Homogeneous HDL-Cholesterol Assay Versus Ultracentrifugation/Dextran Sulfate-Mg$^{2+}$ Precipitation and Dextran Sulfate-Mg$^{2+}$ Precipitation in Healthy Population and in Hemodialysis Patients

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Objectives: To evaluate the analytical performance of a new homogeneous HDL-cholesterol assay (Olympus Diagnostica). To investigate possibly discrepant results in chronic hemodialysis patients who commonly exhibit quantitative and qualitative lipoprotein abnormalities, responsible for atherogenic complications in these patients.

Design and methods: Serum samples were collected from 50 healthy subjects and 65 chronic hemodialysis patients. HDL-C levels measured by the homogeneous assay were compared with the routine dextran sulfate-Mg$^{2+}$ precipitation method and the ultracentrifugation/dextran sulfate-Mg$^{2+}$ precipitation method as reference method.

Results: The homogeneous assay was linear up to at least 220 mg/dL. The analytical precision was estimated with three different sets of commercial controls and one set of human pooled serum control. The within-day CV ranged between 1.7% and 3.8% and the between-day CV ranged between 1.0% and 2.3%. HDL-C values in both populations correlated highly with the dextran sulfate-Mg$^{2+}$ precipitation method and the ultracentrifugation/dextran sulfate-Mg$^{2+}$ precipitation method (r $0.96$, bias between $2^0.9$ and 2.3 mg/dL). Lipemia up to triglyceride concentration of 600 mg/dL did not alter the HDL-C value.

Conclusions: The homogeneous assay for HDL-C (Olympus) uses much less sample, is accurate and convenient to handle, and allows full automation. The test should considerably facilitate the screening of individuals at an increased risk of cardiovascular disease, including hemodialysis patients. Copyright © 1999 The Canadian Society of Clinical Chemists

KEY WORDS: HDL-C; quantification; method comparison; triglycerides; hemodialysis patients.

Introduction

Epidemiological and clinical studies have clearly shown the inverse correlation between serum high density lipoprotein cholesterol (HDL-C) concentration and the risk of atherosclerotic diseases. Therefore, HDL-C concentrations <35 mg/dL are thought to be a cardiovascular risk factor. According to the guidelines of National Cholesterol Education Program Adult Treatment Panel II (NCEPATP II) for the diagnosis and treatment of hypercholesterolemia in adults, HDL-C should be measured along with total cholesterol (TC) at the initial screening stage. Additionally, the HDL-C determination is necessitated for the calculation of low-density lipoprotein cholesterol (LDL-C), which is strongly linked to cardiovascular risk and comprises the main target of hypolipidemic therapy (1–3). Moreover, high HDL-C (>60 mg/dL) is a negative risk factor, thereby compensating for the presence of a positive risk factor. Therefore, it is of particular importance for the clinical laboratory to provide accurate, reliable, and easy-to-perform measurement of HDL-C, especially at the decision points of 35 and 60 mg/dL.

Common methods for separating HDL from other lipoproteins involve chemical precipitation of apo B-containing lipoproteins from fresh plasma or serum, centrifugation, and quantification of the cholesterol in the supernatant, a procedure which is labor-intensive, prevents full automation and requires a large volume of sample. Large samples are not always available, especially in children, and subjects who are often submitted to an extensive laboratory analysis, as with patients with end-stage renal disease on chronic hemodialysis. Additionally, in the presence of high plasma triglycerides (TG), commonly found in patients with renal diseases, apo B-containing particles do not completely precipitate,
accounting for the falsely increased results for HDL-C.

The recently developed direct or homogeneous assays for HDL-C, also called third generation assays, are fully automated and require only a small sample volume. Although the reagent costs are higher than the conventional methods, their advantages in every-day practice and their reliability have been extensively discussed (4–13).

We report our assessment of the accuracy of a direct, third generation, polyanion-polymer/detergent HDL-C assay provided by Olympus Diagnostica (Hamburg, Germany) compared with the routinely used assay of dextran sulfate-Mg\(^{2+}\) precipitation, and the ultracentrifugation/dextran sulfate-Mg\(^{2+}\) precipitation as reference method. We used samples from normal subjects as well as from chronic hemodialysis patients. The latter group commonly exhibit both quantitative abnormalities, such as hypertriglyceremia and low HDL-C, and important qualitative lipoprotein changes which can interfere with the routine laboratory measurements (14–16). Moreover, the applicability of the method in the every day practice in a laboratory of clinical chemistry is also being considered.

