Journal of Clinical Microbiology, May 1996, p. 1337–1339 Vol. 34, No. 5
0095-1137/96/$04.00+0
Copyright © 1996, American Society for Microbiology

# Evaluation of CMV Brite Kit for Detection of Cytomegalovirus pp65 Antigenemia in Peripheral Blood Leukocytes by Immunofluorescence

MARIE L. LANDRY,[1]* DAVID FERGUSON,[1] TERRY STEVENS-AYERS,[2] MARTINUS W. A. de JONGE,[3] and MICHAEL BOECKH[2]

*Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut[1]; Division of Infectious Diseases, Fred Hutchinson Cancer Research Center, Seattle, Washington[2]; and MCA Development, Groningen, The Netherlands[3]*

Received 10 October 1995/Returned for modification 7 December 1995/Accepted 8 February 1996

**The CMV Brite antigenemia kit was compared with culture and an established cytomegalovirus pp65 antigenemia assay (CMV AG). Of 300 clinical specimens tested, 92 were positive by CMV Brite, 83 were positive by CMV AG, and 34 were positive by culture. Discrepancies could be attributed to anticytomegalovirus therapy or low-level antigenemia.**

The cytomegalovirus (CMV) antigenemia assay (4, 9, 10) uses methods familiar to diagnostic-laboratory personnel, yet the time required for preparation and standardization of reagents and the difficulties in obtaining appropriate control slides have hindered widespread use of this technique. In this study, we have compared the new CMV Brite immunofluorescence antigenemia kit (Biotest Diagnostics, Denville, N.J., and Immuno Quality Products, Groningen, The Netherlands) with conventional and shell vial cultures (1, 6) and a reference CMV antigenemia assay (CMV AG).

Five to 10 ml of blood in heparin or EDTA tubes was collected from patients with suspected CMV infection at the Fred Hutchinson Cancer Research Center Seattle, Wash., and at Yale New Haven Hospital, New Haven, Conn. A total of 300 blood samples were analyzed: 159 (53%) from marrow transplant patients, 85 (28%) from human immunodeficiency virus-positive patients, 47 (16%) from solid-organ transplant patients, and 9 (3%) from immunocompetent patients.

The CMV Brite assay was performed according to the manufacturer's instructions. Following dextran separation and erythrocyte lysis, leukocytes were counted in a hemocytometer, centrifuged onto two to three slides per specimen (150,000 cells per slide), fixed, permeabilized, and stained with anti-CMV pp65 (C10/C11) monoclonal antibodies; this was followed by staining with fluorescein isothiocyanate-labeled rabbit anti-mouse immunoglobulin G conjugate and Evans blue. Control slides, consisting of one well of CMV pp65-transfected insect cells mixed with CMV-negative human leukocytes and one well of CMV-negative human leukocytes, were also stained. The CMV AG assay (4, 9) used C10/C11 antibodies (Clonab CMV; Biotest Diagnostics) and a similar procedure as previously described (2, 7). CMV Brite and CMV AG slides were examined by different readers and were read by additional readers if discrepancies were noted. A positive result consisted of one or more CMV-antigen-positive cells per set of duplicate slides.

The results are shown in Table 1. Of the 68 samples positive by CMV Brite and/or CMV AG but negative by culture, 53 (78%) were obtained from patients receiving anti-CMV therapy. The remaining 15 culture-negative samples had low numbers of positive cells. Discrepancies between CMV Brite and CMV AG results occurred only in specimens from patients with low-grade antigenemia (median, one positive cell per two slides). Of the 16 samples positive by CMV Brite only, 14 were identified as true positives by other positive CMV cultures or antigenemia tests or by the presence of CMV disease. However, two results could not be confirmed: one from a marrow transplant recipient and one from a CMV-seropositive kidney transplant recipient. Of the seven samples positive only by CMV AG, six were confirmed as true positives; the remaining patient, a CMV-seronegative host with Bell's palsy, was lost to follow-up. Thus, 99 of 300 samples were from confirmed active CMV infections, and CMV Brite detected 90 of the 99. CMV Brite had a sensitivity, specificity, positive predictive value, and negative predictive value of 90.9, 99, 97.8, and 95.2%, respectively. Culture methods detected only 34 positive samples.

CMV Brite and CMV AG results were also compared by patient risk group (Table 2). For all groups, the mean and median numbers of antigenemia-positive cells were higher in culture-positive samples than in culture-negative samples. Overall, there was no significant difference between the results of CMV Brite and CMV AG, and the two methods showed a high degree of correlation.

