified cutoff and 0.200. This often results in a reversal of a previous serologic classification, as our data show. In our own ELISA, duplicate sample loading and use of a control antigen for each specimen are usually adequate to identify problematic sera requiring further analysis.

The latex agglutination assay is simple and rapid and has been widely advocated for use in donor screening applications (1, 13, 18, 20, 25). Previous laboratory comparisons suggested high accuracy, including reports of 100% sensitivity (2, 14, 23). Unfortunately, we found, during 2 years of actual use of the assay in our transplant program, that occasional (about 5%) seropositive and demonstrably infective donors were missed by this assay. At our center, this resulted in three unexpected primary CMV infections. If ELISA results had been used, the donors would have been appropriately classified. The latex agglutination assay also has an element of subjectivity in reading, and efforts to maximize sensitivity by reading doubtful agglutination as positive may increase the number of false-positive results.

Thus, although the latex agglutination assay is rapid and convenient and we continue to use it, the ELISA provides important corroborative data and may be preferable for donor-recipient matching when time permits, as with living related donors and recipients. However, interpretation of the Abbott ELISA requires attention to possible false-positive results which could result in inappropriate grafting of seropositive organs into recipients who are incorrectly classified as seropositive.

Sera from some donors who transmitted CMV had low positive test results by ELISA. Although the infectivity of weakly seropositive donors has been doubted (24, 25), we were not able to demonstrate any level of seropositivity below which donors could be considered noninfectious. It is, however, very likely that the serologic status of some donors is obscured by antemortem transfusions given before collection of blood for serologic testing. Whether these donors are noninfective and whether latently infected cells in the trans-
fused blood can be transferred indirectly into an organ recipient are points for future study.

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LITERATURE CITED


