

Comparison of Five Methods of Cytomegalovirus Antibody Screening of Blood Donors

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A group of 120 sera from blood donors was screened by complement fixation and commercially available immunofluorescence, solid-phase fluorescence immunoassay, enzyme-linked immunosorbent assay, and indirect hemagglutination tests. Twenty-four of the sera were positive by three or more of the five tests and judged to be true positives; 89 were negative by three or more of the tests and considered to be true negatives. The tests were ranked for accuracy, sensitivity, specificity, false-positive rate, and false-negative rate. The indirect hemagglutination test scored best, followed by enzyme-linked immunosorbent assay, solid-phase fluorescence immunoassay, complement fixation, and immunofluorescence, in that order. When the tests were ranked on the basis of technical demands, turnaround time, requirement for special equipment, and subjectivity in reading, the indirect hemagglutination test again scored best, followed by solid-phase fluorescence immunoassay, enzyme-linked immunosorbent assay, immunofluorescence, and complement fixation in that order. Our findings suggest that the indirect hemagglutination test is the most reliable and effective commercially available test for the identification of those donors who are very unlikely to transmit cytomegalovirus to recipients.

The transmission of cytomegalovirus (CMV) to transplant recipients via transfused blood is well documented and has been extensively reviewed (1, 6). Although blood transfusions may account for most CMV infection in organ transplants (1), neonates (12), and heart surgery (8), there is no unanimity as to the source of the latent virus; it has been reported to be associated with leukocytes (8, 11), predominantly polymorphonuclear (10), and associated with T cells (4). The concentration of virus in blood from a latently infected blood donor is very low, with virus being recoverable from only 3 out of 10^6 T cells (4). The extended period of time required for CMV isolation, by whichever method, including modern techniques such as hybridization, render rapid direct demonstration of infection in a donor impracticable at the present time.

Serology is the only practicable system at present for the selection of blood donors with a high probability of being free of virus. Although there appear to be no data from which to determine the correlation between the transmission of CMV and the antibody titer of donors, it has been shown that there is an association between the presence of antibody in mother's blood and acquisition of CMV during the first year of an infant's life; 24 healthy newborns were assessed

(9). Of 13 infants that did not become infected with CMV, the mothers of 10 had antibody titers of $\leq 1:8$ by complement fixation (CF), and three mothers had CMV antibody titers of 1:16. Of 11 infants proved to be excretors of CMV at some time during the first year of life, the mothers of 7 had CMV antibody titers of 1:16 to 1:64, and four mothers had titers of $\leq 1:8$. These results suggest that the higher the antibody titer in the blood of a subject the greater is the probability that the individual can transmit CMV infection. Also, there was a positive association between the level of CMV serum antibody by CF and excretion of virus in the urine (7). More pertinently, Yeager et al. (12) showed that when using a titer of $\geq 1:8$ by indirect hemagglutination (IHA) as the criterion for seropositivity, infants of seronegative mothers who received blood cells from seronegative donors did not acquire CMV infection. Conversely, infants of CMV seronegative mothers who received red blood cells from seropositive donors had an approximately 20% infection rate when the transfused volume was ≥ 50 ml. These results suggest that donors with an antibody level of $< 1:8$ by IHA most likely lack the potential to transmit CMV infection. It is therefore important that health care facilities use a reliable and

TABLE 1. Distribution of sera by the number of positive CMV antibody tests

No. of positive tests	No. of sera
5	16
4	5
3	3
2	4
1	34
0	51

reproducible method to determine the CMV antibody status of blood donors. The purpose of the study reported here was to compare the accuracy, sensitivity, and specificity of five methods of serological evaluation of the antibody status of blood donors, four of them available as commercial kits.

(The work reported here was carried out in partial fulfilment of the requirements for the degree of Master of Science, University of Ottawa [L.G.]