In this study we have shown that the CMV Brite immuno-

TABLE 1. Correlation of CMV Brite test kit with culture methods and a reference CMV antigenemia assay

| Culture result[a] | CMV AG result[b] | No. of samples | | |
|---|---|---|---|---|
| | | With CMV Brite result | | Total |
| | | Positive | Negative | |
| Positive | Positive | 31 | 0 | 31 |
| Positive | Negative | 0 | 3 | 3 |
| Negative | Positive | 45 | 7[c] | 52 |
| Negative | Negative | 16[d] | 198 | 214 |
| Total | | 92 | 208 | 300 |

[a] Conventional culture and/or shell vial centrifugation culture results.
[b] CMV antigenemia reference method results.
[c] Active CMV infection could not be confirmed in one patient.
[d] Active CMV infection could not be confirmed in two patients.

* Corresponding author. Mailing address: Department of Laboratory Medicine, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510. Phone: (203) 785-3475. Fax: (203) 785-7340.

TABLE 2. Comparison of CMV Brite kit and CMV antigenemia reference method for the detection of quantitative CMV antigenemia in different patient groups

| Patient group | Result of CMV culture | No. of specimens[a] | No. of CMV antigenemia-positive leukocytes[b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | By CMV Brite | | | By CMV AG | | |
| | | | Mean | Median | Range | Mean | Median | Range |
| Bone marrow transplant[c] | Positive | 3 | 72 | 41 | 18–157 | 39 | 29 | 25–63 |
| | Negative | 51 | 20 | 3 | 0–192 | 17 | 3 | 0–204 |
| Solid-organ transplant[d] | Positive | 7 | 30 | 19 | 3–81 | 70 | 12 | 3–187 |
| | Negative | 4 | 2 | 2 | 1–3 | 1 | 1 | 0–2 |
| HIV positive[d] | Positive | 21 | 418 | 13 | 1–>3,000 | 458 | 8 | 1–>3,000 |
| | Negative | 12 | 27 | 3 | 1–130 | 29 | 1 | 0–130 |
| Normal host[d] | Negative | 1 | 0 | 0 | | 2 | 2 | |
| All patients | Positive | 31 | 297 | 28 | 0–>3,000 | 329 | 18 | 0–>3,000 |
| | Negative | 68 | 20 | 3 | 0–192 | 18 | 2 | 0–204 |

[a] Three samples positive by culture only were not included.
[b] Sum of positive cells per two slides examined per sample; differences between CMV Brite and CMV AG were not significant ($P < 0.40$, paired $t$ test; $r = 0.97$).
[c] Samples were tested at Fred Hutchinson Cancer Research Center.
[d] Samples were tested at Yale. HIV, human immunodeficiency virus.

fluorescence antigenemia kit is a highly sensitive, specific, and predictive test for detection of active CMV infection in blood specimens. The kit showed a high degree of correlation ($r = 0.97$, $P < 0.0001$) with a reference antigenemia assay (2, 4, 7) and was significantly more sensitive than nonquantitative shell vial centrifugation and conventional tube cultures.

CMV Brite uses the optimal methodology for detection of CMV antigenemia, including a formalin-based fixative, providing maximal sensitivity and excellent readability (2, 4, 9); cytospin-prepared slides, providing an equal distribution of cells on the slide; and a CMV antibody pool (C10/C11) that has been evaluated in a large number of clinical studies in different patient populations (1, 2, 6–10). We compared CMV Brite with a similar, previously published version of the CMV AG assay. Thus, comparable results were anticipated. Although CMV Brite was slightly more sensitive than the reference CMV AG assay in both the number of positive samples and the median number of positive cells detected per sample, the differences were not statistically significant. Discrepancies occurred only in specimens from patients with low-grade antigenemia (median, one positive cell per set of duplicate slides) and thus may reflect sampling variation rather than a true difference in sensitivity.

Active CMV infection could not be confirmed for two samples positive by CMV Brite. However, a transient low-grade antigenemia that resolves spontaneously often occurs in the face of immunosuppression (3–5). Thus, these samples could well be true positives.

As anticipated, CMV antigenemia was often detected in the absence of positive cultures when patients were receiving anti-CMV therapy or when antigenemia levels were very low. The low percentage (6%) of culture-positive CMV antigenemia samples from marrow transplant recipients in contrast to that in solid-organ transplant and human immunodeficiency virus-infected patients (64%) resulted from early antiviral treatment of antigenemia-positive marrow transplant patients. The highest antigenemia levels were seen in some AIDS patients, in whom high levels of CMV antigenemia were tolerated for a longer period before end-organ disease became apparent and treatment was initiated. Three samples positive by culture only were from patients with low-level subclinical viremia as previously described (1, 6, 8–10).

