Determination of high density lipoprotein cholesterol in venous and capillary whole blood

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Summary A procedure is presented and evaluated for separation of plasma high density lipoprotein from either capillary or venous whole blood. The lipoprotein is separated by adding 50 µl of sample to 250 µl of 0.15 M NaCl solution containing 99.9 g/l polyethyleneglycol 6000, 0.0374 g/l dextran sulfate (M, 15,000) and 2.6 mM Mg²⁺. After gentle mixing for a few minutes and standing 10 min at room temperature, mixtures are centrifuged (1,500 g) for 10 min and cholesterol is measured on 200 µl of supernatant by an enzymatic-colorimetric method. Comparison studies demonstrate a good correlation between high density lipoprotein cholesterol in plasma and capillary or venous whole blood. The procedure is simple, has the advantage of using either K₃-EDTA-anticoagulated whole blood, without the need of centrifugation, or capillary whole blood which can also be collected away from the laboratory.

Materials and methods

Disposable 50-µl calibrated micropipettes (Blaubrand intraMark) were obtained from Carlo Erba, Divisione Chimica Vettric (Milano, Italy). PEG 6000 was purchased from Merck-Schuchard and dextran sulfate M, 15,000 (Dextralipid 15) was from Sochibo (Boulogne, France). For the cholesterol assay we used the enzymatic-colorimetric kit “Monotest Cholesterol-High performance CHOD-PAP” from Boehringer (Mannheim, West Germany).

The HDL precipitation mixture contained PEG 6000, 99.9 g/l; dextran sulfate, 37.4 mg/l; and MgCl₂, 2.6 mmol/l. The working solution was obtained by diluting 1.0 ml of a stock solution to 1000 ml with PEG (100 g/l) in NaCl (0.15 mol/l). The stock solution containing dextran sulfate (37.4 g/l) and MgCl₂ (2.6 mol/l) is stable for at least 6 months at room temperature and the working solution for at least 3 weeks at room temperature.

Samples

HDL-Chol measurements were performed on plasma and venous whole blood collected in K₃-EDTA (1.5 mg/ml) from 219 hospitalized patients who had fasted for 8–10 hr. Capillary and venous blood was drawn from 34 fasting healthy laboratory personnel volunteers.

METHODS

K₃-EDTA-treated samples were processed within 6 hr from the time of venipuncture. Skin puncture was performed according to the guidelines of the National Com-

Abbreviations: HDL, high density lipoproteins; HDL-Chol, high density lipoprotein cholesterol; PEG, polyethyleneglycol; Hct, hematocrit.

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mittee of Clinical Laboratory Standards (NCCLS) (11). Capillary blood was collected with 50-μl calibrated micropipettes and immediately diluted with the proper volume of reagent. Fresh venous whole blood was collected into small conical tubes and then treated in the same way as capillary blood. The effect of standing time on the HDL-Chol assay was investigated in two samples at various hematocrit (Hct) values and serum HDL-Chol concentrations. For this purpose, whole blood samples were mixed with proper volumes of reagent and the mixture was stored 4 hr at room temperature. HDL-Chol determinations were performed at intervals of 30 min.

Hct values were determined by a standard microcentrifugation method.

HDL separation was performed, using Eppendorf conical microtubes, by adding 25 μl of plasma or 50 μl of whole blood to 250 μl of reagent. After gentle mixing for a few seconds and standing for 10 min at room temperature, mixtures were centrifuged (1,500 g) for 10 min at room temperature. For manual HDL-Chol assay, 200 μl of supernatant was added to 1.0 ml of the enzymatic cholesterol reagent and the mixture was incubated 15 min at room temperature in a Beckman microcuvette. Absorbances were read at 500 nm on a Beckman DU-7 spectrophotometer against a reagent blank in which HDL-Chol values on whole blood were corrected for Hct values according to the formula: corrected value (CV) = (obtained value (OV) x 50)/(100 – Hct). Thus, in a whole blood sample with Hct value of 42 and HDL-Chol concentration of 1.20 mmol/l, the corrected value of the analyte was 1.03 mmol/l. In the text, all results are reported as corrected values. The values obtained on whole blood were compared with those obtained on venous K3-EDTA-treated plasma with the same precipitation mixture (PEG 6000, dextran sulfate, MgCl2) and with those obtained in 74 normolipidemic samples by heparin-Mn2+ (Mn2+ = 92 mmol/l) procedure according to Warnick and Albers (2). Linear regression analysis and Student's paired t-test were used for comparison studies.

RESULTS

By using K2-EDTA-anticoagulated whole blood, the within-assay coefficient of variation for the manual procedure was 2.3% (n = 12) and 2.6% (n = 11) at 1.16 and 1.48 mmol/l of HDL-Chol, respectively. The mean values of absorbance were 0.131 ± 0.003 (Hct = 42) and 0.184 ± 0.005 (Hct = 38) in the two series of replicates. By the automated procedure, the within-assay coefficient of variation was 1.7% (n = 27) and 1.5% (n = 18) at 0.82 and 2.21 mmol/l of analyte, respectively. The manual method (x) was compared with the automated procedure (y) and the agreement for 48 samples was good (y = 1.002x + 0.001, Sxy, = 0.042, r = 0.995, not significant by Student's paired t-test).