MATERIALS AND METHODS

Sera. All specimens in the comparison were submitted by the Canadian Red Cross, Ottawa, for assay of CMV antibody. They were stored at -20°C in screw-capped glass vials until tested.

CF test. The CF test used was a microtiter adaptation of the method of Bradstreet and Taylor (3). Sera were titrated from 1:2 to 1:64. A titer of $\geq 1:4$ was considered positive.

IFA. The immunofluorescence assay (IFA) kit and methodology supplied by Electro-Nucleonics Laboratories, Inc., Bethesda, Md. were used. The sera were titrated from 1:2 to 1:32 and reacted with pre-fixed monolayers of infected human fibroblastoid cells. After washing, the slides were treated with fluorescein isothionate-conjugated anti-human immunoglobulin G (IgG). The tests were read by incident UV light illumination with two Leitz Wetzlar SM-LUX microscopes fitted with a comparison bridge. This had a split screen for simultaneous viewing of the degrees of fluorescence in the test and in the control prepara-

tions. Specific intranuclear fluorescence of \pm or greater at $\geq 1:16$ serum dilution was the criterion for positivity.

SFI. For the solid-phase fluorescence immunoassay (SFI), the FIAX CMV IgG assay employs CMV antigen adsorbed onto a solid phase, StiQ sampler. The StiQ sampler is reacted with a dilution of serum. Specifically, bound CMV antibody is then detected with fluorescein-labeled anti-human IgG. The intensity of fluorescence on the solid-phase surface is directly proportional to the concentration of IgG CMV antibody and is measured by using the FIAX fluorometer. The FIAX reagents, methodology, and equipment used for this test were supplied by International Diagnostic Technology, Inc., Santa Clara, Calif. A reading of ≥ 25 FIAX units was considered positive.

EIA. The enzyme-linked immunosorbent assay (EIA) was carried out with a kit and methodology supplied by M.A. Bioproducts, Walkersville, Md. Ten microliters of a serum sample was added to 250 μl of diluent in two wells of a polystyrene plate, one with CMV antigen immobilized on the surface and one with control antigen immobilized on it. After reaction and washing, fixed IgG was detected by reacting it with alkaline phosphatase-conjugated rabbit anti-human IgG and then with *para*-nitrophenyl phosphate as the enzyme substrate. Calibrator sera of predetermined reactivity, and positive and negative control sera were included in the test. The tests were read on an MR 580 Microelisa Auto Reader kindly loaned by Dynatech Laboratories, Inc., Alexandria, Va. The EIA values (test absorbance - control absorbance) of the calibrator sera were plotted against the given Cytomegelisa units, and from the regression equation, the Cytomegelisa value of the test serum was calculated. A Cytomegelisa value of ≥ 0.25 was considered positive. The coefficient of determination for the calibrator sera regression was ≥ 0.95 in all tests.

IHA. The Cetus IHA kit, which uses human group O erythrocytes sensitized with CMV antigen, was used. This was kindly supplied by Cetus Corporation, Berkeley, Calif. A serum titer of $\geq 1:4$ was considered positive.

In all of the commercial tests, the base-line positive titers used were those recommended by the manufacturers. All tests used an antigen or antigens prepared from the AD169 strain of CMV.

Analysis of results. Accuracy, sensitivity, specificity, false-positive rate, and false-negative rate were

TABLE 2. Results of evaluating 24 CMV antibody-positive sera and 89 negative sera by five serological tests

Serum category	Test	No. of sera:		% Accuracy	% Sensitivity	% Specificity	False positive rate (%)	False negative rate (%)
		Positive	Negative					
Positive	CF	18	6	94	75	99	1	25
Negative		1	88					
Positive	IFA	23	1	74	96	69	32	4
Negative		28	61					
Positive	SFI	23	1	89	96	87	12	4
Negative		12	77					
Positive	IHA	24	0	99	100	99	1	0
Negative		1	88					
Positive	EIA	21	3	97	88	100	0	13
Negative		0	89					

TABLE 3. Ranking of five CMV serological tests

Test	Test ranking (1 to 5)					Sum of ranks
	Accuracy	Sensitivity	Specificity	False positive rate	False negative rate	
IHA	1	1	2	2	1	7
EIA	2	4	1	1	4	12
SFI	4	2	4	4	2	16
CF	3	5	2	2	5	17
IFA	5	2	5	5	2	19

calculated by the methods recommended by Griner et al. (5).

