INTRODUCTION

We have designed these tubes, to allow a one-step isolation of mononuclear cells for simplicity and safety.

The BD CPT™ tube is an evacuated tube containing anticoagulant, separation gel, and density gradient liquid. Whole blood is collected, centrifuged and processed entirely within this tube. During centrifugation, the mononuclear cells move from the plasma and are suspended in the density gradient, while the separation gel forms a stable barrier isolating them from the erythrocytes and granulocytes.

The BD CPT™ tube may be used to obtain highly concentrated mononuclear cells and/or plasma for testing.

Maximum PBMC recovery will be obtained by gently inverting the BD CPT™ tube after centrifugation. Transfer the total supernatant, above the gel barrier, into a conical secondary tube for the cell washing steps.

In addition, the mononuclear cells may be resuspended in the plasma to retain viability for storage and transportation. Cell processing for immunotyping, culture, and cell function studies, are faster than with multi-step manual separation methods. The BD CPT™ tube has the added safety of a sterile single-tube closed system.

BD CPT™ tubes are available with either sodium Citrate or sodium Heparin as an anticoagulant. Blood draw capacity is 8 ml. A 4 ml tube is also available (Citrate only).

Please note that BD CPT™ tubes must be centrifuged at 18-25°C (room temperature) in a horizontal rotor (swing-out head) at 1500g (measured at the bottom of the tubes) for 20 minutes. Lesser force and/or time will negatively impact the tube performance. Greater force and/or more time may decrease cell yield and should be used with caution (see package insert). We recommend that centrifugation take place within two hours after blood collection for best results.

How to process BD CPT™ tubes

1 AFTER BLOOD COLLECTION
   Gently invert 8 times

2 CENTRIFUGE
   - At room temperature
   - For 20 minutes
   - In a horizontal rotor (swing-out head; appropriate tube adapters)
   - At 1500g RCF
   For best results, centrifuge within two hours after blood collection

3 AFTER CENTRIFUGATION
   - Plasma
   - Lymphocytes and monocytes
   - Gel barrier
   - Erythrocytes and neutrophils
   - Density gradient fluid

4 FOR TRANSPORTATION
   Gently invert 1 times

   Cell suspension (plasma and mononuclear cells)
APPLICATION AREA
Infectious Diseases

1. HIV RNA QUANTIFICATION RESULTS WITH WHOLE BLOOD.
The tubes were spun down at 2, 8 and 30 hours. The plasma was
frozen down at -70°C. The Chiron assay was run.
The viral load was measured at each time point to evaluate
degradation.

<table>
<thead>
<tr>
<th>HIV RNA assay results</th>
<th>(Cleveland Clinic Foundation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient # 93-299</td>
<td>CD4=2</td>
</tr>
</tbody>
</table>

Conclusion:
Separate PLASMA within a few hours to avoid viral decay.

2. HIV RNA QUANTIFICATION RESULTS WITH CPT™.
CLEVELAND CLINIC, results.
3 (three) sets of BD CPT™ tubes were used.

<table>
<thead>
<tr>
<th>Blue-BD CPT™ tube</th>
<th>spun down within 2 hours at 4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red-BD CPT™ tube</td>
<td>spun down at 8 and 30 hours at 4°C</td>
</tr>
<tr>
<td>Green-BD CPT™ tube</td>
<td>spun down at 8 and 30 hours at room temperature</td>
</tr>
</tbody>
</table>

Plasma was removed, frozen, viral load measured by the Roche
assay.

Conclusion:
BD CPT™ tubes, spun down within 2 hours and held at +4°C, will reduce the degradation of HIV RNA.
APPLICATION AREA

Immune Function


C.H.J. Siebelink, Ph. H. Rothbarth, University Hospital Rotterdam, Dept. Of Virology, Rotterdam, The Netherlands

