Technical methods

Cytomegalovirus (CMV) antibody screening in blood donors: modification of new latex agglutination test compared with two standard methods

A PUCKETT J E DAVIS From the Regional Blood Transfusion Centre, John Radcliffe Hospital, Headington, Oxford, England

Infection with cytomegalovirus (CMV) is common, and between 50 and 100% of adults may show evidence of infection.1 The transmission of the virus by blood transfusion2 and, therefore, the need to screen donations intended for at risk groups such as immunocompromised patients3 4 and neonates5 7 is now well established.

The techniques used for routine screening of blood donations have traditionally been complement fixation tests, immunofluorescence, and enzyme linked immunosorbent assays (ELISA). A commercial indirect haemagglutination assay (IHA) was recently introduced and was the subject of a comparative study by Hunt et al.8 We chose the IHA for routine antibody screening because of cost, convenience, and its good correlation with the traditional methods of complement fixation tests and immunofluorescence. The quality of the reagents for the IHA was not maintained, however, and thus immunofluorescence has remained the routine screening method at this centre.

Recently, a commercial latex agglutination test (LAT) kit was introduced and a local modification of this kit was devised, based on the miniaturisation described by Barbara et al.9

The purpose of this study was to compare the latex agglutination test (LAT) with two standard methods of CMV antibody detection, immunofluorescence, and complement fixation tests.

Materials and methods

All specimens were obtained from a random sample of healthy blood donors. The blood was allowed to clot overnight at 4°C. Serum samples were separated on the next working day and either tested the same day or frozen at −30°C for later assay. Sera for complement fixation tests were inactivated by heating at 56°C for 30 minutes.

Complement fixation tests were performed according to established methods,10 11 except that microtitre plates were used instead of World Health Organisation trays. For maximum sensitivity an initial serum dilution of 1/4 was used. The antigen preparation used was a CMV complement fixation test antigen supplied by either Flow Laboratories Ltd, Irvine, Scotland, or the Central Public Health Laboratory, Colindale, England. Guinea pig complements were supplied by Wellcome Diagnostics, Dartford, England, or Don Whitly Scientific Ltd, Shipley, England. Complement fixation tests were performed using the following CMV antigen and complement combinations: (1) PHLS CMV antigen + Wellcome Diagnostics complement. (2) PHLS CMV antigen + Don Whitly complement, and (3) Flow Laboratories CMV antigen + Wellcome Diagnostics complement.

Immunofluorescence tests were performed according to a standard method,12 13 using substrate slides of CMV infected (Westwood strain) fibroblasts provided by the Oxford Public Health Laboratory.

CMV Scan passive latex agglutination kits were supplied by Hynson, Westcott and Dunning, Maryland, USA (UK agents, Becton Dickinson, Oxford). A 500 test kit comprised five bottles of CMV antigen coated latex particles, five bottles of card dilution buffer, and negative, low reactive, and high reactive controls, cards, and stirrers.

Latex particles that have been sensitised with CMV viral antigens will agglutinate in the presence of CMV antibody. This is visible to the naked eye against the dark background of the card. Tests were rotated on a Macro-Vue card test rotator Model 54 supplied by the company.

The recommended test procedure is as follows: 25 µl test serum is placed in a well of the test card using a pipette and spread over the entire circle. 15 µl latex antigen is then dropped on to each well and the card is rotated for eight minutes at 100 rpm. The results are read macroscopically under a high intensity incandescent lamp. Barbara et al9 miniaturised this technique (a) to use 15 µl serum and 5 µl latex antigen, and (b) dilute the latex antigen 2:1 in the card dilution buffer.

In our study the LAT was modified to use 10 µl serum and 5 µl latex antigen, which is closer to the serum:latex antigen ratio in the recommended test procedure. When using such small volumes of reagents, it is particularly important to use the humidifier lid supplied with the rotator. Extra cards and stirrers are required for this modification, and are obtainable from the company. Tests were performed using CMV latex antigen both undiluted and diluted 2:1.
Table 1  Discrepant results among LAT, complement fixation tests, and immunofluorescence in 514 tests

<table>
<thead>
<tr>
<th>Result</th>
<th>LAT</th>
<th>IF</th>
<th>CFT</th>
<th>No of discrepancies</th>
<th>Percentage of total No of sera tested</th>
<th>Combined discrepancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrepant</td>
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<td>-</td>
<td>+</td>
<td>6</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>LAT results</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Discrepant</td>
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<td>-</td>
<td>+</td>
<td>8</td>
<td>1.6</td>
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<tr>
<td>IF results</td>
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<td>+</td>
<td>3</td>
<td>0.6</td>
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<tr>
<td>Discrepant</td>
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<td>-</td>
<td>+</td>
<td>8</td>
<td>1.6</td>
<td>1.8</td>
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<tr>
<td>CFT results</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1</td>
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</tr>
<tr>
<td>Totals</td>
<td>32</td>
<td></td>
<td></td>
<td>32</td>
<td></td>
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</tr>
</tbody>
</table>

CFT, complement fixation tests; IF, immunofluorescence.

