Letters to the Editor

Comparison of Cytomegalovirus Antigenemia Assay with Shell Vial-Indirect Immunofluorescence Assay for Rapid Detection of Cytomegalovirus in Blood

The recent article by Mazzulli et al. (4) further demonstrates the increasing interest of virologists and microbiologists in the cytomegalovirus antigenemia (CMV-Ag) assay. Mazzulli and coworkers report that the CMV-Ag assay is more sensitive than the shell vial-indirect immunofluorescence assay (SVA-IFA), perhaps implying that the direct antigen detection assay should be considered the method of choice for the rapid detection of CMV in peripheral blood. Most clinical virologists and microbiologists, however, would agree that the SVA-IFA remains the most commonly used methodology in the general diagnostic virology laboratory for 1- (or 2-) day detection of the infectious agent in blood. Presented data which support the CMV-Ag assay having improved sensitivity over the SVA-IFA must be carefully scrutinized, as such information will have a major impact on the test system used in diagnostic laboratories involved with samples from patients suspected of a treatable CMV infection.

The CMV-Ag assay and the SVA-IFA have recently been compared in our laboratory (3). Among 186 peripheral blood specimens collected from our immunocompromised patients, we found no significant difference between the detection rate of CMV in blood \(2 \times 10^6\) polymorphonuclear leukocytes [PMNLs]) by the CMV-Ag assay and that by the SVA-IFA. Perhaps we are misreading Mazzulli and coworkers’ methods, but several points in their execution of the SVA-IFA might need to be clarified. First, was parallel testing performed on blood specimens when the CMV-Ag assay was compared with the SVA-IFA? Specifically, the CMV-Ag assay was noted to have used PMNLs (6). Were PMNLs used solely in assessing the performance of the SVA-IFA, or were shell vials inoculated with leukocyte populations consisting of PMNLs plus monocytes and macrophages? Additionally, was the number of cells used in the preparation of slides for the CMV-Ag assay equal to that total number inoculated into shell vials? Lastly, Mazzulli et al. centrifuged their shell vials for 40 min at temperatures ranging from 20 to 30°C. In our laboratory, shell vials are routinely centrifuged for 60 min at a temperature of 36 ± 1°C (2, 3). We cannot help but question whether the centrifugation period at the relatively low temperatures of 20 to 30°C might have adversely affected virus penetration and resulted in suboptimal yields of the CMV immediate-early and early nuclear antigens. CMV penetration of host cell fibroblasts and the recovery of virus from centrifuged and noncentrifuged assay systems are acknowledged to occur optimally at temperatures slightly above the mid-30°C range (1, 5–7).

The study by Mazzulli et al. is an important contribution. Their investigation adds credence to the applicability of the CMV-Ag assay as a sensitive and simple to perform methodology for same-day detection of CMV in the blood. However, additional parallel studies comparing this assay with the SVA-IFA are needed before the medical community may accept the suggested superiority of the CMV-Ag assay, except where patients on CMV antiviral drugs are concerned.

REFERENCES


Author's Reply

We agree with Lipson et al. that the SVA-IFA remains the most commonly used method in the general diagnostic virology laboratory for the rapid detection of viral agents in blood. However, the sensitivity of the SVA-IFA for the detection of CMV in blood specimens remains suboptimal (6, 8). There is a need for assay systems with greater sensitivity. To date, all studies but one (7) have consistently shown that the CMV-Ag assay has a greater sensitivity than SVA-IFA for the detection of CMV in blood (2, 5, 9, 10). This is in agreement with our results.