Abstract

Isolation of mononuclear cells from peripheral blood by density gradient centrifugation using Lymphoprep™ (Nycodenz) was compared with that of the newly developed VACUTAINER® Cell Preparation Tube (CPT™, Becton Dickinson). The yield of peripheral blood mononuclear cells (PBMC) isolated within two hours after blood collection with the CPT™ Heparin was higher than that of PBMC isolated using Lymphoprep™ density gradient fluid (2.0 x 106/ml blood vs 0.8 x 106/ml blood). After storage of whole blood for 16 hours at room temperature no significantly differences were found in the yield of PBMC isolated with Lymphoprep™, CPT™ Heparin and CPT™ Citrate (per ml blood 1.0 x 106, 1.1 x 106, and 1.4 x 106 respectively). After freezing and storage of PBMC at -135°C the yield, viability and mitogen induced proliferative responses were not significantly different between the PBMC isolated with the different methods. Centrifugation of CPT™ Heparin within two hours followed by storage at room temperature for 16 hours decreased the yield, viability and proliferative responses of PBMC significantly.

Introduction

Isolation of PBMC is laborious and time consuming due the layering of the blood on the density gradient fluid. The newly developed VACUTAINER® CPT™ is an one step system in which peripheral blood collection and density gradient centrifugation for PBMC isolation in the same tube.

Materials and Methods

PBMC. Peripheral blood from 10 healthy donors was collected in two sodium-heparin tubes, three CPT™ Heparin and one CPT™ Citrate. Two and 16 hours after blood collection the PBMC were isolated according to table 1. Blood collected in the sodium-heparin tubes was layered on Lymphoprep™ density gradient fluid and centrifuged for 20 minutes at 100 x g. The CPT™ were centrifuged 20 minutes at 1500 x g. The interface containing the PBMC was collected and the cells were washed three times with phosphate buffered saline. One CPT™ Heparin was centrifuged within two hours and the cells were collected after 16 hours (group 6). The PBMC were frozen and stored at -135°C in RPMI supplemented with antibiotics, 10% foetal bovine serum and 10% DMSO.

Table 1: Blood collection and isolation of PBMC

<table>
<thead>
<tr>
<th>Group</th>
<th>Isolation</th>
<th>Hours after collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lymphoprep™</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>CPT™ Heparin</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Lymphoprep™</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>CPT™ Heparin</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>CPT™ Citrate</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>CPT™ Heparin</td>
<td>2/16</td>
</tr>
</tbody>
</table>

Viability: The viability of PBMC after thawing was determined by trypan blue-staining.

Proliferative responses. In triplicate wells of a 96-well tissue culture plate 105 PBMC were stimulated with PHA-L (1 µg/ml), ConA (5 µg/ml) or PWM (1 µg/ml) for 72 hours. Proliferation was measured by the incorporation of 3H-thymidine for 16 hours and is expressed as counts per minute (CPM).

Results

Fig. 1. Mean number of mononuclear cells separated per ml peripheral blood of 10 individuals using Lymphoprep™ and VACUTAINER-CPT™ at 2 or 16 hours after blood collection

Fig. 2. Mean number of frozen and thawed mononuclear cells per ml peripheral blood of 10 individuals using Lymphoprep™ and VACUTAINER-CPT™ at 2 or 16 hours after blood collection

Fig. 3. Viability of mononuclear cells separated from peripheral blood of 10 individuals using Lymphoprep™ and VACUTAINER-CPT™ at 2 or 16 hours after blood collection and stored at -135°C

Fig. 4. Mitogen-induced proliferative responses of mononuclear cells separated from peripheral blood of 10 individuals using Lymphoprep™ and VACUTAINER-CPT™ at 2 or 16 hours after blood collection and stored at -135°C

Conclusions:

The yield, viability and mitogen-induced proliferative responses of the PBMC isolated with CPT™-Heparin or CPT™-Citrate are comparable with those of PBMC isolated with Lymphoprep™. The use of Citrate as a anti-coagulant may be used and is advantageous in case the plasma will be used for PCR purposes. Blood may be stored overnight at room temperature in the CPT™ tubes before centrifugation.

APPLICATION AREA

Micrometastasis

References:

Enhanced reverse transcriptase-polymerase chain reaction for prostate specific antigen as an indicator of true pathologic stage in patients with prostate cancer.

Comparison of Three Methods for Isolating Prostate Cancer Cells in Whole Blood for Reverse Transcription Polymerase Chain Reaction of Prostate Specific Antigen. 
G. Chong, R. Rabeo, E. MacNamara, Medical Biochemistry, McGill University - Jewish General Hospital, Montreal, Qc. Canada.

