Comparison of Culture and the Antigenemia Assay for Detection of Cytomegalovirus in Blood Specimens Submitted to a Reference Laboratory

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We compared the antigenemia assay (AA) with tandem shell vial cultures (SVCs) and tube cultures (TCs) for detection of cytomegalovirus (CMV) in 343 blood specimens. For 249 specimens, the AA was performed in duplicate with two different commercially available monoclonal antibody reagents (Biotest Diagnostic Corporation and Argene Biosoft). Specimens considered true positives were positive in either culture system or both AAs. Only specimens which were negative in both cultures and positive in a single AA were tested retrospectively with a CMV PCR assay. CMV recovery rates were also calculated to determine if increased specimen age resulted in decreased positivity. CMV recovery rates for the AA and the combination of both cultures were 20.0 and 5.0% at 3 to 18 h, 20.2 and 14.0% at 18 to 35 h, 12.5 and 7.8% at 36 to 52 h, and 18.8 and 6.3% at 64 to 75 h, respectively. The sensitivities and specificities of the Biotest AA, the Argene AA, SVC, and TC were 100.0, 100.0 and 99.6, 44.4 and 100.0, and 46.0 and 100.0%, respectively. The AA was significantly more sensitive than either culture method alone and was also more sensitive than the two culture methods used in tandem (the tandem culture sensitivity was 63.5%); the Argene AA identified more positives than the Biotest AA.

Monitoring cytomegalovirus (CMV) antigenemia in patients who are immunocompromised because of organ transplantation or AIDS has been reported to be a useful marker of disease progression and treatment efficacy (2, 3, 7, 9, 10, 17–21). Although the antigenemia assay (AA) is very sensitive for detection of CMV, delays of as little as 6 h in transporting and processing specimens have been reported to reduce antigen detection rates as much as 80% (1). Recent studies have indicated that antigenemia positivity can remain in stored specimens for up to 24 h or longer (7, 11); consequently, the effect of delayed specimen processing on the AA is controversial (8, 10, 17, 18, 21). It is generally accepted, however, that viral infectivity for cultures persists for 48 h during cold storage (6). Specimens submitted to a reference laboratory are often at least 24 h old when received because they are shipped from different parts of the country. Specimens are maintained and shipped at refrigerated temperatures (2 to 8°C) to preserve virus viability for culture (16). Thus, the ability of the reference laboratory to adequately perform the AA depends on the effects of cold storage on antigen-positive cells.

We tested 343 blood specimens received in our laboratory for CMV diagnosis from July 1995 through February 1996 with both the AA and culture. Because the shell vial culture (SVC) for CMV from blood has been reported to have low sensitivity (12), all specimens were cultured in SVCs and tube cultures (TCs). Our objectives were to determine (i) if an AA could replace both SVC and TC, (ii) which AA performed better, and (iii) the effects of delayed specimen processing on the AA.

MATERIALS AND METHODS

Specimens. Specimens were collected from transplant recipients, AIDS patients, and other individuals. The specific patient clinical history was not provided in most cases. Blood (5 to 10 ml) was collected in tubes containing sodium heparinate or EDTA as an anticoagulant. Immediately after collection, specimens were refrigerated (2 to 8°C) and shipped on a cold pack. The time between collection and processing of specimens ranged from 3 to 75 h. Of the 343 specimens, 20 (5.8%) were 3 to 18 h old, 243 (71.1%) were 18 to 35 h old, 64 (18.7%) were 36 to 52 h old, and 16 (4.4%) were 64 to 75 h old upon receipt. Leukocytes were separated by dextran sedimentation (4:1 ratio of blood and 5% dextran). The dextran solution was prepared in phosphate-buffered saline (PBS) at pH 7.4. Leukocytes were pelleted by centrifugation at 300 × g for 10 min. Pellets showing visible erythrocyte contamination were lysed with 0.8% ammonium chloride. Leukocytes were then washed once in PBS and suspended in Eagle’s minimum essential medium with 10% fetal bovine serum. The leukocyte suspension was divided into equal volumes for TC, SVC, antigenemia testing, and freezer storage (−85°C) for later testing by PCR if required. Of the 343 specimens examined with the various assays, only 249 of the 343 specimens were tested with the Biotest AA.