To clarify the points raised by Lipson et al. in regards to the methodology used in our laboratory for the SVA-IFA, we make the following points. Two tubes of blood were collected from the patients and processed in parallel. One tube was used for the CMV-Ag assay, and one was used for the SVA-IFA and conventional tube culture. Shell vials were inoculated with mixed-leukocyte populations, which yield higher isolation rates of CMV than PMNLs alone (2, 4). Cell counts were not performed on the cell suspensions used for inoculating the shell vials, and thus direct comparison of the number of cells...
used for preparation of slides for the CMV-Ag assay (2 × 10^5 cells) and the number of cells used for inoculating the shell vials cannot be made. Although others have used similar inoculation techniques for the SVA-IFA, recently Buller et al. reported a greater yield in SVA-IFA by using a standard inoculum to avoid toxicity (1). We have since adopted this method. The lower centrifugation time and temperatures used in our laboratory are in keeping with those in other published reports, including reference texts which recommend centrifuging at room temperature (1, 3, 5, 11), and are those recommended by the manufacturer (Syva). Following centrifugation, shell vials were incubated at 36 ± 1°C. Therefore, it is unlikely that our methodology for processing the SVA-IFA would account for the difference in sensitivity between the two assays. Why Lipson et al. have found no difference between the detection rate of CMV in blood using the CMV-Ag assay and that using the SVA-IFA is not clear, but we look forward to reading their article. As more studies comparing these assays are reported, greater insight into their optimal utilization will be gained.

REFERENCES

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**Evaluation of Clearview Chlamydia**

Previous studies (1, 4, 9, 11–13) of the performance of Clearview Chlamydia with both low- and high-prevalence populations have proved the test to show excellent performance in clinical settings. These data are summarized in Table 1. In these independent, peer-reviewed evaluations, the sensitivity of the Clearview test ranged from 79.0 to 93.5% and the specificity ranged from 98.0 to 100%.

However, results of a study by Klyutmans et al. (5) involving 724 women attending a sexually transmitted diseases clinic who were tested for the presence of chlamydia with Clearview and whose results were compared with those by cell culture showed that Clearview had a sensitivity of 67.3% (35 of 52 samples) and a specificity of 99.7% (662 of 664 samples). The prevalence of infection in women was fairly low at 7.5% but was within the range in these other studies.

Compared with that of cell culture, in the same study the sensitivities of the Magic Lite Chlamydia test (Ciba Corning) and a PCR test were also lower than expected, at 48.1 and 77.8%, respectively, with specimens from women. As has happened with Clearview Chlamydia, other workers have reported much higher sensitivities for these tests (2, 3, 6, 7, 10).

Klyutmans et al. also report a confirmatory method for analysis of discordant results. By this technique, the sensitivities of the three tests in the evaluation were revised to 62.3% for Clearview, 50.9% for Magic Lite, and 79.2% for PCR. Note that no confirmatory tests on the Clearview swab could be carried out and no independent assessment of swab quality, e.g., immunofluorescence, was made. The possible effect of the order of the swabs taken on the Clearview results is not considered. In a previous study (9) six out of seven false-negative results by Clearview were from swabs taken last; and immunofluorescence slides were also negative. Different swabs

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**TABLE 1. Performance of Clearview Chlamydia versus that of cell culture in previously published studies**

<table>
<thead>
<tr>
<th>Study no. (reference)</th>
<th>No. of specimens with the following result by culture</th>
<th>Prevalence (%)</th>
<th>Clearview Chlamydia</th>
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<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Sensitivity (%)</td>
</tr>
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<td>1 (1)</td>
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<td>2 (13)</td>
<td>42</td>
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<tr>
<td>3 (9)</td>
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<td>6.2</td>
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<td>4 (12)</td>
<td>40</td>
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<td>6.2</td>
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<tr>
<td>5 (11)</td>
<td>43</td>
<td>922</td>
<td>4.5</td>
</tr>
<tr>
<td>6 (4)</td>
<td>47</td>
<td>459</td>
<td>9.3</td>
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</table>
Cytomegalovirus Antigenemia: Clinical Correlations in Transplant Recipients and in Persons with AIDS

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We evaluated a rapid immunoperoxidase technique for the detection of cytomegalovirus (CMV) antigenemia in peripheral blood neutrophils of 56 transplant recipients (117 specimens) and 36 persons with AIDS (59 specimens). Antigenemia was 92% sensitive and 98% specific for the detection of clinical CMV infection in transplant recipients and 100% sensitive and 86% specific in persons with AIDS. Overall, CMV antigenemia was a more rapid and sensitive method for the detection of clinical CMV infection than either shell vial culture or conventional tube culture of blood.

Infection with human cytomegalovirus (CMV) continues to be a major cause of morbidity and mortality in immunocompromised patients. Twenty to 60% of recipients of all types of allografts and 25 to 90% of patients with AIDS may develop active CMV infection (2, 15). With the introduction of effective anti-CMV therapies, early and rapid diagnosis is essential for proper management, because the benefit of therapy is substantially increased by early administration (7).

