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_r 15,000) and 2.6 mM Mg^{2+}. 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 K_3-EDTA-anticoagulated whole blood, without the need of centrifugation, or capillary whole blood which can also be collected away from the laboratory.—**Lippi, U., M. S. Graziani, M. Schinella, F. Manzato, and R. Bazzani.** Determination of high density lipoprotein cholesterol in venous and capillary whole blood. *J. Lipid Res.* 1988. **29:** 112–115.

Supplementary key words HDL separation • HDL-cholesterol

Several chemical procedures for plasma or serum HDL separation have been proposed (1–7) and evaluated (8, 9). However, no methods have been reported for HDL separation and HDL-cholesterol (HDL-Chol) assay directly on capillary or venous whole blood.

In this report we describe a simple and rapid procedure for HDL separation and HDL-Chol determination using 50 μl of capillary or venous whole blood and a PEG-dextran sulfate-Mg^{2+} mixture. The procedure is very effective using either normo- or hypertriglyceridemic samples. The concentrations of the reagents were previously selected from a "titration curve" using normo- and hyperlipidemic samples in which cholesterol concentrations were determined in HDL separated by the reference method (ultracentrifugation). The procedure showed good agreement with the PEG standard method (5) and with quantitative determination of HDL-Chol by agarose gel electrophoresis (10) in normal as well as in hypertriglyceridemic sera (6).

MATERIALS AND METHODS

Materials and reagents

Disposable 50-μl calibrated micropipettes (Blaubrand intraMark) were obtained from Carlo Erba, Divisione Chimica Vetrerie (Milano, Italy). PEG 6000 was purchased from Merck-Schuchard and dextran sulfate M_r 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 $MgCl_2$, 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 $MgCl_2$ (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 K_3-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

K_3-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 precipitation mixture was used instead of supernatant solution. Cholesterol measurement was standardized using "normal" reference sera in which target values of the analyte were assigned by the Abell-Kendall reference method (12). Reference sera were diluted 1:30 with NaCl (0.15 mol/l) and processed in the same way as the supernatant solutions. HDL-Chol was alternatively measured at 30°C on a Technicon RA-1000 random-access analyzer with the following instrument setting: Type 2, % Smp vol 60, Filter pos 4 wl 500, Delay 9, % Rgt vol 70. HDL-Chol values on whole blood were corrected for Hct values according to the formula: corrected value (CV) = (obtained value (OV) × 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 K_3-EDTA-treated plasma with the same precipitation mixture (PEG 6000, dextran sulfate, $MgCl_2$) and with those obtained in 74 normolipidemic samples by heparin-Mn^{2+} (Mn^{2+} = 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 K_3-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 ($y = 1.002x + 0.001$, $S_{y.x} = 0.042$, $r = 0.995$, 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 K_3-EDTA, a correlation was made between HDL-Chol

TABLE 1. Comparison of 219 determinations of HDL-Chol in plasma and whole blood at various ranges of analyte and hematocrit values

Variables	n	HDL-Chol		$S_{y.x}$[a]	Intercept	Slope	*r*	Student's Paired *t*-Test
		K_3-EDTA Plasma	K_3-EDTA Whole Blood					
		mmol/l						
A. Plasma HDL-Chol (mmol/l)								
≤ 1.00	97	0.76 ± 0.019[b]	0.77 ± 0.019[b]	0.030	0.006	1.005	0.987	NS
1.10–1.50	86	1.24 ± 0.014	1.23 ± 0.015	0.044	0.021	0.980	0.947	NS
1.51–2.00	30	1.70 ± 0.025	1.69 ± 0.026	0.053	0.103	0.935	0.926	NS
≥ 2.10	6	2.35 ± 0.164	2.32 ± 0.155	0.076	0.148	0.926	0.983	NS
0.13–3.08	219	1.12 ± 0.029	1.12 ± 0.028	0.041	0.025	0.992	0.996	NS
B. Hematocrit (%)								
≤ 21	16	0.87 ± 0.074	0.87 ± 0.077	0.034	−0.010	1.033	0.994	NS
22–30	30	0.92 ± 0.061	0.91 ± 0.059	0.028	−0.017	0.971	0.996	NS
31–40	79	1.14 ± 0.051	1.15 ± 0.052	0.041	0.020	0.984	0.995	NS
41–51	94	1.20 ± 0.042	1.21 ± 0.041	0.045	0.035	0.969	0.993	NS