Results

COMPARISON OF IMMUNOFLUORESCENCE, COMPLEMENT FIXATION TESTS, AND LAT (UNDILUTED ANTIGEN)

Five hundred and fourteen sera were tested by the three methods. Initially, the complement fixation test system included the PHLS CMV antigen and Wellcome complement combination, but the results showed an unexpectedly poor correlation by complement fixation test and immunofluorescence (9-4% discrepancies) in contrast to the good correlation (0-8% discrepancies) found by Hunt et al. If immunofluorescence is taken as the definitive test then 68% of these discrepancies are falsely negative and 32% falsely positive by complement fixation tests. To resolve this difference the complement fixation tests were repeated twice, once with a different complement (PHLS antigen and Don Whit-ley complement combination) which yielded 18% discrepancies, and once with a different antigen (Flow antigen and Wellcome complement combination) which yielded 3-9% discrepancies. This accords with the findings of Hunt et al.

Of 514 sera tested by LAT, immunofluorescence, and complement fixation tests (using the Flow CMV antigen and Wellcome complement combination), 482 (93.6%) gave similar results in all three tests. There were discrepancies in 32 (6.4%), and an analysis of these is shown in table 1.

DILUTION OF CMV LATEX ANTIGEN

Neat latex antigen was compared with antigen diluted 2:1 in card dilution buffer. Of 359 sera tested, 328 (91.4%) gave similar results, while 31 (8.6%) were different. Assuming that the results obtained with undiluted antigen are correct (as they agree with complement fixation tests and immunofluorescence results), there were 7.2% false negative and 1.4% false positive results using diluted antigen. Most of the differing results were obtained with antigen that had been diluted for more than two days so the discrepancies were analysed daily (table 2). With increasing dilution time the false negative results (as a percentage of total discrepancies) rose from 0% on day 1 to 32.5% on day 5, while the percentage of false positive results fell from 3.8% to 0%.

Discussion

Provision of blood free of CMV for neonates and immunocompromised patients is a continuing problem for the Blood Transfusion Service. The development of techniques for population screening has been hampered by long operator time, technical complexity, lack of sensitivity and specificity, and
cost. In this study a new latex agglutination test was compared with immunofluorescence and complement fixation using these criteria.

LAT is a simple technique requiring little specialised equipment, and it is rapid; 50 CMV antibody tests can easily be performed in 20 minutes. In contrast, both complement fixation tests and immunofluorescence require a good deal of time and technical expertise. Immunofluorescence takes about three hours and need a fluorescence microscope. Complement fixation tests need little specialised equipment, but are time consuming and require overnight incubation. Shorter incubation times have been used, but with noticeably inferior sensitivity. The quality of complement fixation test results also seem to depend on the source of reagents.

The combined discrepancy rates for the three tests (complement fixation tests, 1.8%; immunofluorescence 2.2%; LAT 2.4%) compare favourably with previously reported discrepancy rates for complement fixation tests (0.8–25.0%), immunofluorescence (0.8–36.0%), ELISA (0.0–13.0%), IHA (0.8–3.6%) and radioimmunoassay (0.6%). The number of false negative results recorded for LAT (1.2%), however, is higher than those recorded for complement fixation tests (0.2%) and immunofluorescence (0.6%), indicating a slightly reduced sensitivity.

Using the manufacturer’s recommended volumes, the cost of LAT in October 1986 was 79 pence per test. By reducing the volume to a third of that recommended, the cost was reduced to 26 pence per test. This compared with 12 pence per test for complement fixation tests and 3.4 pence per test for immunofluorescence (excluding costs of substrate slides and microscope). The cost of LAT could be further reduced to about 17 pence per test by diluting the antigen. We found, however, that this yielded false positive results when freshly diluted, and false negative results after it had been diluted a few days, and it was decided to use undiluted antigen in this centre.

LAT has clear advantages in terms of technical simplicity and speed, and we regard the slightly reduced sensitivity as being within acceptable limits. The cost per test appeared to be high but after considering operator and training times, capital costs, and the ability to test much larger batches we decided to use LAT at this centre for CMV testing.

We thank Dr CC Entwistle for his help.

References
3 Lang DJ, Ebert PA, Rodgers BM, Boggess HP, Ritse RS. Reduction of postperfusion cytomegalovirus infections following the use of leukocyte depleted blood. Transfusion 1977;17:391–95.