Materials

Samples

The population studied consisted of 50 healthy subjects, mainly staff of the University Hospital of Ioannina, and 65 patients undergoing hemodialysis (HD). Clinical and laboratory parameters of the study population are shown in Table 1.

Blood samples were obtained in the morning after an overnight fast from all subjects. In hemodialysis patients, samples were taken before the dialysis session. Serum was isolated within 2 h (centrifugation at 1500 g for 15 min) and was stored at 4° C until analysis (generally within 3 days).

Controls

To assess the precision of the homogeneous HDL-C assay, we used three different sets of commercial controls and one set of human pooled serum control. The first set was the Liquichek™ lipids control (Levels 1,2) from Bio-Rad (Anaheim, CA, USA) available in frozen form. The second set was the QCS® (Levels 1,2) controls from Ciba Corning Diagnostics (Medfield, MA, USA), which need reconstitution before use, and the third set was the liquid “ready to use” controls, Decision® (Levels 1,2,3) from Beckman (Fullerton, CA, USA). The Decision® controls contain ethylene glycol and thus HDL-C cannot be determined by conventional precipitation assays. However, the direct method used gives reliable measurements. The two pooled serum samples (Pool 1,2) were prepared from normal human serum and stored in aliquots at −80° C. Each sample was assayed in duplicate for 20 successive days.

Methods

Dextran Sulfate-Mg\(^{2+}\) Assay

This assay uses dextran sulfate and MgCl\(_2\) to precipitate all lipoproteins except HDL, which remains in the supernatant and is assayed. The precipitation was performed according to Warnick et al. (17). Sample of 500 µL was mixed with 50 µL of a solution containing 500 mmol/L MgCl\(_2\) and 10 g/L dextran sulfate (M, 50 000) (Sigma). After precipitation at room temperature for 10 min, the reaction mixture was centrifuged at 1500 g for 30 min at 4° C and the supernatant was collected for enzymatic determination of HDL-C using the reagents for total cholesterol (Olympus Diagnostica).

Ultracentrifugation/Dextran Sulfate-Mg\(^{2+}\) Assay

In this assay VLDL and chylomicrons are removed by ultracentrifugation before LDL is precipitated by dextran sulfate-MgCl\(_2\). HDL-C is measured in the supernatant as noted (17). Sample of 3 mL were ultracentrifuged at \(d = 1.006\) Kg/L in a Beckman L7-65 ultracentrifuge at 40 000 rpm, 14 °C for 10 h, using a type NVT 65 rotor (Beckman Instruments). After ultracentrifugation, the tubes were sliced to remove the floated VLDL and chylomicron particles. The volume of the infranate was then adjusted to 3 mL by adding a solution of 9 g/L NaCl and HDL-C was determined as above after precipitation of the apo B-containing lipoproteins (mainly LDL) by dextran sulfate-MgCl\(_2\).
The assay protocol for cholesterol that we initially used to determine HDL-C after precipitation with Dextran sulfate-Mg\(^{2+}\) has a calibration value (Calibrant I) of 198 mg/dL and it is linear within a concentration range of 25 to 700 mg/dL. This assay is designed to measure both total and HDL cholesterol levels. During the performance of the present study, we observed that the HDL-C values of most hemodialysis patients were particularly low. Moreover, according to the Dextran sulfate-Mg\(^{2+}\) precipitation method, as it is described above, the serum sample is diluted by the precipitation reagent resulting in a cholesterol value which is lower by 10%. Therefore, as a consequence, the HDL-C values that had to be measured in hemodialysis patients were close to the detection limit of cholesterol assay. For the accuracy of the measurement in such low cholesterol values, in the same analyzer, with the same reagents, calibrator and controls, we designed another assay protocol with a calibration value of 98 mg/dL (Calibrant II) that, in addition, is close to the calibration value of the direct HDL-C assay (60 mg/dL). Calibrant II, 98 mg/dL, was prepared by a 2-fold dilution of Calibrant I with saline (9 g/L NaCl). Controls of low cholesterol values were prepared by 2-, 5-, and 10-fold dilution of Beckman controls with saline. Table 2 shows the ranges with Calibrant I, there was a distortion in the low values. However, at these levels Calibrant II was very accurate.

