

## Comparison of Four Techniques for Detection of Antibodies to Cytomegalovirus

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**Four serological methods were compared and evaluated for use in detecting cytomegalovirus antibody in blood and organ donors. Western blotting (immunoblotting), latex agglutination, enzyme-linked immunosorbent assay, and a recent available microparticle enzyme immunosorbent assay were used. The microparticle enzyme immunoassay appears to compare favorably with each of the other three assays tested for screening blood and organ donors for a previous cytomegalovirus infection.**

Cytomegalovirus (CMV) is a ubiquitous human viral pathogen belonging to the family *Herpesviridae*. Infection with CMV is usually asymptomatic in the immunocompetent host but can cause dramatic disease in immunosuppressed patients (9). After primary infection, the virus persists in the host as a chronic or latent infection, which can periodically reactivate to an active infection.

CMV is known to be transmitted through blood transfusions (1) and transplanted organs (6). This can result, especially in immunosuppressed seronegative patients, in severe CMV disease (3, 13). The presence of specific CMV antibodies as evidence of past or present infection is the most valuable indicator of a potentially infective donor (12). The serological screening of blood and organ donors for antibodies against CMV antigens is therefore an essential step in the prevention of acquired CMV disease (4, 7).

The serological demonstration of antibodies to CMV for screening purposes is still a matter of some controversy (7, 10). The method of choice used for screening antibodies should be efficient and technically easy to perform, and, ideally, the assay should be highly sensitive and have a low incidence of false-negative results to avoid CMV transmission to recipients. The latex agglutination test, which is used in most laboratories, is a simple and rapid method for the determination of CMV immune status (2, 8). However, there is some doubt as to whether all seronegative donors have never really had previous CMV infection, and the subsequent possibility of the presence of latent virus (7, 16).

Recently, a new commercial microparticle enzyme immunoassay (MEIA) (Abbott Laboratories, North Chicago, Ill.) has become available. This prompted us to do a comparative study with the aim of evaluating the performance and practicability of several screening tests for the detection of CMV antibodies. The serological tests investigated were the Western blotting (immunoblotting) technique (IB), the latex agglutination test (LA), the enzyme-linked immunosorbent assay (ELISA), and the MEIA. A total of 90 serum samples were studied.

The procedure for the IB has been described in detail elsewhere (5, 11). Purified CMV (strain AD169) was used as antigenic material. A serum specimen was considered posi-

tive for CMV by the IB if antibodies reactive to one or more of the major structural proteins with relative mobilities of 150, 82, 66, 55, 38, or 28 kDa were present. In particular, reactivity to the CMV 150-kDa polypeptide is enough to indicate seropositivity (11). An ELISA procedure that has been described previously (14, 15) was used. Antigen material was derived from CMV (strain AD169)-infected human embryonal fibroblasts. After extensive testing, we considered sera with a positive/negative ratio above 2.1 (mean plus two times the standard deviation) to be positive, while sera with a positive/negative ratio below this value were considered to be negative for CMV. For the LA, the CMV Scan of Becton Dickinson Microbiology Systems, (Cockeysville, Md.) was used. The MEIA was done by using the IMx CMV immunoglobulin G antibody assay of Abbott Laboratories.

Each method was compared with the other three assays; i.e., comparison was based on concordance with two or more of the other assays. Table 1 lists the positive and negative results of the four assays. Table 2 summarizes the sensitivity, specificity, and overall agreement, as well as false-positive and -negative result rates, for each assay. No significant differences were found.

As has been indicated by several studies (4, 7, 12), it is important to use a dependable serological assay for screening blood and organ donors to achieve a reduction of transmitted CMV infection and CMV disease in seronegative recipients at risk of primary infection. Comparison of the assays showed a moderate number of false-positive results, with a range of 1.1% for the ELISA, 4.4% for the IB, 7.8% for the LA, and 10.0% for the MEIA. This high number of false-positive results by the LA and the MEIA can be explained in two ways. Firstly, the low serum dilution used

TABLE 1. Results of testing 90 sera for antibody against CMV by four serological assays

Assay	Test results									
IB	-	+	-	+	-	-	+	+	-	-
LA	-	+	+	-	+	-	+	-	-	+
ELISA	-	+	-	+	-	-	-	-	+	+
MEIA	-	+	+	+	-	+	+	-	+	+
Total no. with result	45	25	4	3	3	2	2	2	2	1

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TABLE 2. Correlation of IB, LA, ELISA, and MEIA for CMV antibody detection<sup>a</sup>

Assay	Sensitivity <sup>b</sup> (%)	Specificity <sup>c</sup> (%)	Overall agreement <sup>d</sup> (%)	False result rate (%)	
				Positive	Negative
IB	83.3	92.6	88.9	4.4	6.7
LA	82.3	87.5	85.7	7.8	6.7
ELISA	78.4	98.1	90.0	1.1	8.9
MEIA	100.0	84.8	90.0	10.0	0.0

<sup>a</sup> Based on concordance of two or more of the four methods.