One advantage of the CMV Brite kit is the provision of control slides consisting of one well of fixed pp65-transfected insect cells mixed with CMV antigen-negative human leukocytes and one well of CMV antigen-negative human leukocytes. For our CMV AG reference procedure, positive control slides are prepared from CMV antigenemia-positive patient blood samples, which may not be readily available in many laboratories. In the CMV Brite kit, control slides are separate and any number of samples can be processed in parallel with a single set of controls. An additional advantage of the kit is the provision of the formaldehyde fixative, as well as all necessary reagents. However, the laboratory must have access to both an immunofluorescence microscope and a cytospin centrifuge. While the cytospin centrifuge and funnels are expensive, cytospin-prepared slides provide a more uniform distribution of cells and thus greater readability.

In conclusion, the CMV Brite kit is a sensitive, specific, and predictive test for detection of CMV infection in blood specimens. Results obtained with CMV Brite correlated highly with results of a CMV AG reference method. Thus, the kit was not only more sensitive than CMV blood cultures but also equivalent to published antigenemia assays. Multicenter treatment studies of CMV infection would greatly benefit from the availability of a standardized, rapid technique for quantifying viral load that is within the capabilities of a routine clinical laboratory. CMV Brite provides such a methodology and should allow implementation of antigenemia assays in both clinical laboratories and research settings.

We thank Maike Ankermann and the Clinical Virology Laboratory staffs at Yale New Haven Hospital and the Fred Hutchinson Cancer Research Center for technical assistance. We are indebted to Emmanuel Lerner for help with statistical analysis.

This study was supported in part by Biotest Diagnostics, Denville, N.J. Michael Boeckh was supported in part by the National Institutes of Health (CA 18029).

## REFERENCES

1. **Boeckh, M., R. A. Bowden, J. M. Goodrich, M. Pettinger, and J. D. Meyers.** 1992. Cytomegalovirus antigen detection in peripheral blood leukocytes after allogeneic marrow transplantation. Blood **80:**1358–1364.
2. **Boeckh, M., P. M. Woogerd, T. Stevens-Ayers, C. G. Ray, and R. A. Bowden.** 1994. Factors influencing detection of quantitative cytomegalovirus antigenemia. J. Clin. Microbiol. **32:**832–834.
3. **Gerna, G., P. Maurizio, E. Percivalle, D. Zipeto, E. Silini, G. Barbarini, and G. Milanesi.** 1990. Human cytomegalovirus viraemia in HIV-1-seropositive patients at various clinical stages of infection. AIDS **4:**1027–1031.
4. **Gerna, G., M. G. Revello, E. Percivalle, and F. Morini.** 1992. Comparison of different immunostaining techniques and monoclonal antibodies to the lower matrix phosphoprotein (pp65) for optimal quantitation of human cytomegalovirus antigenemia. J. Clin. Microbiol. **30:**1232–1237.
5. **Gerna, G., D. Zipeto, M. Parea, M. G. Revello, E. Silini, E. Percivalle, M. Zavattoni, P. Grossi, and G. Milanesi.** 1991. Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant recipients by determination of viremia, antigenemia, and DNAemia. J. Infect. Dis. **164:**488–498.
6. **Landry, M. L., and D. Ferguson.** 1993. Comparison of quantitative cytomegalovirus antigenemia assay with culture methods and correlation with clinical disease. J. Clin. Microbiol. **31:**2851–2856.
7. **Landry, M. L., D. Ferguson, S. Cohen, K. Huber, and P. Wetherill.** 1995. Effect of delayed specimen processing on cytomegalovirus antigenemia test results. J. Clin. Microbiol. **33:**257–259.
8. **The, T. H., M. van der Ploeg, A. P. van der Berg, A. M. Vlieger, M. van der Giessen, and W. J. van Son.** 1992. Direct detection of cytomegalovirus in peripheral blood leukocytes—a review of the antigenemia assay and polymerase chain reaction. Transplantation **54:**193–198.
9. **van der Bij, W., J. Schirm, R. Torensma, W. J. van Son, A. M. Tegzess, and T. H. The.** 1988. Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. J. Clin. Microbiol. **26:**2531–2535.
10. **van der Bij, W., R. Torensma, W. J. van Son, J. Anema, J. Schirm, A. M. Tegzess, and T. H. The.** 1988. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leukocytes. J. Med. Virol. **25:**179–188.