[a] Estimated standard deviation from regression.
[b] 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 **Fig. 1**. The effect of standing time on the HDL-Chol determination, after mixing whole blood samples with the reagent, is presented in **Fig. 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 μl), 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 5.0% of variation in the final concentration of the mixture components.

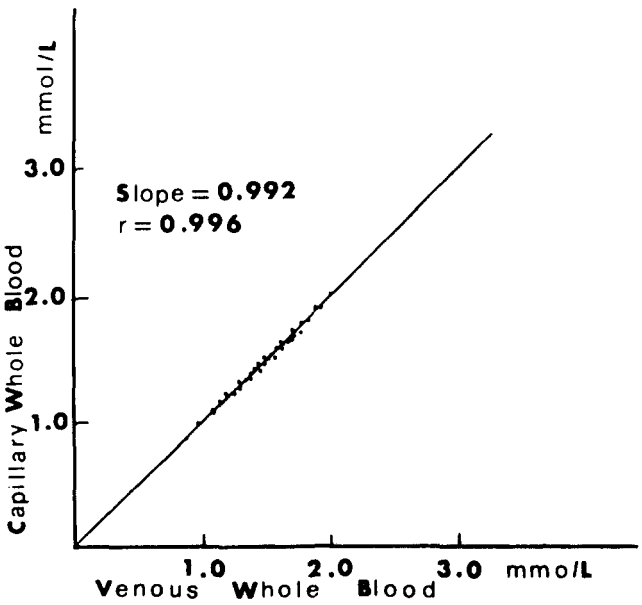

Fig. 1. Comparison of HDL-Chol in venous whole blood and capillary whole blood drawn from the same healthy laboratory personnel volunteers (n = 34).

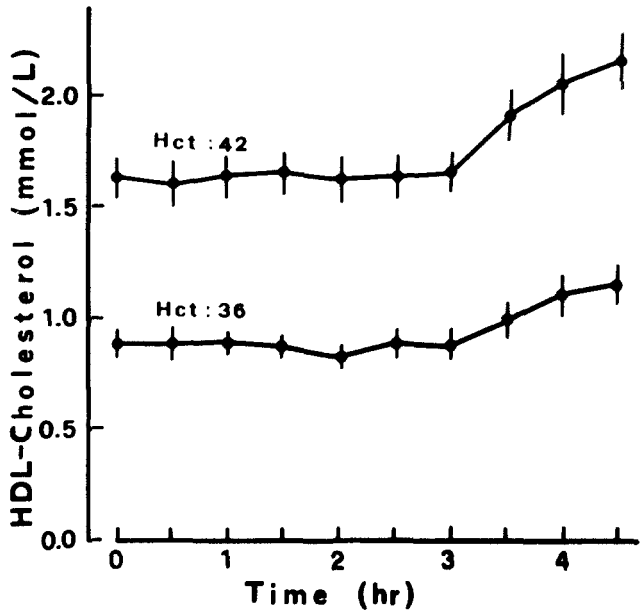

Fig. 2. Effect of standing time on HDL-Chol values in two mixtures of whole blood and reagent stored at room temperature up to 4 hr prior to centrifugation.

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 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

TABLE 2. Comparison of 74 determinations of HDL-Chol in whole blood and in supernatant solutions obtained by heparin-Mn^{2+} procedure (2)

Sample	HDL-Chol	$S_{y \cdot x}$[a]	Intercept	Slope	r	Student's Paired t-Test
	mmol/l					
Whole blood	1.36 ± 0.017[b]	0.042	0.028	0.988	0.985	NS
Plasma	1.39 ± 0.028					

[a] Estimated standard deviation from regression.
[b] Mean ± SEM.

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 K_3-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 K_3-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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