The detection of CMV in blood specimens (CMV viremia) has been shown to be the most specific indicator of significant CMV infection (10, 16). However, conventional tube cultures (CCs) of blood may not yield results for several weeks. Although shell vial culture (SV) techniques have shortened the detection time for CMV infection considerably (5, 6), they may be negative in 37% or more of blood specimens that are positive by CC (11).

Recently, a diagnostic technique was developed that allows for the rapid and quantitative detection of CMV antigenemia in peripheral blood polymorphonuclear leukocytes (PMNLs) (1, 3, 13, 18, 19, 21–23). This technique uses a pool of monoclonal antibodies directed against what was initially thought to be CMV immediate early antigen but is now recognized as being a lower-matrix phosphoprotein (pp65) (8, 12). Using these monoclonal antibodies, we evaluated an immunoperoxidase staining technique for the diagnosis of CMV antigenemia in transplant recipients and human immunodeficiency virus-infected patients, comparing the results with those from SV and CC of blood. The results were correlated with the presence or absence of clinical CMV disease.

Between December 1991 and December 1992, blood specimens from all organ transplant recipients with unexplained fever 3 weeks to 3 months posttransplantation and persons with AIDS with CD4+ lymphocyte counts of <100 cells per mm3 were examined for the presence of CMV antigenemia and viremia. Interpretation of the antigenemia assay slides was made independent of the results of SV, CC, and the clinical status of the patients. Transplant recipients were deemed to have clinical CMV infection if they had no other cause of their fever identified, had evidence of CMV by conventional culture or tissue biopsy, and responded to ganciclovir therapy. Persons with AIDS were deemed to have clinical CMV infection if they had signs or symptoms (e.g., retinitis) suggestive of CMV disease and conventional culture or tissue biopsy evidence of CMV. Among the transplant recipients, patients receiving ganciclovir therapy for ≥24 h at the time the blood specimen was drawn were excluded from the study.

Preparation and staining of PMNL cytoplasts were carried out in accordance with methods described by van der Bij et al. (22), with slight modification. Cytospin preparations were made with 100 μl of a suspension of 2 × 106 cells per ml (Shandon cytospin III; Shandon/Lipshaw, Pittsburgh, Pa.), and only monoclonal antibodies C10 and C11 (22) were used. Specimens known to contain CMV antigen-positive cells were used as positive controls. Negative control PMNL slides were prepared from healthy donors.

For SV and CC, 5 ml of EDTA-treated blood was allowed to sediment by gravity and the leukocyte-rich plasma was removed and centrifuged. The pellet was washed twice with minimal essential media (Bio Whittaker, Walkersville, Md.) and then was resuspended in 2 ml of minimal essential medium with 10% fetal bovine serum. CCs with human embryonic lung fibroblast (HEL 299) cells (American Type Culture Collection) were inoculated with 1 ml of the cell suspension. Three SVs, prepared weekly in-house, with MRC-5 cells (Viromed Laboratories, Minneapolis, Minn.) were inoculated with 0.3 ml of the cell suspension and centrifuged at 700 × g for 40 min at 20 to 30°C. CCs were maintained for 4 weeks (or longer if there was evidence of CMV-induced cytopathic effect). Coverslips from two SVs were fixed, stained (with fluorescein isothiocyanate-labelled CMV monoclonal antibody [Syva MicroTrak, Palo Alto, Calif.]), and examined at 16 to 20 h, and the third SV was examined at 48 h. Any specimen that was SV negative but showed the characteristic cytopathic effect in CC was confirmed with the fluorescein isothiocyanate-labelled monoclonal antibody.

One hundred seventy-six specimens from 56 transplant recipients (117 specimens) and 36 persons with AIDS (59

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specimens) were evaluated for antigenemia. For 49 specimens, SV was not performed, and for 18 of these specimens CC was not performed. For an additional 10 SV specimens, the inoculum was toxic for the fibroblast monolayer, making them uninterpretable. Of the transplant recipients, there were 19 liver, 19 kidney, 8 lung, and 10 heart allografts. Thirty-five percent of transplant recipients and 30% of the persons with AIDS were women.