Cultures. SVC was performed with MRC-5 cells as previously described (4, 12) with the following modifications. Vials were incubated for a minimum of 26 h and stained by an indirect immunofluorescence assay (IFA) with a mouse monoclonal antibody (MAb) specific for the immediate-early antigen of CMV (Chemicon International, Inc., Temecula, Calif.) and fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse polyclonal antibodies (Sigma Chemical Company, St. Louis, Mo.). TC was performed with duplicate human fetal tonsil cells by standard methodology with one blind passage (6). Both cell lines were found to have comparable sensitivities for the isolation of CMV from leukocytes in previous studies performed in our laboratory (data not shown). Cells (AD 169 ATCC VR-538) was used as the positive control in both culture systems. Tubes and shell vial monolayers inoculated and fed with cell maintenance medium were stained by an indirect immunofluorescence assay (IFA) with mouse monoclonal antibody (MAb) specific for the immediate-early antigen of CMV (Chemicon International, Inc., Temecula, Calif.) and fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse polyclonal antibodies (Sigma Chemical Company, St. Louis, Mo.).

Antigenemia. For the AA, leukocytes suspended as described above were pelleted again, washed once in PBS, and suspended in PBS. Cells were counted by hand with a hemacytometer, and the cell concentration was adjusted to 2.0 × 106 cells/ml. Slides were prepared by cytocentrifugation (2 × 105 cells/slide) with a Cytospin-2 centrifuge (Shandon Scientific, Runcorn, England). Slides were stained by an indirect immunofluorescence assay (IFA) with a mouse monoclonal antibody (MAb) specific for the immediate-early antigen of CMV (Chemicon International, Inc., Temecula, Calif.) and fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse polyclonal antibodies (Sigma Chemical Company, St. Louis, Mo.).
then fixed with 5% formaldehyde in PBS, washed first for 5 to 10 s in PBS and then for 5 to 10 s in deionized water, and immersed in 0.5% Nonidet P-40 detergent in PBS for 5 to 10 min. Slides were washed again in PBS and water as described above and were either stained immediately or air dried and stored at −20°C until staining. All slides were stained by IFA. The two MAb reagents evaluated were Enolab Biotest CMV C-10/C-11 (pp65; Dreieich, Germany) and Argene Biosoft CMV IC3 (p65; Varilhes, France) (5, 13). FITC-conjugated sheep anti-mouse polyclonal antibodies (Sigma) were used with the Biotest MAb, and FITC-conjugated goat anti-mouse polyclonal antibodies (Argene) were used with the Argene MAb. The MAb dilutions were in PBS, and the conjugates were diluted in PBS with 0.006% Evan’s blue at the manufacturer’s recommended dilution, except for the sheep anti-mouse antibodies, for which titration determined the optimal dilution to be 1:20. Positive and negative controls were included with each stain run. Positive controls were prepared from uninfected fibroblasts infected with CMV AD169. Negative controls were prepared from uninfected fibroblasts. Slides were incubated with primary MAbs and conjugates for 30 min each and washed for 5 to 10 s with PBS and deionized water between stain steps and after stain completion. Slides were air dried and mounted with buffered glycerol at pH 8.5 ± 0.3. Slides were read, without knowledge of culture results, with a Zeiss Axioscope fluorescence microscope at a ×400 magnification. Specimens having the optimal number of cells (2 × 10⁵ cells/slide) and staining red because of the Evan’s blue counterstain were considered negative. Specimens containing one or more leukocytes with homogenized granular nuclear staining were considered positive. The morphology of antigen-positive cells was confirmed at ×1,000.

PCR. Discrepant specimens which were negative in both cultures but positive in a single AA were resolved with a CMV PCR assay. Specimens positive by both antigen-positive cells was confirmed at 1,000.

### RESULTS

Of the 343 specimens, a total of 64 (18.7%) specimens were positive in one or more test systems. For determination of performance characteristics (Table 1), specimens which were positive in either culture or both AAs were counted as true positives. Nine specimens which were positive only in the Argene AA were tested retrospectively by PCR for result resolution. Eight of the nine specimens were confirmed as true-positive specimens. One Argene AA-positive specimen was PCR negative and was therefore counted as a false positive. Specimens negative in all test systems were counted as true negatives. The positive Argene AA specimen which was PCR negative was counted as a true negative by both culture methods and the Biotest AA. Specimens which were negative by one method but positive by two or more methods were considered false negatives. The eight samples which were confirmed by PCR as true positives for the Argene AA, were considered to be false negatives for both culture methods and the Biotest AA. The sensitivity and specificity of the Biotest AA, Argene AA, SVC, TC, and combined cultures were 84.4 and 100.0, 100.00 and 96.6, 44.4 and 100.0, 46.0 and 100.0, and 63.5 and 100.0%, respectively.