RESULTS

Sera from 120 healthy blood donors were evaluated by all of the tests. Two sera were anticomplementary by CF, one showed nuclear antibody by IFA, and four agglutinated the control cells in the IHA test. These sera were excluded from the comparison so that 113 sera were used to compare the five tests being evaluated.

Criterion of positivity. Short of demonstrating seroconversion, no absolute determination of whether an individual had experienced CMV infection and, therefore, of seropositivity was available. For the purpose of this study, a serum was considered to be antibody positive if it gave a specific positive reaction in three or more of the tests under comparison. Table 1 shows the distribution of the sera by positive tests. Twenty-four sera were considered positive and 89 were considered negative.

Table 2 shows the results of the comparison. The greatest accuracy was achieved with the IHA test (99%), followed by EIA (97%). The poorest accuracy (74%) was found when the IFA test was used. The highest sensitivity was shown by the IHA test (100%), with IFA and SFI close at 96%. The sensitivity of the EIA test was low at 88%. EIA, IHA, and CF all showed high specificity (EIA, 100%; IHA and CF, 99%). IFA showed a high proportion of false-positive results (32%), and SFI showed a 12% false-positive rate. EIA gave no false-positive results,

whereas IHA and CF showed 1% each. CF gave a high false-negative rate of 25%, and EIA also showed a high false-negative rate at 13%. The only test to give no false-negative results was IHA; SFI and IFA each showed 4% false-negative results.

When the results in each evaluation category were ranked on a scale of 1 to 5, the results were as shown in Table 3. IHA ranked first in three out of five categories and second in the remaining two. Although EIA ranked second overall, it scored poorly in terms of sensitivity and false-negative rate. IFA gave the poorest overall results; CF and SFI were ranked close to each other and to IFA.

The five tests were also ranked in terms of practicality. The results are shown in Table 4. The figures in parentheses are the subjective rankings. The IHA test ranked first, closely followed by SFI and EIA. The IFA test ranked lower on account of the subjectivity and effort of reading. The CF test has a turnaround time of 18 h and is reliable only when carried out on an ongoing basis by a skilled and experienced technical staff.

Finally, four replicate IHA tests of a panel of eight sera, of which four were CMV antibody negative and four were CMV antibody positive, carried out on different days showed perfect reproducibility.

DISCUSSION

This study concentrated on commercially available tests since the screening of blood donors for CMV antibody status will frequently be

TABLE 4. Ranking of five CMV serological tests on the basis of practicalities

Test	Test ranking by category ^a				Sum of ranks
	Technical demands	Turnaround time (h)	Special equipment	Subjectivity of reading	
IHA	(1)	2-4 (1)	No (1)	Yes (3)	6
SFI	(1)	2 (1)	Yes (5)	No (1)	8
EIA	(3)	3 (1)	Yes (4)	No (1)	9
IFA	(3)	2 (1)	Yes (3)	Yes (5)	12
CF	(5)	24 (5)	Yes (2)	Yes (3)	15

^a Numbers in parentheses indicate ranking.

carried out in laboratories which do not have the virological resources or available expertise to construct and standardize their own tests from basic materials.

Therefore, the critical considerations were time, false-positive results, and false-negative results.