Table 1 shows that in both transplant recipients and persons with AIDS, the antigenemia assay was highly sensitive (92 to 100%) and specific (86 to 98%) for the detection of clinical CMV infection. The sensitivities of SVs and CCs were very low (23 to 40% and 59 to 67%, respectively). CC was positive a mean of 24 days (median, 20 days; range, 7 to 57 days) after the specimen was drawn.

The sensitivities of the antigenemia assay and SV for the detection of CMV viremia by CC were 92 and 41%, respectively, for transplant recipients and 100 and 44%, respectively, for patients with AIDS. The specificities were 78 and 100%, respectively, for transplant recipients and 74 and 100%, respectively, for patients with AIDS (Table 2).

The mean number of antigen-positive cells per slide in the six specimens from patients without clinical CMV infection was 2.1 cells (range, 1 to 4; median, 2), compared with a mean of 76 antigen-positive cells in the 68 specimens from patients with clinical CMV infection (range, 1 to 600; median, 14) (P < 0.005, Mann-Whitney U test). The transplant recipient in whom the antigenemia assay was positive, but who had no evidence of clinical CMV infection, was CMV seropositive and had received a liver from a CMV-seropositive donor 30 days prior to the antigenemia test. Of the five patients with AIDS with a positive antigenemia test but no evidence of clinical CMV infection at the time of the test, two patients had a blood culture positive for CMV, two other patients had positive blood cultures on a subsequent specimen, and the final patient developed CMV colitis within 2 months of the positive antigenemia result.

Of the four transplant recipients with negative antigenemia but evidence of clinical CMV infection, all had negative SVs, two had positive CCs (on days 10 and 57 of culture), and three had positive antigenemia detected on a blood specimen drawn within 10 days of the first negative assay. Three of these patients had fever and leukopenia, and the fourth had fever and elevated liver function tests. Forty-five specimens from 26 transplant recipients with 36 episodes of clinical CMV infection were antigenemia positive. Of these 26 patients, 15 (21 episodes) had fever and leukopenia (28 antigen-positive specimens, 14 positive CCs of blood for CMV, 8 positive urine and/or throat cultures for CMV, 2 biopsies of the gastric antrum positive for CMV), 7 (11 episodes) had fever and elevated liver function tests with or without leukopenia (13 antigen-positive specimens, 4 positive CCs of blood for CMV, 6 histologic and/or culture-positive blood biopsies for CMV, 6 positive urine and/or throat cultures), and 4 (4 episodes) had fever and pneumonitis (4 antigen-positive specimens, 4 positive CCs of blood for CMV, 2 bronchoalveolar lavage specimens positive for CMV).

Antigenemia was positive in all 23 specimens from 13 persons with AIDS who had clinical CMV infection. Of these 13 persons, 9 had CMV retinitis (15 antigen-positive specimens, 9 positive CCs of blood for CMV), 2 had CMV colitis (4 antigen-positive specimens, 1 positive CC of blood for CMV), 1 had CMV pneumonitis (1 antigen-positive specimen, 1 positive blood culture, and 1 positive bronchoalveolar lavage culture for CMV), and 1 had unexplained fever (3 antigen-positive specimens, 3 positive CCs of blood for CMV). In all cases of CMV retinitis, the diagnosis was made by an ophthalmologist. The diagnosis of CMV colitis was based on endoscopic findings and histologic examination of biopsy specimens.

In nine transplant recipients (11 episodes of clinical CMV infection) from whom a blood specimen was examined during or immediately after ganciclovir therapy, antigenemia levels fell rapidly and corresponded to clinical improvement. In two persons with AIDS and CMV retinitis, a rapid rise in antigenemia level while receiving ganciclovir therapy paralleled the development of progressive CMV disease.

