Enzyme linked immunosorbent assay (ELISA) for cytomegalovirus antibody in donor plasma

S RAMSKILL, P SENIOR, P BARLOW, L A D TOVEY *Leeds Regional Transfusion Centre, Leeds*

SUMMARY An adaptation of an enzyme immunoassay technique was developed to screen donor plasma for high titres of antibodies to cytomegalovirus (CMV). The technique uses microtitre plates treated with glutaraldehyde and coated with CMV antigen and an anti-IgG alkaline phosphatase conjugate to detect the captured antibody. Using an anti-CMV standard with a 1/64 titre by complement fixation, 34 (6.8%) of 500 sera were shown to have an antibody titre that was acceptable to the Blood Products Laboratory in England for anti-CMV immunoglobulin production.

Human anti-cytomegalovirus (CMV) immunoglobulin is in demand for both prophylaxis and treatment of immunosuppressed patients, particularly those receiving bone marrow transplants. Although published results are somewhat contradictory,^{1,4} there is sufficient optimism to warrant further trials. Anti-CMV immunoglobulin is prepared from high titre anti-CMV plasma obtained from blood donors, and the Blood Products Laboratory at Elstree request plasma with a titre of 1 in 64 by complement fixation technique.

Material and methods

ENZYME IMMUNOASSAY

- 1 *Microtitre plates:* Sterilin M24A 'U' well type.
- 2 *Coating buffer:* 0.79 g of Na₂CO₃; 1.46 g of NaHCO₃ made up to 500 ml with distilled water (pH 9.8).
- 3 *Glutaraldehyde solution:* 0.002 ml of a stock of 25% aqueous solution of glutaraldehyde in 500 ml of coating buffer.
- 4 *Wash buffer:* phosphate buffered saline (pH 7.4) with 0.05% Tween 20 (BDH) added.
- 5 *Serum dilution buffer:* 400 ml of wash buffer solution with 100 ml of goat serum (Tissue Culture Services) added.
- 6 *Coating antigen:* CMV antigen (Northumbria Biologicals Ltd, Northumberland—Code 1130), reconstituted with distilled water and frozen in 0.1 ml aliquots. Each aliquot is diluted in 10 ml of coating buffer before use. This is sufficient for one microtitre plate.
- 7 *Conjugate:* goat anti-IgG plus alkaline phosphatase (Sigma Labs—Code A3150). 0.01 ml diluted

in 10 ml of serum dilution buffer is sufficient for one microtitre plate, and is prepared immediately before use.

8 *Substrate buffer:* 10% diethanolamine (pH 9.8), containing 0.01% (w/v) magnesium chloride and 0.02% (w/v) sodium azide.

9 *Substrate solution:* p-nitrophenyl phosphate tablets (Sigma Laboratories code 104-105). Two tablets dissolved in 10 ml of substrate buffer is sufficient for one microtitre plate. This solution is prepared just before use.

10 *Serum samples:* clotted samples were taken from donors who supplied up to 500 ml of plasma donated on a Haemonetics model 50 cell separator unit. The samples were stored overnight at 4°C and serum tested the following day.

11 *Stop solution for the enzyme reaction:* 3M NaOH.

12 *Plate reading equipment:* Multiskan MCC (Flow Laboratories).

COMPLEMENT FIXATION

- 1 CMV antigen (as above).
- 2 Rabbit haemolytic serum (Tissue Culture Services).
- 3 Guinea pig complement in 20% Richardson's preservative (Tissue Culture Services).
- 4 Sheep's red cells in Alsever's solution (Tissue Culture Services).

EIA FOR IgG ANTI-CMV

The glutaraldehyde solution was distributed into each well of a blank microtitre plate in 0.1 aliquots.⁶ The plate was covered and floated in a 56°C waterbath for two hours, after which it was washed by aspirating the fluid and then filling and emptying the wells with wash buffer four times in succession. (0.1 ml) CMV antigen diluted in coating buffer was pipetted into each well.

The plate was covered and incubated for 22 hours at 4°C. Excess antigen solution was removed from the wells and the wash sequence repeated, but this time a one minute soak after the first wash was included (the plate was left standing for one minute with buffer in the wells before aspiration took place).

Each serum to be tested was diluted in serum dilution buffer and 0.1 ml of this solution added to a coated well. The plate was covered and incubated in a humidified 45°C incubator for 90 minutes. After incubation the plate was washed four times (including the soak phase as previously described). The diluted conjugate (0.1 ml) was added to each well, and the plate covered and further incubated for 90 minutes in the same 45°C incubator. The plate was again washed four times with a soak phase as before. Substrate solution (0.1 ml) was added to each well and the plate left at 22°C for 30 minutes for colour to develop. The reaction could be fixed by adding 0.05 ml of 3M NaOH to the wells. The optical density of the contents of each well was measured at 405 nm in the plate-reading equipment. In each test plate a column of wells was set aside to introduce known CMV antibody positive and negative sera. The positive sera were initially obtained from the Blood Products Laboratory, Elstree, and had a defined titre of CMV antibody. Subsequently pools of donor sera with suitable titres of reactivity were used as standards, while unreactive sera were pooled for our negative controls.

The complement fixation test procedure was the microtitre technique described by Casey.⁷

Results

Five hundred sera from plasma donors were tested by this enzyme immunoassay method for CMV antibody. Fig 1 shows the distribution of optical densities achieved at 405 nm. A standard serum containing a titre of 1/64 of anti-CMV by complement fixation was

Fig 1 Distribution of optical densities obtained when testing 500 plasma donor samples for CMV antibody by EIA method.

used to determine the cut off point for optical density. The mean optical density value for this standard was 1.6. Thirty four (6.8%) of samples gave values above this cut off. These 34 samples were further tested and 28 (82%) showed complement fixation titres of 64 or over. Of the remaining six, only one gave a titre of less than 32.

Ninety one sera representing a wide range of optical densities were then titred by enzyme immunoassay and complement fixation techniques. An acceptable correlation of these two methods ($r = 0.926$) was seen, even though enzyme immunoassay is about 10 times more sensitive than complement fixation (fig 2).

Fig 2 Correlation between EIA titre and complement fixation titre of anti-CMV on 91 sera from plasma donors.

Discussion

Although Blood Products Laboratory require a specific titre of anti-CMV for immunoglobulin production based on complement fixation titre, we prefer the use of ELISA as a routine screening technique. ELISA has several advantages, in particular the stability of reagents, the simplicity of the test, and the semiquantitative nature of the end point using a single test.

Complement fixation, on the other hand, is less reliable as the many variables in the test are difficult to control, and the end point depends on subjective evaluation.

Latex agglutination has also been used by several

laboratories for the selection of CMV immune plasma, but this commercial test (CMV Scan, Becton Dickinson, Oxford) is costly and does not fit into the routine microplate configuration which is consistent with contemporary technology.

The Transfusion Centre at Leeds has been selecting suitable plasma since early 1983 using the "in-house" ELISA method, and during this time 1750 donations of plasma (about 890 litres) have been sent for fractionation.

As 6.8% of our plasma donors have suitable amounts of high titre anti-CMV this type of screening is very productive and has proved to be a worthwhile effort.

References

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Requests for reprints to: Mr S J Ramskill, Microbiology Laboratory, National Blood Transfusion Service, Regional Transfusion Centre, Bridle Path, Leeds LS15 7TW, England.