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2-Hour Cytomegalovirus pp65 Antigenemia Assay for Rapid Quantitation of Cytomegalovirus in Blood Samples

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Of 109 blood samples tested for cytomegalovirus (CMV) antigenemia, 18 (16.5%) were positive. CMV Brite detected 13 and CMV Brite Turbo detected 16 of the 18 positives. There was no significant difference in the number of positive cells detected per sample. The seven discrepant samples contained a median of only one positive cell.

Cytomegalovirus (CMV) is a serious pathogen in immunocompromised hosts, and rapid diagnosis allows prompt institution of antiviral therapy. Thus, the introduction of the CMV antigenemia assay, which allows direct detection and quantitation of CMV-infected peripheral blood leukocytes (PBLs), has been of great benefit to patient management (6). Standard test methodology requires approximately 4 h to complete and utilizes dextran sedimentation to separate PBLs, followed by immunofluorescence staining of PBLs with antibodies directed against CMV lower matrix protein pp65 (2).

A new commercial CMV antigenemia kit (CMV Brite Turbo) has recently been developed that can be completed within 2 h. This rapid method utilizes direct erythrocyte lysis to separate PBLs from whole blood and shorter incubation times.

In this study, the new CMV Brite Turbo kit was compared with the standard CMV Brite antigenemia test for detection and quantitation of CMV in clinical samples.

One hundred nine blood samples in EDTA submitted to the Yale New Haven Hospital Clinical Virology Laboratory for CMV antigenemia testing were aliquoted and processed within 6 h of collection by using both the standard CMV Brite kit and the CMV Turbo kit (both from Biotest Diagnostics, Denville, N.J.) in accordance with the manufacturer's instructions.

For the standard assay, PBLs were separated by dextran sedimentation and 150,000 cells were applied to each of two slides by cytocentrifugation (Cytospin, Shandon, Inc., Pittsburgh, Pa.). Cells were fixed in paraformaldehyde, permeabilized with Nonidet P-40 (NP-40), stained with CMV pp65 antibodies (C10 and C11), and examined under an epifluorescence microscope. For the rapid CMV Brite Turbo assay, PBLs were separated by whole-blood lysis. Two slides with 200,000 cells each were prepared by using fixation and staining times shorter than those recommended for the standard kit (Table 1). Stained slides were examined independently by two readers, and CMV-positive neutrophils were enumerated.

Positive results obtained with both kits were considered true positives. Discrepant samples were defined as samples with CMV pp65-positive cells detected by one kit but not by the other kit.

Statistical analysis was performed by using the Wilcoxon signed rank test to compare quantitative differences between paired samples and the McNemar test to compare qualitative differences.

Of 109 samples tested, 18 (16.5%) were positive by either or both of the CMV antigenemia tests. CMV Brite detected 13 and CMV Turbo detected 16 of the 18 positives ($P = 0.257$ by McNemar's test). The range of positive cells detected was 1 to >2,000 for both tests, and numbers of positive cells detected in each sample were not significantly different between the two kits ($P = 0.25$ by the Wilcoxon signed rank test). The mean numbers of cells positive by the standard and Turbo kits were 132 and 155, respectively, and the medians were 3 and 2. However, higher numbers of positive cells were detected in 11 (61%) of the 18 positive samples by the Turbo kit and in only 5 (28%) of the 18 by the standard CMV Brite kit. The seven discrepant samples contained a median of one positive cell per two slides examined.

The quality of the stained slides was comparable between the two methods, with low nonspecific background staining and excellent leukocyte morphology, and the technical hands-on time for the Turbo kit was shorter.

CMV antigenemia has become an essential test in the management of immunocompromised hosts. The ability to rapidly quantitate CMV-infected leukocytes, rather than report merely a positive or negative result, is a critical factor in determining the need to institute therapy. With the standard assay, our laboratory separates leukocytes and prepares and fixes slides on the day of sample receipt but stains the slides on the following morning (4). However, waiting even 1 day for a result can be detrimental for critically ill patients and we have received an increasing number of requests for same-day antigenemia test results for critically ill patients.

Thus, the availability of a commercial assay that can be completed in 2 h is of great benefit to the laboratory, as well as to patient care. Importantly, the 2-h assay provided quantitative results equivalent to or slightly better than those of the standard test. The reason for this difference is most likely that 33% more cells were applied to slides for the rapid test, as recommended by the manufacturer. The justification for the increased number of cells is that standard dextran sedimentation for separation of leukocytes enriches for neutrophils, the cells predominantly expressing the CMV pp65 antigen in the peripheral blood. With direct erythrocyte lysis, there is presumably no such enrichment. Therefore, to obtain equivalent numbers of neutrophils, a greater number of cells must be examined. However, Ho et al. reported that differential counts of PBLs separated by dextran sedimentation versus direct

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TABLE 1. Comparison of the CMV Brite and CMV Brite Turbo methods

Factor	CMV Brite	CMV Brite Turbo
PBL isolation method	Dextran sedimentation	Whole-blood lysis
No. of PBL isolation steps	6	3
Minimum PBL isolation time (min)	56	15
Fixation method	Paraformaldehyde–NP-40	Paraformaldehyde–NP-40
Fixation time (min)	25	14
CMV pp65 antibodies	C10, C11	C10, C11
Minimum staining time (min)	74	48
Nonspecific staining	Rare	Rare
No. of PBLs applied to slides	300,000	400,000

erythrocyte lysis were, in fact, similar (3). We confirmed this finding. Thus, the Turbo kit, in fact, increases the number of neutrophils examined.

A variety of sophisticated molecular tests are now available for CMV. In general, the reagents are more expensive and special equipment and/or separate rooms are often required. Many of these assays, although sensitive, are not quantitative (1) and viral load measurement is important in assessing risk of disease. Because of the time and expense involved in performing quantitative nucleic acid detection methods, single or even small numbers of samples are usually not tested. Rather, samples are batch tested once or several times per week. DNA-based methods may be advantageous for reference laboratories with high test volumes and processing delays due to specimen transport (5). However, in the hospital laboratory, CMV antigenemia has many advantages. None of the molecular tests can provide a quantitative result with a 2-h turnaround time.

CMV antigenemia is within the capabilities of routine virology laboratories. However, it requires attention to detail. It is critical that a high-quality microscope with a good bulb be utilized, that the specified numbers of PBLs be examined on the final slides, that cell morphology be well preserved, and that the technologists be precise in their identification and quantitation of characteristically stained cells.

The rapid CMV Brite Turbo assay provided performance characteristics and results equivalent to those of the standard CMV Brite method. The availability of a 2-h CMV viral load test will be of great benefit to laboratories and clinicians managing critically ill, immunocompromised hosts. The effect of direct erythrocyte lysis using the Turbo kit on the ability to isolate CMV from blood leukocytes is currently under study.

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