Overall CMV recovery rates were calculated according to specimen age to determine if positive rates decreased with increasing storage time. Specimens were divided into four main groups: those 3 to 18, 18 to 35, 36 to 52, and 64 to 75 h old upon receipt (all positives were counted here). The maximum numbers of specimens tested in each group were 20 at 3 to 18 h, 243 at 18 to 35 h, 64 at 36 to 52 h, and 16 at 64 to 75 h. The combined recovery rates were determined for both AAs and both cultures, when either or both of the tests were positive for CMV. The combined recovery rates for both AAs and both cultures were 20.0 and 5.0% at 3 to 18 h, 20.2 and 14.0% at 18 to 35 h, 12.5 and 7.8% at 36 to 52 h, and 18.8 and 6.3% at 64 to 75 h, respectively. The results for each of the groups are outlined in Table 2.
DISCUSSION

The AA was more sensitive than either culture method alone and was also more sensitive than both culture methods in tandem. The sensitivity of the Argene AA compared to tandem cultures remained at 100%, no matter how old the specimen. Therefore, the AA is clearly superior to culture for diagnosis of CMV in blood specimens submitted to a reference laboratory, despite the fact that specimens are almost always older than 6 h when received (only one specimen was less than 6 h old when received). Culture sensitivity could potentially have been enhanced by standardizing the number of cells used in both culture assays. Also, specimens positive by AA may have been negative by culture because of a sampling error (49% of the positive antigenemia specimens had less than 10 antigen-positive cells).

The overall positive rate for the AA did not show any clear trend according to specimen age. The rate appeared to drop from 18- to 35-h-old to 36- to 52-h-old specimens (from 20.2% to 12.5%, respectively) and to rise again for 64- to 75-h-old specimens (to 18.8%). However, there were not enough specimens tested in the 36- to 52- and 64- to 75-h-old groups (n = 64 and 16, respectively) to draw any firm conclusions about whether specimens older than 36 h should be tested by AA. Since the same group of specimens was not tested repeatedly over 75 h, the possibility of increasing false-negative AA results due to specimen age cannot be ruled out. A few specimens which were 96 h old were examined (data not shown); almost none had sufficient cells for analysis.

The qualitative detection of CMV by the AA does not appear to be adversely affected by the delay in processing due to specimen shipment. In the present study, no experiments regarding the quantitation of antigen-positive cells were performed. Quantitation of the number of positive cells from an antigenemia specimen can be useful in differentiating asymptomatic infection from disease or progression to disease based on changes in the antigenemia level over time. We do not know what effects processing delays have on quantitative antigenemia levels. Testing the samples repeatedly over a number of days would be a better indicator of whether significant decreases in quantitative antigenemia levels occur as blood ages.

The positive rate for cultures dropped significantly for specimens older than 35 h (from 14.0% at 18 to 35 h to 7.8% at 36 to 52 h and 6.3% at 64 to 75 h). Therefore, it would seem that specimens may actually be more stable for the AA than for culture when refrigerated over time. More testing is needed to determine if this is indeed true, since specimen numbers in the 3- to 18-, 36- to 52-, and 64- to 75-h groups were low. Periodic testing of a single group of specimens over time would provide additional information about virus stability for culture.

The Argene Biosoft reagent identified more positives than the Biotest diagnostic reagent (sensitivities of 100.0 and 84.4%, respectively). Not only was there less background staining observed with the Argene reagent than with the Biotest reagent, but also positive cells stained brighter with the Argene reagent than with the Biotest reagent. However, both reagents outperformed culture dramatically. The tandem culture sensitivity was 63.5%, compared to a “gold standard” of positivity in either or both cultures, both AAs, or one AA and PCR. The sensitivities of single-culture methods used alone were even lower at 44.4% and 46.0% for SVCs and TCs, respectively.

The AA offered enhanced test performance and a significantly quicker turnaround time over culture for recovery of CMV from blood specimens submitted to a reference laboratory. Processing delays necessitated by specimen shipment did not adversely affect test performance compared to that of culture.

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REFERENCES