Evaluation of a Leukocyte Stabilization Reagent for Use in the Cytomegalovirus pp65 Antigenemia Assay

CHARLENE E. BUSH* AND JULIA A. SLUCHAK-CARLSEN

Division of Infectious Diseases, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan

Received 15 May 1998/Returned for modification 7 July 1998/Accepted 19 August 1998

New erythrocyte lysis and leukocyte stabilization reagents (Streck Laboratories, Inc.) were tested in the cytomegalovirus pp65 antigenemia assay, to determine if whole-blood processing time could be delayed to 24 h postdraw. The combination of these reagents gave results comparable to those for patient samples processed immediately after blood draw.

Cytomegalovirus (CMV) remains one of the most important agents causing opportunistic disease in immunocompromised patients, especially human immunodeficiency virus (HIV)-infected patients and transplant recipients. CMV causes an array of disease manifestations, including retinitis, allograft rejection, pneumonitis, vasculitis and neurological syndromes (9, 10). Three antiviral drugs are currently available to treat CMV disease: ganciclovir, foscarnet, and cidofovir. These drugs have demonstrated the ability to delay disease progression; however, despite prolonged antiviral therapy many patients experience relapse due to viral reactivation (7). This is particularly serious in HIV-infected patients with CMV retinitis who risk irreversible eye damage and blindness with disease progression (6). With the extended survival of HIV-infected patients with CMV disease, an increase in the number of transplants being performed, and the use of preemptive antiviral therapy in patients at increased risk for CMV disease, it is imperative that simple, inexpensive, and reliable clinical tests be developed to monitor CMV.

Studies have shown that the quantitative CMV pp65 antigenemia assay is a rapid and simple test that can be useful in identifying patients with clinical disease and monitoring therapeutic responses to antiviral drugs (1, 2). The major limitation of this test, however, is the need to perform rapid blood processing to avoid peripheral blood leukocyte (PBL) autolysis which leads to loss of signal and false-negative reports (4). This requirement for rapid manual blood processing has increased the clinical workload, increased the cost of the assay, and restricted the use of this assay in studies which require sample transport to distant sites.

The adverse effect of delaying the blood processing time on the results of the CMV pp65 antigenemia assay has been established (4). Landry and collaborators quantitatively showed that the reduction in CMV antigenemia-positive cells was approximately 50% in blood from HIV-infected patients held at room temperature or 4°C for 24 h (8). This reduction in the number of antigenemia-positive cells led to approximately 14% false-negative test results. Their attempts to preserve the number of CMV antigenemia-positive cells for 24 h using cell separation and holding in viral transport media or by the addition of protease inhibitors were unsuccessful.

Recently, Ho developed a modified CMV pp65 antigenemia assay which reliably detected CMV antigenemia-positive cells from freshly drawn blood in less than 3 h (5). This compares favorably with an assay time of approximately 5 h using dextran sedimentation and commercial pp65 antigenemia assay procedures. Although this new assay developed by Ho reduces the hands-on time of a clinical technician, it does not address the problem of leukocyte stability, which would be required for shipment of patient samples to reference laboratories and to ease the workload in the clinical laboratory by allowing for delayed processing and sample batching.

The purpose of this study was to determine if a new erythrocyte (RBC) lysis reagent and leukocyte stabilization reagent (Streck Laboratories Inc., Omaha, Nebr.) could be easily incorporated into the CMV pp65 antigenemia assay and allow for delayed blood processing time with combined maintenance of signal integrity and ease of handling.

Blood samples anticoagulated with EDTA from 35 HIV-infected patients preselected as culture positive for CMV. This study was approved by the institutional review board, and all participants gave oral informed consent. Each blood sample was divided into four aliquots. The first aliquot was immediately processed by dextran sedimentation according to the instructions of a commercially available CMV antigenemia kit (CMV Brite pp65 antigenemia kit; Biotest Diagnostics, Denville, N.J.). The second aliquot was immediately processed according to instructions from Streck Laboratories. This aliquot was mixed 1:4 with Streck RBC lysis reagent, incubated at room temperature for 15 min, and centrifuged at 200 × g for 10 min. The PBLs from both aliquots were washed once in phosphate-buffered saline (PBS), counted, fixed on a slide, and stained by the Biotest kit procedure. The number of CMV antigenemia-positive leukocytes was determined by indirect immunofluorescence staining. These fractions served as baselines to determine the number of PBLs isolated by both separation techniques and the number of pp65 antigenemia-positive cells.

The third and fourth blood aliquots were evaluated after 24-h storage at 4°C. The third aliquot was processed by dextran sedimentation using the Biotest procedure. The fourth aliquot was mixed 1:1 with Streck leukocyte stabilization reagent prior to refrigeration. After 24 h, this aliquot was mixed 1:4 with Streck RBC lysis reagent, incubated at room temperature for 15 min, and centrifuged as before. The leukocytes from these aliquots were washed once in PBS, counted, fixed on a slide, and stained by the Biotest kit procedure. The number of CMV antigenemia-positive leukocytes was determined as before. These fractions served as delayed-processing samples to determine the number of PBLs isolated with and without leukocyte stabilization and by both separation techniques and the num-

* Corresponding author. Mailing address: Infectious Diseases Research Laboratory, 7069 E & R Building, Henry Ford Hospital, 2799 W. Grand Blvd., Detroit, MI 48202. Phone: (313) 876-9412. Fax: (313) 556-8737. E-mail: CEBUSHDON@AOL.COM.
The Streck stabilization reagent did not effect the ability to culture CMV virus from leukocytes from four of four patients (100%) treated for 24 h with the stabilization reagent. There was, however, an approximately 14% reduction (range, 10 to 18%) in the amount of detectable infectious virus recovered from leukocytes incubated with Streck stabilization reagent compared with fresh leukocytes from four different patients.

We conclude that the Streck Laboratories RBC lysis solution and stabilization reagents significantly improved the recovery of PBLs and the number of CMV pp65 antigenemia-positive cells from HIV-infected patients. The availability of these reagents should help expand the applications for the CMV pp65 antigenemia assay and reduce the cost for patient sample processing.

This work was supported in part by a grant from Streck Laboratories Inc.

REFERENCES