Table 1 shows HDL-Chol values for plasma and whole blood samples. The measurements were performed by the automated procedure. There was a highly significant correlation between the two sets of data over large ranges of HDL-Chol concentrations (0.13–3.08 mmol/l) and Hct values (21–51). In order to investigate the possible effects of K2-EDTA, a correlation was made between HDL-Chol

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<tr>
<th>TABLE 1. Comparison of 219 determinations of HDL-Chol in plasma and whole blood at various ranges of analyte and hematocrit values</th>
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<tr>
<td>Variables</td>
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<td>A. Plasma HDL-Chol (mmol/l)</td>
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<td>1.10–1.50</td>
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<td>B. Hematocrit (%)</td>
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<td>31–40</td>
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<td>41–51</td>
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*Estimated standard deviation from regression.

Mean ± SEM.
values \( n = 31 \) obtained on fresh blood \((y)\) and K\(_3\)-EDTA-anticoagulated whole blood \((x)\): no significant differences were found by Student's paired \( t \)-test \((y = 1.003x + 0.002, S_{y.x} = 0.051, r = 0.998)\).

The HDL-Chol values on the 34 capillary blood samples were compared with the values obtained on K\(_3\)-EDTA-anticoagulated venous whole blood drawn from the same subjects. No differences were seen, as shown in Figure 1. The effect of standing time on the HDL-Chol determination, after mixing whole blood samples with the reagent, is presented in Figure 2.

Table 2 shows the results of comparison studies between HDL separations and HDL-Chol determinations on whole blood and on K\(_3\)-EDTA-treated plasma by the method of Warnick and Albers (2), a commonly used and highly standardized procedure.

**DISCUSSION**

The aim of this study was to investigate the reliability of a procedure for plasma HDL separation and HDL-Chol assay on capillary and venous whole blood samples. We assumed that blood cells are inert particles and so we used a double volume of sample \((50 \mu l)\), a departure from a former method for serum or plasma HDL separation (6). The first problem concerned the effects of the precipitation mixture on blood cells with regard to possible hemolysis and to the release of cholesterol. Hemolysis was not detectable by visual inspection even though free hemoglobin was present in the supernatants after centrifugation. The good agreement between HDL-Chol values in venous whole blood and in plasma excluded any interference to the HDL-Chol assay as a result of hemolysis and release of cholesterol from the blood cells to the precipitation mixture. The correlation between HDL-Chol values in plasma and in venous whole blood was verified over a large range of hematocrits demonstrating an effective HDL separation independently of the changes in reagent/sample ratios induced by Hct variations (Table 1). The high reagent/sample ratio of the procedure allowed effective HDL separation up to 3.0\% of variation in the final concentration of the mixture components.

K\(_3\)-EDTA did not interfere with HDL separation and HDL-Chol values were the same in either anticoagulated or fresh venous or capillary whole blood.

As previously reported (13), mean HDL-Chol values in serum drawn by skin puncture were 4.1\% lower than in venous blood serum. Surprisingly, our study demonstrated that capillary and venous whole blood can be used interchangeably and there is no problem in specifying new reference values.

In preliminary studies we attempted plasma HDL separation in whole blood by other chemical methods. By using phosphotungstate-Mg\(^{2+}\) (1), heparin-Mn\(^{2+}\) \((92 \text{ mmol/l})\) (2), and dextran sulfate-Mg\(^{2+}\) (4) as precipitating mixtures, severe hemolysis of red blood cells was observed, resulting in unreliable assay of cholesterol in the supernatant solutions. Moreover, these methods required a high sample/reagent ratio and did not allow the use of small volumes of sample. With the use of PEG 6000 (5) and PEG 20,000 (7), hemolysis did not occur. However, the...
final concentration of these polymers is critical and Hct variations consistently caused large changes in the sample/reagent ratio (1:2 and 1:3, refs. 5 and 7, respectively) resulting in ineffective HDL separation.

The high reagent/sample ratio of the proposed procedure accounts for the effectiveness of HDL separation by virtue of the minimal changes in the final concentration of the precipitant components induced by Hct variations.

In conclusion, our results demonstrate the reliability of a simple and rapid procedure for plasma HDL-Chol assay on venous and capillary whole blood. Its advantages are obvious: a) using K2-EDTA-anticoagulated whole blood, centrifugation is not required; b) using capillary whole blood, samples can be drawn without venipuncture, which is often performed with difficulty in newborns, infants, and sometimes even in adults; and, c) samples taken by skin puncture can be mixed with the reagent in the place of sampling, away from the laboratory. The mixture can stand up to 3 hr prior to centrifugation. After this time a significant increase of HDL-Chol values is seen. The standing may cause a spurious release of cholesterol from blood cells to the reagent.

The sole limitation of this procedure is the need for Hct determination. In our experience, the same values are obtained on capillary or K2-EDTA-anticoagulated venous whole blood. The HDL-Chol determinations on either venous or capillary whole blood provide the same results as those obtained using a standardized method (2); they allow the use of the same reference values, which makes them suitable for a clinical setting.

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REFERENCES