As hemodialysis patients are often sampled for blood, we did not repeat the analysis in the patients where it was already done, instead we performed it with both calibrants on the remaining population. Thus, in 60 samples (22 controls and 38 hemodialysis patients) in the ultracentrifugation/dextran sulfate-Mg\(^{2+}\) precipitation assay, the enzymatic determination of HDL-C in the infranate was performed twice using the two different calibrants. After ultracentrifugation of each serum, the infranatant was divided into two batches, and apo B-containing lipoproteins were precipitated by dextran sulfate-Mg\(^{2+}\). The samples were centrifuged and cholesterol was measured in the two supernatants, twice with Calibrant I and twice with Calibrant II. The resulting HDL-C concentration was, therefore, the mean of 4 measurements for each calibrant. The analysis was performed in duplicate in the limited population in order to ensure that there is a remarkable difference in the serum samples as it was noticed in the experiment with the diluted controls (Table 2).

**DIRECT (HOMOGENEOUS) ASSAY**

The direct Olympus assay was performed according to the manufacturer’s instructions on the Olympus AU560 clinical chemistry analyzer. Reagent 1 contains synthetic polymers and polyanions which stabilise VLDL, LDL, and chylomicrons and shield their cholesterol; in reagent 2 a detergent destroys the HDL structure releasing cholesterol, which is then quantified by cholesterol esterase, cholesterol oxidase, peroxidase, and 4-aminoantipyrine present, as detailed in the manufacturer’s instructions (Olympus Diagnostica). Both reagents are ready to use. The calibration value for cholesterol standard is 60 mg/dL and is included in the routine Olympus System Calibrator. The required serum sample volume is 12 μL.

**TOTAL CHOLESTEROL AND TRIGLYCERIDES MEASUREMENTS**

Concentrations of total cholesterol (TC) and triglycerides (TG) were determined enzymatically on the Olympus AU560 Clinical Chemistry analyzer. Our laboratory is currently participating in the Murex Clinical Chemistry Quality Assessment Programme. Our CV values in this program, in the past 2 years (four cycles) have ranged between 0.99 and 2.14% for cholesterol, and between 1.73 and 3.36% for triglycerides.

**APOLIPOPROTEINS**

Apo A-I and Apo B were measured with a Behring Nephelometer BN100, and reagents (antibodies and calibrators) from Behring Diagnostics GmbH. The assays were calibrated according to the IFCC standards.
Isolation of VLDL, LDL, and HDL

Lipoproteins were isolated by sequential ultracentrifugation at the following densities: \( d < 1.006 \text{ Kg/L} \) for VLDL, \( d = 1.019 \) to \( 1.063 \text{ Kg/L} \) for LDL, and \( d = 1.063 \) to \( 1.210 \text{ Kg/L} \) for HDL. All ultracentrifugations were performed in a Beckman L7-65 ultracentrifuge at 40,000 rpm, 14° C for 24 h, using a type SW41 Ti rotor.

Statistical analysis

The mean and SD of all parameters were calculated by using Microsoft Excel Version 7.0 (Microsoft, Redmond, WA, USA). Student’s t-test and linear regression analysis by the least-squares method were performed using the Statistica Version 5.0 statistical program. The t-tests were judged significant at \( p \leq 0.05 \). Assay bias was calculated as the test method result minus the reference method result. Total error was calculated by combining systematic error and random error. At an HDL-C concentration of \( x_c \), systematic error was equal to \( y_c - x_c \) where \( y_c = bx_c + a \) (linear regression equation). Random error was \( 1.96 \times \text{SD} \) from the between-day precision study (18,19). \( S_{yx} \) represents the standard deviation of the residuals. Precision data were calculated according to recommendations of the NCCLS evaluation protocol EP5-T (20).

Results

Analytical performance

To assess the precision of the new homogeneous HDL-C assay, the three sets of commercial control sera and the set of pooled serum samples, referred to in Materials, were used (Table 3). Within-day CV for the four sets of controls ranged from 1.7% to 3.8% and between-day CV ranged from 1% to 2.3%. The precision of the assay in all controls fell within the 1998 precision goals established by the NCEP (21, 22) (CV \( \leq 4\% \) at concentrations of 42 mg/dL or higher and SD \( \leq 1.7 \text{ mg/dL} \) at concentrations <42 mg/dL). The total imprecision ranged from 2.0% to 4.2%.