Several studies using reverse transcription polymerase chain reaction (RT-PCR) have shown that the presence of tumor cells in the circulation may predict disease recurrence and metastases. In these studies, density gradient procedures are often used to isolate cancer cells in peripheral blood. The aim of the present study was to compare three methods for isolating prostate cancer cells in whole blood. The CPT<sup>™</sup> system from Beckton Dickinson (BD) consists of an evacuated tube containing anticoagulant, separation gel, and density gradient liquid. The immunomagnetic bead isolation system from Dynal uses an epithelium-specific monoclonal antibody bound to magnetic beads. They were compared with a Ficoll-Paque (Pharmacia) density gradient procedure. The prostate carcinoma cell line LNCaP was used as the source of cancer cells. LNCaP cells were serially diluted into whole blood to give dilutions of 2, 5, 10, 20, 50, 500, and 2000 LNCaP cells/mL. The detection limit of each method was established by RT-PCR of PSA on total RNA or mRNA obtained from the cell dilution. The primers used for the PCR assays were previously described (1). The 710 bp PCR product was detected by agarose gel electrophoresis and ethidium bromide staining. The detection limit for both the BD CPT<sup>™</sup> system and the Ficoll-Paque method was approximately 0.5 LNCaP cells/1 million white cells. The limit of detection for the Dynal immunobead method was 10 LNCaP cell/1 million white cells.

All three methods are reliable for the isolation of epithelial cancer cells in whole blood. While it is usual to manipulate the RT-PCR step to improve sensitivity (nested primers, increased cycles, etc.), this may affect specificity. We show that the choice of an appropriate cell isolation procedure is a simple step to improve cell recovery ad thus the overall sensitivity of the test system.

**Conclusion:**
The BD CPT<sup>™</sup> system provided consistently better yield of total RNA.

---

**APPLICATION AREA**

**HLA Determinations**

Evaluation of a Novel Mononuclear Cell Isolation Procedure for Serological HLA Typing (Published at the Clinical and Diagnostic Laboratory Immunology, Nov. 1998, p. 808-813)

P. Schlecker<sup>1</sup>, H. Klöter<sup>1</sup>, M. Müller-Stehnhardt<sup>1</sup>, H-J. Hammer<sup>1</sup>, K. Borcher<sup>1</sup>, G. Reim<sup>1</sup> - Institute of Immunology and Transfusion Medicine, University of Luebeck School of Medicine, Luebeck, Germany<sup>1</sup> - Institute of Clinical Immunology and Transfusion Medicine, University of Giessen, Germany<sup>1</sup>

Despite recent advances in DNA-based genotyping, the microcytotoxicity test is still broadly used for the determination of human leukocyte class I antigens in patients as well as organ donors and also for the detection of HLA antibodies. Excellent purity and viability of peripheral blood mononuclear cells (PBMC) are essential for reliable HLA typing results. Background staining and cell loss can contribute to impaired typing results or even cause misinterpretations. A novel isolation procedure using cell preparation tubes (CPT) with pre-filled Ficoll was compared with the standard Ficoll gradient. We determined the recovery, purity, and viability of the PBMC after several periods of storage. Finally, the isolated cells were used for HLA class I typing, and background reactivities were scored. By using the CPT method, the recovery of PBMC was significantly higher than recovery with the standard technique (P < 0.001). Contamination by granulocytes increased considerably during the storage time for the standard protocol, whereas purity remained stable when CPT was used (P < 0.001). With both methods, lymphocyte viability declined markedly over time. We found significantly more dead cells by using the CPT methods. Due to high background scores, HLA typing was impossible after 48 h. The isolation of PBMC by the CPT method resulted in a higher yield and improved purity compared to those obtained with the standard gradient technique. The decreasing viability after 48 h limits the use of both methods for HLA typing and HLA antibody screening.

