Comparison of the Histopaque-1119 Method with the Plasmagel Method for Separation of Blood Leukocytes for Cytomegalovirus Isolation

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Histopaque-1119 (Sigma Chemical Co., St. Louis, Mo.) and Plasmagel (Cellular Products, Inc., Buffalo, N.Y.) were compared as density gradient separation reagents for the separation of polymorphonuclear leukocytes and mononuclear cells from blood for the isolation of cytomegalovirus (CMV). Of 200 peripheral blood specimens examined, CMV was recovered from 51 by both methods. The time of detection of immunofluorescent sites or a cytopathic effect associated with CMV was similar by each method. The Histopaque-1119 method was less time-consuming than the Plasmagel method since it did not require a precentrifugation step for the settling of erythrocytes. The use of Histopaque-1119 will permit an effective alternative single-step method for the separation of blood leukocytes for the isolation of CMV.

Cytomegalovirus (CMV), when present in the blood, is associated with the polymorphonuclear and mononuclear blood cell fractions (4, 7, 11, 12). The first method for the isolation of leukocytes from whole blood used low-speed centrifugation (2, 3). It was later demonstrated that certain plant phytohemagglutinins could be used to sediment erythrocytes (9). Various high-molecular-weight substrates including dextran, synthetic sucrose polymers, and other high-molecular-weight solutions are used to isolate leukocytes through the formation of density gradients (1, 8, 10, 13). It is well established that a much higher yield of CMV from buffy coats may be obtained with the application of various density gradient systems (10).

The Ficoll-Paque/Macrodex procedure requires the use of two separate reagents for the respective concentration of polymorphonuclear and mononuclear fractions (6, 8). In contrast, each of the Plasmagel and the Sepracell-MN density gradient separation methods yields both of the mononuclear and polymorphonuclear cell components with a single reagent (10, 13).

Histopaque-1119 (Sigma Chemical Co., St. Louis, Mo.) is a solution composed of a polysaccharide and sodium diatrizoate adjusted to a density of 1.119. When combined with Histopaque-1077, it can be used to separate both mononuclear and granulocytic cells (procedure no. 1119; Sigma Diagnostics, St. Louis, Mo.) (5).

In this study, we report the application of Histopaque-1119 in order to obtain leukocyte fractions for the detection of CMV in cell culture. This study compared the recovery of CMV from leukocyte preparations from 200 blood specimens processed by the Histopaque-1119 and the Plasmagel methods.

An evaluation of two blood specimen-processing methods for the detection of CMV in cell culture was conducted over a 13-month period (January 1991 to February 1992). On requests for a viral blood culture, a phlebotomist obtained 10 ml of blood in a heparinized tube. The blood samples were immediately transported to the virology laboratory for proc-

essing. The majority of the blood specimens were obtained from patients in the bone marrow and renal transplant units of Allegheny General Hospital. Several blood specimens from many of these patients were processed for CMV detection.

The leukocyte fractions of the blood specimens were separated by using both the Histopaque-1119 and the Plasmagel methods. For the Histopaque-1119 method, 4 ml of the blood specimen was layered onto 4 ml of the Histopaque-1119 solution in a sterile 15-ml centrifuge tube. The tube was capped and then centrifuged in a tabletop centrifuge at 400 × g for 30 min at ambient temperature. A diffuse band of leukocytes occurred above the erythrocyte pellet. This layer of cells was aseptically removed with a pipette and transferred to a sterile 15-ml centrifuge tube.

For the Plasmagel method (13), 4 ml of heparinized blood was mixed with 1 ml of Plasmagel in a 10-ml round-bottom sterile glass or plastic tube, and the tube was capped. The cells were allowed to settle at room temperature for 45 min. The upper plasma layer containing the mixed leukocyte fraction was removed and centrifuged at 400 × g for 30 min. The plasma layer was then decanted, leaving the leukocytes at the bottom of the tube. The leukocytes and contaminating erythrocytes harvested by either method were counted simultaneously by using a hemacytometer.

The mixed leukocyte populations obtained by each of the harvesting methods were mixed with 10 ml of Eagle minimal essential medium with Earle's salts (MEM; Bio Whittaker, Walkersville, Md.). The cells were washed by a 10-min centrifugation at ambient temperature. The supernatant was removed, and the cells were washed for a second time. Two ml of MEM containing 2% calf serum was added to the cell pellet, and the solution was mixed in order to suspend the cells. Three shell vials of MRC-5 cells and three shell vials of HF cells (Bartels Diagnostics Division, Baxter, Bellevue, Wash.) were each inoculated with 0.2 ml from each cell mixture, and 1.0 ml of medium was added.

After overnight adsorption at 35°C, the culture medium and loose cells of the cells that were inoculated were removed, and fresh medium was added to the monolayers. At 72 h postinoculation, coverslip cultures from one inocu-

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lated MRC-5 cell and one HF cell shell-vial culture from each set of inoculated shell vials were fixed in cold acetone and stained with a direct immunofluorescence reagent that detects early and late CMV nuclear and cytoplasmic antigens. The procedure suggested by the manufacturer (Baxter, Bartels Diagnostic Division) was followed. Shell-vial cultures not positive for specific immunofluorescent foci associated with CMV were incubated for an additional 14 days and were examined daily with an inverted microscope for the presence of cytopathogenic effects (CPEs) characteristic of CMV. Confirmation of a CPE characteristic of CMV was obtained by the immunofluorescence test.