Linearity

HDL isolated by sequential ultracentrifugation was added at increasing amounts to a serum pool initially containing 136 mg/dL TC, 86 mg/dL TG and 30.6 mg/dL HDL-C. The direct HDL-C assay is linear up to 220 mg/dL and the linear regression equation is \( y = 1.08x - 2.1 \).

Specificity

The specificity of the assay was evaluated by adding various amounts of VLDL and LDL, isolated by sequential ultracentrifugation, to a serum pool. For the specificity of VLDL, serum pool containing initially 168 mg/dL TC, 160 mg/dL TG, and 41 mg/dL HDL-C was supplemented with given amounts of VLDL to yield a final concentration of TG up to 1660 mg/dL (Figure 1A). The supplementation did not appreciably alter the HDL-C value up to a concentration of TG of 600 mg/dL [mean % HDL-C recovery 102.2 \( \pm 1.47 \) (\( r = 0.1 \), \( p = \text{NS} \)]. After this value an increase which reached 15% at 1660 mg/dL was noticeable. Specifically, at TG concentration 601 \( \pm 1050 \) mg/dL the mean % HDL-C recovery was 105.5 \( \pm 1.7 \) (\( r = 0.4 \), \( p = \text{NS} \)), which differs significantly from 102.2 \( \pm 1.47 \) (\( p = 0.03 \)). Finally, at TG concentration 1051 \( \pm 1660 \) mg/dL mean % HDL-C recovery was 111.5 \( \pm 3.1 \) (\( r = 0.99 \), \( p = 0.006 \)) which differs significantly from 102.2 \( \pm 1.47 \) (\( p = 0.0001 \)) and 105.5 \( \pm 1.7 \) (\( p = 0.002 \)).

For LDL specificity, a serum pool containing TC 204 mg/dL, TG 123 mg/dL, and HDL-C 31 mg/dL

### Analytical Performance for HDL-C

<table>
<thead>
<tr>
<th>Control</th>
<th>HDL-C (mg/dL)</th>
<th>Within-Day</th>
<th>Between-Day</th>
<th>Totala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SDw (mg/dL)</td>
<td>CV (%)</td>
<td>SDb (mg/dL)</td>
</tr>
<tr>
<td>Liquichek</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td>26.2</td>
<td>0.6</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Level 2</td>
<td>75.1</td>
<td>1.6</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Corning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QCS1</td>
<td>39.1</td>
<td>0.9</td>
<td>2.3</td>
<td>0.8</td>
</tr>
<tr>
<td>QCS2</td>
<td>52.1</td>
<td>1.3</td>
<td>2.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Beckman</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td>35.0</td>
<td>1.2</td>
<td>3.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Level 2</td>
<td>39.8</td>
<td>1.5</td>
<td>3.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Level 3</td>
<td>54.5</td>
<td>0.9</td>
<td>1.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Pooled serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pool 1</td>
<td>31.3</td>
<td>1.1</td>
<td>3.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Pool 2</td>
<td>46.5</td>
<td>1.3</td>
<td>2.8</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\[ SD_T^2 = SD_W^2 + SD_B^2 \]
was supplemented with LDL-C up to 330 mg/dL to yield a final concentration increasing to 480 mg/dL (Figure 1B). The elevated levels of LDL-C did not alter the apparent HDL-C concentration [mean % HDL-C recovery 99.6 ± 1.24 (r = 0.1, p = NS)].

**INTERMETHOD COMPARISON**

The homogeneous HDL-C assay was compared with the ultracentrifugation/dextran sulfate-Mg\(^{2+}\) method, as reference method and the conventional dextran sulfate-Mg\(^{2+}\)-based precipitation method, in 50 healthy subjects and 65 hemodialysis patients, who exhibited a wide range of triglyceride levels from 43 to 560 mg/dL and HDL-C levels from 19 to 79 mg/dL (Table 4).

The direct (homogeneous) assay was in good agreement with the ultracentrifugation/dextran sulfate-Mg\(^{2+}\) method with correlation coefficients 0.96 for the healthy subjects and 0.98 for the hemodialysis patients (Figure 2 A,D). When we estimated the parameters of the regression lines, slopes were 0.98 and 0.99, intercepts 1.8 and 2.6 mg/dL and standard deviation 3.7 and 2.2, respectively. The mean bias was 1.0 and 2.3 mg/dL.