**Conclusion:**
The BD CPT<sup>™</sup> method recovers reproducibly more viable and pure lymphocytes during the first 24 hours after blood drawing than the standard ficoll gradient method.
APPLICATION AREA

Cell Stimulation Assays - Lymphocyte Proliferation Assays

Effect of Shipment, Storage, Anticoagulant, and Cell Separation on Lymphocyte Proliferation Assays for Human Immunodeficiency Virus-Infected Patients (Published at the Clinical and Immunodeficiency Laboratory Immunology, Nov. 1998, p. 804-807)
A. Weinberg, R. A. Betensky, L. Zhang, G. Roy - University of Colorado School of Medicine, Denver, Colorado 1 - Harvard School of Public Health, Boston, Massachusetts

Lymphocyte proliferation assays (LPA), which can provide important information regarding the immune reconstitution of human immunodeficiency virus (HIV)-infected patients on highly active antiretroviral therapy, frequently involve shipment of specimens to central laboratories. In this study, we examine the effect of stimulant, anticoagulant, cell separation, storage, and transportation on LPA results. LPA responses of whole blood and separated peripheral blood mononuclear cells (PBMC) to different stimulants (cytomegalovirus, varicella-zoster virus, candida and tetanus toxoid antigens, and phytohemagglutinin) were measured using fresh specimens shipped overnight and frozen specimens collected in heparin, acid citrate dextrose (ACD), and citrate cell preparation tubes (CPT) from 12 HIV-infected patients and uninfected controls. Odds ratios for positive LPA responses were significantly higher in separated PBMC than in whole blood from ACD- and heparin-anticoagulated samples obtained from HIV-infected patients and from ACD-anticoagulated samples from uninfected controls. On separated PBMC, positive responses were significantly more frequent in fresh samples compared with overnight transportation for all antigens and compared with cryopreservation for the candida and tetanus antigens. In addition, viral antigen LPA responses were better preserved in frozen PBMC compared with specimens shipped overnight. CPT tubes yielded significantly more positive LPA results for all antigens, irrespective of the HIV patient status compared with ACD, but only for the candida and tetanus antigens and only in HIV-negative controls compared with heparin. Although HIV-infected patients had a significantly lower number of positive antigen-driven LPA responses compared with uninfected controls, most of the specimen processing variables had similar effects on HIV-positive and negative samples. We conclude that LPA should be performed on site, whenever feasible, by using separated PBMC from fresh blood samples collected in either heparin or ACD. However, if on-site testing is not available, optimal transportation conditions should be established for specific antigens.

Conclusion:
Superior preservation of LPA responses in CPT tubes may be ascribed to separation of PBMC from erythrocytes and granulocytes during transport.

CPT™ a Practical Tool For Routine Lymphocyte Separation for HLA Typing.
A. Amarosa, M. Tacconella, L. Calosso, Transplantation Immunology Center - Dept. of Genetic, Biology and Medical Chemistry - University of Torino, Italy

Ideally, samples for HLA typing should be processed, stored, transported and analyzed in a standardized manner.

In this study design we compared the expression of HLA antigens on lymphocytes collected and processed in VACUTAINER® CPT™ tubes with the current traditional method used in our routine.

Blood samples were collected from consenting subjects into Citrate buffered VACUTAINER® CPT™ tubes (ref. 362761).

The CPT™ tubes contain a gel barrier and Ficoll to allow for separation of PBMC and plasma from erythrocytes and granulocytes following a single centrifugation step. The evaluation of HLA molecule expression on lymphocyte populations purified using CPT™ technique was made by indirect immunofluorescence using the following antibodies: R.1.30/3.8/3E11 (HLA-Class I), aB2, 9Gb18R (HLA-Class II), CB39/D4 (CD25), CBT3G (CD3), BU12 (CD19), FACS analysis and “Lysis II” software were also utilized. Total lymphocyte amount was similar after CPT™ treatment to that obtained with traditional methods (Lymphoprep™). The two methods also gave similar amounts of B and T cells. The cells expressing HLA Class I molecules decreased by 10% after CPT™ treatment, while Class II expression was not influenced.

The lymphocytes belonging to 8 different individuals were evaluated in complement-dependent cytotoxicity test for HLA typing.

Conclusion:
Cell viability and typing results were similar when lymphocytes were purified with either CPT™ or traditional methods.