The screening of donors is a special application of diagnostic serology that identifies individuals whose CMV antibody titer is of such a level that they are very unlikely to transmit CMV to a recipient. Such a test is often required to produce a result within 1 working day when blood donors are urgently required after a bone marrow transplant, so that a turnaround time of ≤ 6 h is a necessary characteristic of the test. This factor rules out direct culture methods since these will not give a presumptive negative result in less than 2 weeks, and the results when looking for evidence of latent CMV infection are unlikely to be reliable (6). The CF test, using overnight fixation at 4°C, is also eliminated on the basis of a turnaround time of 24 h, as also is an EIA test using overnight binding at 4°C (2).

The costs of false results are an important consideration. The most costly errors inherent in a test methodology are false-negative results. A test giving a significant proportion of false-negative results will cause donors to be considered for blood or organ donation who are potential transmitters of CMV infection. In our study, two tests fell into this category, CF (false-negative rate, 25%) and the standard EIA (false-negative rate, 13%). We would emphasize, however, that we tested only one commercial EIA kit and used only one reader. Other EIA test kits may vary in this respect. Also, we did not compare these results with overnight EIA (2) results, since that was outside of this study. Of the remaining three tests, IHA was the only one which gave a 0% false-negative rate. False-positive results are less costly. In this case, a proportion of negative individuals will be excluded from the donor pool, but no hazard is posed to the recipient. It is preferable, however, to keep the proportion of false-positive results to a minimum to maximize the number of donors available for cross-matching. In our study, IFA gave an unacceptably high false-positive rate (32%), and SFI gave a false-positive rate of 12%. The IHA test showed only a 1% false-positive rate.

The test of choice then, from our results, is the IHA test. It gave no false-negative results, minimizing the possibility of CMV being transmitted to the recipient, and it gave only a 1% false-positive rate, maximizing the number of available donors; it gave the best ranking in terms of practicality and appeared to give reproducible results. The test does not require special

instrumentation, is technically simple to perform, and has a short turnaround time. It has, however, two disadvantages. The test is not easy to read after only 90 min of incubation; the patterns are unclear. It is preferable to read the test after 4 to 18 h; however, in checking several hundred sera in routine work, we have not found any discrepancy between the 2-h and 18-h readings. Also, nonspecific results were obtained with four sera (3%) due to nonspecific agglutination in the erythrocyte controls. Continued routine use has confirmed this level of nonspecific reactors. This means a loss of 3 to 4% of the CMV-negative donors from an available pool, which we consider acceptable.

The experiments reported above were undertaken to select the best commercially available test for the purpose of minimizing the transmission of CMV from latently infected blood donors to recipients. However, the added hazard of the viremic acute or convalescent CMV case has to be considered. In a convalescent case, antibody can be expected to be detectable by 8 to 12 days after onset of clinical disease by CF (determined from a review of 10 cases diagnosed by rising CF titer in our own laboratory), and these cases would be eliminated on the basis of detected antibody. We emphasize, however, that the performance of the IHA test used here has not yet been evaluated on sera from known early seroconversions; however, so far we have not been able to detect CMV IgM antibody in three sera by using the kit in parallel with IFA to test immunoglobulin fractions separated on sucrose density gradients (unpublished data). Cases with clinical signs and symptoms would, hopefully, be eliminated on clinical grounds. This leaves an indeterminate number of subclinical cases in the initial phase of a CMV infection and without detectable CMV antibodies who may pose a serious hazard to a recipient but cannot rapidly and economically be identified at the present time.

There is presently no direct and easy method of demonstrating latent CMV infection in organ donors, and it is known (1) that renal allograft recipients who are seronegative before transplantation and receive a kidney from a donor who is seronegative never shed CMV after transplantation, whereas recipients, whether seropositive or seronegative, who receive a kidney from a seropositive donor usually shed virus after transplant. The IHA test has the potential to be applied as a screening test to organ donors and recipients.

ACKNOWLEDGMENTS

We thank the technical and secretarial staff of the Regional Virology Laboratory for invaluable assistance with this work.

and Gail Rock and V. Blanchette of the Canadian Red Cross, Ottawa, for helpful suggestions.

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