The results of our study show that an antigenemia assay for the detection of CMV in PMNLs is a sensitive and rapid test for the diagnosis of CMV infection and disease. The antigenemia assay provided positive results in 27 specimens with negative SV and CC results. Clinical CMV infection was present in all but four of these specimen donors at the time. Others have also reported greater sensitivity of the

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### TABLE 1. Comparison of antigenemia assay, SV, and CC of blood for detection of clinical CMV infection

<table>
<thead>
<tr>
<th>Assay</th>
<th>Transplant recipients</th>
<th></th>
<th></th>
<th>Persons with AIDS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinical CMV infection (no. positive/no. tested)</td>
<td>No clinical CMV infection (no. positive/no. tested)</td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td>Clinical CMV infection (no. positive/no. tested)</td>
</tr>
<tr>
<td>Antigenemia</td>
<td>45/49</td>
<td>1/68</td>
<td>92</td>
<td>98</td>
<td>23/23</td>
</tr>
<tr>
<td>SV</td>
<td>9/40</td>
<td>0/57</td>
<td>23</td>
<td>100</td>
<td>4/10</td>
</tr>
<tr>
<td>CC</td>
<td>24/41</td>
<td>0/62</td>
<td>59</td>
<td>100</td>
<td>14/21</td>
</tr>
</tbody>
</table>

* Values are for specimens tested.

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### TABLE 2. Comparison of antigenemia assay and SV with CC for detection of positive CMV blood culture

<table>
<thead>
<tr>
<th>Assay result</th>
<th>No. of transplant recipients:</th>
<th>No. of persons with AIDS:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Antigenemia</td>
<td>+</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>SV</td>
<td>+</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>13</td>
</tr>
</tbody>
</table>

* The sensitivity and specificity of the antigenemia assay for the detection of CMV viremia were 92 and 78%, respectively, in transplant recipients and 100 and 74%, respectively, in persons with AIDS. The sensitivity and specificity of the SV for the detection of CMV viremia were 41 and 100%, respectively, in transplant recipients and 44 and 100%, respectively, in persons with AIDS.
antigenemia assay compared with both SV and CC techniques (4, 14). Only one study thus far has reported poor results with an antigenemia assay, detecting no CMV-positive transplant recipients (9). The poor results in this study may have been due to less frequent testing than in other studies, delays in processing specimens, differences in host immunocompetence, and the use of different monoclonal antibodies (9).

Patients in our study who were antigenemia positive and had evidence of clinical CMV infection had a mean number of antigen-positive cells per slide that was significantly higher than those patients who had no evidence of clinical CMV infection. Others have also reported that the presence or absence of CMV disease is closely related to the number of antigen-positive cells (18, 20). However, as in our study, some patients with clinical CMV infection may have low levels of antigenemia, suggesting that host factors have an important role in determining the clinical impact of a given level of antigenemia.

The quantitative nature of the antigenemia assay may give an estimate of viral load, and this may be useful for monitoring patients before, during, and after therapy (18, 19). Serial testing with the antigenemia assay of patients at risk for CMV infection and disease may allow the detection of important changes in the antigenemia level. Early positive or rising antigenemia levels may signal the onset of active CMV disease and allow early preemptive therapy to be initiated, particularly in transplant recipients (18, 19). In our study, transplant recipients with clinical CMV infection treated with ganciclovir showed a rapid decline in antigenemia levels which paralleled clinical improvement. A persistently high or rising level of antigenemia despite appropriate CMV therapy may signal progressive CMV disease or the development of viral resistance. Further study, however, is needed to determine the significance of persistently elevated or rising antigenemia levels during or immediately after therapy (4).

The CMV antigenemia assay is relatively simple to perform and may be completed in 5 to 6 h (22). Interpretation of slides, however, may be difficult if there is a great deal of background endogenous peroxidase staining (usually the result of eosinophilin in the preparation) or if the morphology of the PMNLs is distorted (17). Despite these potential problems, the CMV antigenemia assay appears to give accurate results, making it useful for the rapid detection of CMV disease in blood specimens from patients at risk. Because the antigenemia assay may occasionally miss some cases of clinical CMV infection, repeat testing should be considered for patients for whom there is strong clinical suspicion of active CMV infection. As was noted in our study, the antigenemia assay was positive on a repeat specimen drawn within 10 days of the initial negative result in three of four patients with clinical CMV infection. The lower level of sensitivity and lengthy time (an average of 24 days in our study) before CC of blood for CMV becomes positive makes it clinically less useful than the antigenemia assay in managing patients at risk for CMV disease.

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