<sup>b</sup> Sensitivity = [(true positives)/(true positives + false negatives)] × 100

<sup>c</sup> Specificity = [(true negatives)/(true negatives + false positives)] × 100

<sup>d</sup> Overall agreement = [(true positives + true negatives)/(total number tested)] × 100

in these tests can facilitate the binding of nonspecific antibodies of the immunoglobulin G class to the antigen that is present. In contrast, in the ELISA procedure, this problem was circumvented by the use of much lower serum concentrations. Secondly, the presence of nonstructural polypeptides in the antigenic material used in the LA and in the MEIA may contribute to the number of false-positive results. In the IB, purified virus, which contains the great majority of important structural viral antigens and is proba-

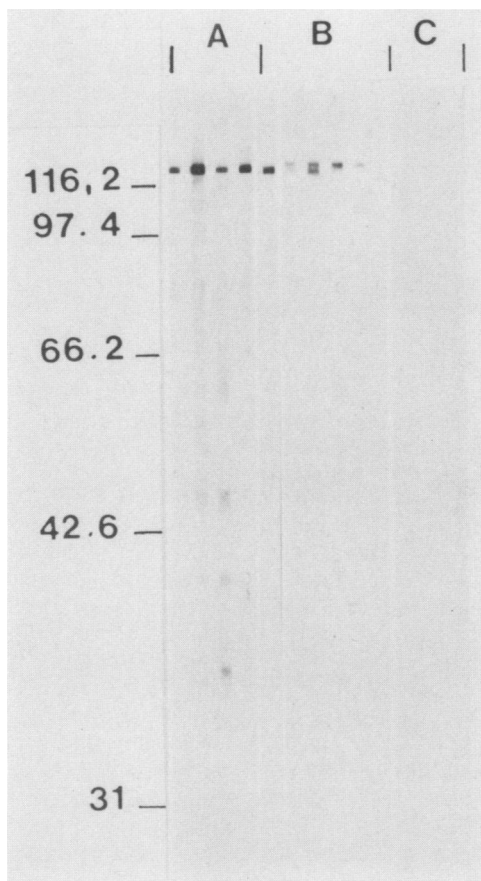


FIG. 1. Results from IB detection of antibodies to CMV proteins in sera of healthy persons. (A) Four lanes of sera with specific antibody reactivity for several proteins; (B) five lanes of sera with positive reaction only for the 150-kDa protein; (C) three lanes of sera without antibodies reacting to CMV proteins.

bly not contaminated with cellular proteins, was used as antigenic material. However, since sera containing antibody reactive with CMV structural proteins also contain antibodies reactive with nonstructural CMV proteins, the difference in the composition of antigenic material in all likelihood does not contribute much to the number of false-positive results.

Comparison of the assays also shows a moderate incidence of false-negative results, ranging from 0.0% for the MEIA and 6.7% for the IB and the LA to 8.9% for the ELISA. These false-negative results are explained by the low sensitivities of the ELISA (78.4%) and of the IB and LA (83.3 and 82.3%, respectively). The absence of false-negative results by the MEIA is due to the high sensitivity of this test.

The results show that the IB technique divides the CMV antibody-positive sera into two groups, depending on the reaction pattern, as demonstrated in Fig. 1. In the first group, reactivity with several structural viral proteins with an apparent molecular weight of 150 kDa, but also with those of 82, 66, 55, 38, and 28 kDa, was present. In the second group, only reactivity with a CMV polypeptide of 150 kDa was present. In our study, sera reactive with only the structural polypeptide of 150 kDa and sera reactive with several structural virion proteins are randomly distributed over the range of sera, resulting in discrepant test results. Despite the small number of sera (90 samples) tested, the conclusion may be drawn that the MEIA using the IMx apparatus (Abbott Laboratories) is a highly sensitive and specific serological method for determining antibodies to CMV, with a low number of false-negative results. The assay is easy to perform and reliable, and it takes approximately 40 min to run 24 samples. The MEIA allows the avoidance of many problems caused by mismatching CMV antibody-positive blood or organ donors with seronegative recipients. A larger study should be started to further evaluate the differences between all the serological techniques available for detection of CMV antibodies.