The direct method correlated well with the dextran sulfate-Mg\(^{2+}\) method with correlation coefficients 0.98 and 0.99, slopes 0.98 and 1.02, intercepts 3.1 and 1.0 mg/dL, standard deviation 2.8 and 2.0 and mean bias 1.9 and 1.8 mg/dL, respectively, for the two groups studied (Figure 2 B,E). As expected, the dextran sulfate-Mg\(^{2+}\) method correlated well with the reference method in both cases (Figure 2 C,F) (22).

In the 60 serum samples (22 controls and 38 hemodialysis patients), the results of the ultracen-

**Figure 1** — Specificity of the homogeneous assay for HDL-C after adding (A) TG up to 1500 mg/dL (final TG concentration 1660 mg/dL) and (B) LDL-C up to 350 mg/dL (final LDL-C concentration 500 mg/dL).

| Table 4 | Comparison of HDL-C Data from Different Methods |
|-----------------------------------------------|
| Sample size                          | Healthy Subjects | Hemodialysis Patients | Total |
| HDL-C, mean ± SD (mg/dL) | 50 | 65 | 115 |
| Direct\(^a\)          | 51.4 ± 13.7 | 37.1 ± 11.6 | 43.3 ± 14.4 |
| Precipitation\(^b\) | 49.1 ± 13.8 | 35.3 ± 11.2 | 41.2 ± 14.1 |
| Ultracentrifugation\(^c\) | 50.3 ± 13.4 | 34.9 ± 11.5 | 41.6 ± 14.5 |
| Bias, mean ± SD        | 1.0 ± 3.6 | 2.3 ± 2.2 | 1.7 ± 2.9 |
| Direct-Ultracentrifugation | 1.9 ± 2.7 | 1.8 ± 2.0 | 1.8 ± 2.3 |
| Direct-Precipitation   | −0.9 ± 2.8 | 0.5 ± 2.3 | −0.1 ± 2.6 |

**Linear regression\(^d\)**

| Direct-Ultracentrifugation | 0.98x + 1.8 (0.96) | 0.99x + 2.6 (0.98) | 0.97x + 3.0 (0.98) |
| Direct-Precipitation      | 0.98x + 3.1 (0.98) | 1.02x + 1.0 (0.99) | 1.00x + 1.9 (0.99) |
| Precipitation-Ultracentrifugation | 1.00x − 1.1 (0.98) | 0.95x + 2.1 (0.98) | 0.96x + 1.6 (0.98) |

\(^a\)Direct: direct (homogeneous) HDL-cholesterol assay.

\(^b\)Precipitation: dextran sulfate-Mg\(^{2+}\) precipitation method (routine method).

\(^c\)Ultracentrifugation: ultracentrifugation/dextran sulfate-Mg\(^{2+}\) precipitation method (reference method).

\(^d\)y = field method; x = reference method; y = bx + a (r).
trifugation/dextran sulfate-Mg²⁺ obtained by each calibrant were compared with the direct assay performed in duplicate and gave the following statistical parameters: Calibrant I: \( y = 0.97x + 2.9, r = 0.99 \) Calibrant II: \( y = 0.97x + 1.3, r = 0.99 \).

These results demonstrate that homogeneous HDL-C assay which uses a calibrant value close to that of Calibrant II is in much better agreement with the ultracentrifugation/dextran sulfate-Mg²⁺ in low HDL-C concentrations presented by the hemodialysis patients. The difference in the intercept looks similar to that observed in the control experiment in Table 2.

Figure 3 shows the assay bias data [direct method result minus reference method result (Figure 3A) and dextran sulfate-Mg²⁺ method result minus reference method result (Figure 3B)] plotted as a function of serum TG concentration (43–564 mg/dL) in all 115 samples studied. No correlation between the assay bias and the serum TG concentration was evident for either the homogeneous (Figure 3A) or the dextran sulfate-Mg²⁺ (Figure 3B) assays. These results are in agreement with the results of the VLDL specificity experiment (Figure 1A), where TG levels up to 600 mg/dL did not interfere with the direct HDL-C measurement.

**Total Error**

Total error, a measure of the overall analytical performance of the assay, was calculated by combining the systematic error and the random error. Systematic error (the absolute value of \( y - x \)) of the homogeneous assay was calculated by the linear regression formula derived by comparing the assay with the reference method separately in the two populations studied. Random error was calculated as 1.96 × SD of the between-day precision of the Liquichek controls. The total error in low HDL-C (26 mg/dL) was 7.9% for the healthy subjects and 12.0% for the hemodialysis patients. In high HDL-C (72 mg/dL) was 3.2% and 5.3%, respectively. All four calculated total error percentages meet the NCEP performance goals for 1998 (≤13%) (22).