A total of 200 blood specimens were submitted over a 13-month period and were tested in parallel by the Plasmagel and Histopaque-1119 methods. CMV was detected in 51 (25.5%) of the blood specimens by both of these separation procedures. Of the 51 specimens, 41 (80%) were first detected in shell vials by immunofluorescence at 72 h postinoculation. In the other 10 specimens, the first indication of the presence of CMV was made by the observation of CPEs characteristic of that virus. A CPE associated with CMV was confirmed by specific immunofluorescence. CMV was detected from 3 to 12 days postinoculation. The period of time of detection of immunofluorescent foci or a CPE associated with CMV was quite similar by each blood leukocyte separation method. The average number of fluorescent sites in the positive vials was also very similar by each of the Plasmagel or the Histopaque-1119 methods (12.5 versus 13.5). CMV was generally detected more often in the 51 shell-vial cultures of HF cells (51 [100%] and 48 [94%] shell vials by the Histopaque-1119 and Plasmagel methods, respectively) than in shell-vial cultures of MRC-5 cells (33 [65%] and 30 [59%] shell vials by the Histopaque-1119 and Plasmagel methods, respectively) by either the Histopaque-1119 or Plasmagel method.

When Histopaque-1119 was used, 18 (9%) of the leukocyte preparations were toxic to MRC-5 cell monolayers and 9 (4.5%) of the leukocyte preparations were toxic to both MRC-5 and HF cell monolayers. In contrast, 30 (15%) of the leukocyte preparations obtained by the Plasmagel method were toxic to MRC-5 cells, while 15 (7.5%) leukocyte preparations obtained by using the Plasmagel reagent were toxic to both MRC-5 and HF cell monolayers.

The leukocyte fraction produced by the Histopaque-1119 method was observed as one diffuse band of cells above the pelleted erythrocytes. On the other hand, the leukocyte fraction separated by the Plasmagel method was distributed throughout the entire plasma layer above the sedimented erythrocytes. Total leukocyte harvests by both separation methods generally yielded comparable cell concentrations. The erythrocyte contamination in the Plasmagel preparations was approximately five times higher than that in the Histopaque-1119 preparations (Table 1).

Various methods have been used to prepare pure populations of leukocytes from human blood (1, 5, 6). Histopaque-1077 is a solution of Ficoll and sodium diatrizoate that is adjusted to a density of about 1.077. When blood is overlaid on this reagent and the solution is centrifuged, mononuclear cells concentrate at the plasma-reagent interface (1). Histopaque-1119 was previously shown to be of use in yielding granulocytes from blood specimens (5). The centrifugation of whole blood placed on a double gradient, which is formed by layering an equal volume of Histopaque-1077 over Histopaque-1119, yields a band of granulocytic cells at the interphase of the two reagents. The mononuclear cells

TABLE 1. Comparison of leukocyte and erythrocyte cell harvests from Histopaque-1119 and Plasmagel preparations[a]

Cell type	No. (%) of cells/ml by the following method:	
	Histopaque-1119	Plasmagel
Erythrocyte	1,113	5,435
Leukocyte	5,455 (100)	5,831 (100)
Granulocyte	2,563 (47)	1,808 (31)
Lymphocyte	2,291 (42)	2,915 (50)
Monocyte	545 (10)	641 (11)

[a] Results are based on the average count from 20 specimens.

concentrated at the plasma–Histopaque-1077 interface (procedure no. 1119; Sigma Diagnostics).

By the method used in the present study, centrifugation of whole blood on a layer of Histopaque-1119 was demonstrated to result in a diffuse band of leukocytes that contained both granulocytes and monocytes. The present investigation also demonstrated that both the Histopaque-1119 and the Plasmagel methods are equally effective in yielding the same rate of detection of CMV from the respective buffy coat preparations. There were no significant differences in the number of CMV-associated immunofluorescent sites obtained by either method. In a previous evaluation (13), Plasmagel was demonstrated to be able to separate both mononuclear and polymorphonuclear cell fractions in a single step. That method, however, requires a 45-min period to allow erythrocytes to settle out from the leukocyte fraction; this is followed by a 30-min centrifugation step. Thus, after approximately 100 min, the harvested cells from the Plasmagel preparation are ready to be inoculated into the tissue culture monolayers. In contrast, after about 50 min, the leukocyte preparation obtained by the Histopaque-1119 method is ready for inoculation. The Histopaque-1119 method can be completed in a shorter period of time because a single centrifugation step is used to separate the leukocyte fraction from the erythrocytes. The leukocyte harvest obtained by the Plasmagel method also contained more erythrocytes than did that obtained by the Histopaque-1119 method. A relatively high concentration of erythrocytes on a cell culture monolayer could interfere with the detection of virus-associated cytopathogenic changes. The various components of Plasmagel may be associated in part with a relatively high rate of cell toxicity. Sepracell-MN is a colloidal silica medium that was reported (10) to have a higher toxicity to cells than the dextran components associated with the Ficoll-Paque/Macrodex components.

The cost of one bottle of Histopaque-1119 is $12.50. Processing of one buffy coat for virus isolation requires 4 ml of Histopaque-1119 and costs $0.50. The relatively low toxicity associated with leukocyte fractions and minimal specimen manipulation indicate the distinct advantage of the Histopaque-1119 method. The Sepracell-MN reagent is no longer commercially available, while Plasmagel is no longer available from a distributor within the United States. The Histopaque-1119 method is an effective alternative approach that permits a relatively rapid and sensitive method for the detection of CMV in blood specimens.

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