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#### REFERENCES

- Adler, S. P. 1983. Transfusion-associated CMV infection. *Rev. Infect. Dis.* 5:977-993.
- Adler, S. P., M. McVoy, V. G. Biro, W. J. Britt, P. Hider, and D. Marshall. 1985. Detection of cytomegalovirus antibody with latex agglutination. *J. Clin. Microbiol.* 22:68-70.
- Barkholt, L. M., B. G. Briczon, A. Ehrnst, M. Forgren, and J. P. Anderson. 1990. Cytomegalovirus infections in liver transplant patients: incidence and outcome. *Transplant. Proc.* 22:235-237.
- Bowden, R. A., M. Sayers, N. Flournoy, B. Newton, M. Ranajii, E. D. Thomas, and J. D. Meyers. 1986. CMV immunoglobulin and seronegative blood products to prevent primary CMV infection after marrow transplantation. *N. Engl. J. Med.* 314:1006-1010.
- Braun, D. K., L. Pereira, B. Norrild, and B. Roizman. 1983. Application of denaturated, electrophoretically separated and immobilized lysates of herpes simplex virus-infected cells for detection of monoclonal antibodies and for studies of the properties of viral proteins. *J. Virol.* 46:103-112.
- Fox, A. S., M. D. Tolpin, A. L. Baker, C. E. Broelsch, P. F. Whittington, T. Jackson, J. R. Thislethwaite, and F. P. Stuart. 1988. Seropositivity in liver transplant recipients as a predictor of CMV disease. *J. Infect. Dis.* 157:383-385.
- Gleaves, C. A., S. F. Wendt, D. R. Dobbs, and J. D. Meyers. 1990. Evaluation of the CMV-CUBE assay for detection of cytomegalovirus serologic status in marrow transplant patients

- and marrow donors. *J. Clin. Microbiol.* **28**:841-842.
8. **Gray, J. J., B. Alvey, D. J. Smith, and T. G. Wreghitt.** 1987. Evaluation of a commercial latex agglutination test for detecting antibodies to CMV in organ donors and transplant recipients. *J. Virol. Methods* **16**:13-19.
  9. **Ho, M.** 1982. *Cytomegalovirus: biology and infection*, p. 9-32. Plenum Medical Book Co., New York.
  10. **Landini, M. P., G. Mirolo, P. Coppolecchia, M. C. Re, and M. La Placa.** 1986. Serum antibodies to individual CMV structural polypeptides in renal transplant recipients during viral infection. *Microbiol. Immunol.* **30**:683-695.
  11. **Landini, M. P., M. C. Re, G. Mirolo, B. Baldassarri, and M. La Placa.** 1985. Human immune response to CMV structural polypeptides studied by immunoblotting. *J. Med. Virol.* **17**:303-311.
  12. **McHugh, T. M., G. H. Savavant, J. C. Wilber, and D. P. Stites.** 1985. Comparison of six methods for detection of antibody to cytomegalovirus. *J. Clin. Microbiol.* **22**:1014-1019.
  13. **Metselaar, H. J., and W. Weimer.** 1989. Cytomegalovirus infection and renal transplantation. *J. Antimicrob. Chemother.* **23**(Suppl. E):37-47.
  14. **van Loon, A. M., F. W. A. Heessen, J. T. H. van der Logt, and J. van der Veen.** 1981. Direct enzyme-linked immunosorbent assay that uses peroxidase-labeled antigen for determination of immunoglobulin M antibody to cytomegalovirus. *J. Clin. Microbiol.* **13**:416-422.
  15. **van Loon, A. M., J. T. H. van der Logt, and J. van der Veen.** 1981. Enzyme linked immunosorbent assay for measurement of antibody against CMV and rubella virus in a single serum dilution. *J. Clin. Pathol.* **34**:665-669.
  16. **Ronco, P., S. Kadereit, S. Michelson, B. Mougnot, P. Thibault, P. Verroust, F. Mignon, and R. Colimon.** 1990. Polymerase chain reaction assay for the detection of cytomegalovirus genome in 22 kidney biopsies; a correlation with the serological status. Abstracts part 2, no. 17. *Eur. J. Clin. Invest.* **20**:A7.