**Discussion**

Conventional HDL-C assays in the clinical laboratory include a chemical precipitation step, which requires both centrifugation and recovery of the supernatant. Even though such precipitation-based methods are precise and give results in agreement with those of ultracentrifugation methods, they are...
time-consuming, require a large volume of serum, and are not amenable to automated analysis. We evaluated a homogeneous polyanion-polymer/detergent assay for HDL-C which eliminates the sample precipitation step and we confirmed that this method allows a direct and fully automated determination of HDL-C. The method is valid not only in normal subjects but most importantly in hemodialysis patients who exhibit a wide range of HDL-C and triglyceride levels at low and high values, respectively (16). The linearity of the new homogeneous HDL-C assay is excellent up to at least 220 mg/dL and the precision of the assay in all commercial controls fell within the 1998 precision goals established by the NCEP at low and at high HDL-C levels.

The method is fully automated and fast (12 minutes), requires only 12 \( \mu \)L of serum sample and reagents do not require reconstitution. For the everyday control of the accuracy, it was shown that the "ready to use" controls containing ethylene glycol can be used. In addition, there is no interference from reducing agents such as bilirubin or ascorbic acid, as reported by the manufacturer and previous studies (6).

The direct assay correlated highly with the ultracentrifugation/dextran sulfate-Mg\(^{2+}\) precipitation method as well as with the conventional dextran sulfate-Mg\(^{2+}\) precipitation procedure in both normal subjects and in hemodialysis patients. Interestingly, the mean bias for the assay was 1.0 and 2.3 compared to ultracentrifugation procedure and 1.9 and 1.8 compared to dextran sulfate-Mg\(^{2+}\) precipitation in the two populations studied.

The total errors calculated in low HDL-C levels (12.0%) and in high HDL-C levels (5.3%) meet the 1998 NCEP performance goal (\(\leq 13\%\)) (22).

The principle of this method is based on the “wrapping” of chylomicrons, VLDL and LDL by synthetic polymers and polyanions. If the wrapping is incomplete, a falsely high HDL-C could be measured leading to a reciprocal error in LDL-C levels, which could be a problem in patients with end-stage renal disease who commonly present with increased concentrations of triglyceride-rich lipoproteins. In this respect, elevated TG levels up to 600 mg/dL did not interfere with HDL-C and the deviation at higher concentration of TG was found to be lower than in other methods. In addition, the assay bias data plotted as a function of TG concentration did not show a significant correlation. Moreover, high LDL-C levels up to 480 mg/dL did not alter the HDL-C concentration.

In routine analysis, in order to be able to assay a wide cholesterol range a single calibration value is used and the instrument assumes linearity between low and very high cholesterol values. A two-point calibration would represent the data better in that the instrument could draw a calibration curve, but it would be limited at the upper range by the high calibrant value chosen. In routine assays for TC a calibrant of 198 mg/dL covers the expected TC range. The assay kit for HDL-C contains a low range calibrant (60 mg/dL). Our results indicate that, when the dextran sulfate-Mg\(^{2+}\) method is used for HDL-C, the routine calibrant of 198 mg/dL gives results which are lower as much as 16.8% (Table 2). The use of a calibrant at a low cholesterol value (60 mg/dL) in the direct HDL-C assay obviates this problem. The information on Calibrant I in Table 2 is useful. It clearly demonstrates that Calibrant I (high cholesterol value) gives an increasing % difference at low cholesterol values, compared with the expected values of the diluted samples. The value of Calibrant II is closer to the calibrant value used by the direct method (60 mg/dL). These are results...
demonstrating the usefulness of the direct method with its own calibrant. It draws attention to the obvious but often neglected fact that the calibrant to be used should be close to the values of the sample.

Conclusion

In the present study, it has been shown that direct HDL-C assay correlates well with the ultracentrifugation/dextran sulfate-Mg2+ assay and dextran sulfate-Mg2+ method, with particular focus on hemodialysis patients, who often present high triglycerides and low HDL-C levels.

This direct (homogeneous) assay for HDL cholesterol uses much less serum sample. It is accurate, fast, convenient to handle and tolerant of high concentrations of triglycerides. It can considerably facilitate the screening of individuals at an increased risk of cardiovascular disease, including hemodialysis patients.

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