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A simple turbidimetric assay designed for the routine screening as well as therapeutic monitoring of native LDL particles

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Abstract

We describe the development and performance of a homogeneous assay for the direct turbidimetric determination of LDL particles in human serum. The assay is based upon the specific agglutination of LDL particles by the polyanion PAMPS. The co-agglutination of VLDL is avoided by the addition of a zwitterionic detergent. Yielding results within 10 min, the assay requires only a small sample volume taken directly from primary serum tubes, i.e., no pretreatment of the sample is necessary. It can be easily applied to routine clinical chemistry analyzers. The results are highly correlated with LDL cholesterol determinations by ultracentrifugation ($r>0.95$) and dextran sulfate precipitation ($r>0.95$), but an increased recovery of small, high density LDL particles is observed, which more adequately reflects the atherogenic risk of LDL. The assay provides excellent intra- and inter-assay CVs in the range of 0.6–1.6 and 1.7–2.4%, respectively, on Roche Diagnostics/Hitachi analyzers. The method is well suited to the high-throughput screening of LDL cholesterol levels.

Keywords: Low density lipoprotein; LDL; LDL particle; Cholesterol

1. Introduction

Low density lipoprotein cholesterol (LDL-C) is well established as the primary risk factor for the development of coronary heart disease (CHD). Epidemiological and clinical studies have demonstrated a strong positive correlation between the concentration of LDL-C in serum and the incidence of CHD [1,2]. Intervention studies have further shown that the lowering of LDL-C levels results in a decrease in the incidence and severity of CHD [3,4], a decrease by 1% in the LDL-C level being thought to produce a decrease by 2% in the risk of CHD [3]. Due to this strong correlation between LDL-C and CHD, the

Abbreviations: ApoB, apolipoprotein B; BSA, bovine serum albumin; CHD, coronary heart disease; FFA, free fatty acids; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; IDL, intermediate density lipoprotein; IDL-C, intermediate density lipoprotein cholesterol; LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; Lp(a), Lipoprotein (a); NCEP, National Cholesterol Education Program; NIH, National Institutes of Health, Bethesda, MD, USA; PAMPS, poly-(2-acrylamido-2-methyl-1-propane sulfonic acid); SDS, sodium dodecylsulfate TG, triglyceride; VLDL, very low density lipoprotein; VLDL-C, very low density lipoprotein cholesterol

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NIH expert panels on the detection, evaluation and treatment of high blood cholesterol in adults have continued to identify LDL-C as ''the primary target of cholesterol-lowering therapy'' [5].

Low density lipoprotein (LDL) comprises a heterogeneous spectrum of particle subclasses differing in size, density, and chemical composition, e.g., cholesterol content [6]. Several case–control studies have established that a predominance of small, dense LDL is associated with an increased risk of myocardial infarction [7,8] and angiographically assessed coronary artery disease [9–11]. In addition, small, dense LDL is also an independent risk factor in diseases commonly associated with atherogenesis, such as non-insulin-dependent diabetes mellitus and familial combined hyperlipidemia [12]. Most recently, a prospective study showed that the presence of small, dense LDL particles is associated with an increased risk of subsequently developing ischemic heart disease (IHD) in men [13].

The current reference method for LDL-C relies on ultracentrifugation and is referred to as β-quantification [14]. This multistep procedure is time consuming and requires expensive equipment and a high level of expertise. It is not suitable for the routine measurement of samples. These comments also hold true for electrophoretic and chromatographic methods [15].

Precipitation methods are also available whereby LDL-C is precipitated by the addition of polyanions such as dextran sulfate [16], polyvinyl sulfate [17,18] or heparin [19] and subsequently removed by centrifugation. The VLDL-C and HDL-C remaining in the supernatant are measured and LDL-C is calculated as the difference between total cholesterol in the sample and cholesterol in the supernatant. These methods, while being widely used for the routine determination of LDL-C, suffer from systematic errors and are sensitive to interferences. Due to these problems another widely used method for the routine determination of LDL-C is its calculation using the Friedewald formula [20]. A major disadvantage of this calculation is the need for three separate determinations, i.e., total cholesterol, HDL cholesterol and triglycerides, which reduces overall accuracy and precision [21]. It also requires the use of samples from fasting patients and it is very sensitive to elevated triglyceride levels, concentrations above 200 mg/dl often leading to false results [22,23].

A recent method for the direct measurement of LDL-C utilizes immunoabsorption to remove HDL-C and VLDL-C from the sample, the remaining LDL-C then being measured directly [24]. However, this method again requires a centrifugation step and this, combined with the high cost per measurement, precludes its use for the screening of large numbers of samples.

All the methods described so far need several determinations and/or labour intensive sample pretreatment steps. One of the first methods designed for the rapid determination of serum LDL without special sample pretreatment was described in 1983 based on the turbidimetric measurement of LDL particles agglutinated by heparin [25]. This method, which can be considered as a precursor of the method described here, showed only an unacceptable correlation to established methods, however.

Reaction principles for the direct determination of LDL-C by enzymatic methods have been described more recently, which are making use of the differences between the different lipoprotein classes to allow the specific determination of LDL-C [26,27].

It has also been proposed to substitute the determination of LDL cholesterol by the determination of total ApoB to circumvent the problem, that the atherogenic risk caused by small, dense LDL particles with their low LDL-C content of only approximately 40% instead of normally approximately 55% is underestimated [6]. However, total ApoB determinations are biased by the ApoB present in VLDL.

The aim of this study was therefore to develop a simple assay for large scale routine screening of LDL particles and LDL-C, respectively. It should be able to rapidly measure LDL particles and the corresponding LDL-C directly from serum without pretreatment of the sample, be applicable to routine clinical chemical analyzers, demonstrate a high level of precision and be insensitive to interference from factors such as lipemia.

2. Materials and methods

2.1. Apparatus

All measurements of LDL-homogeneous, cholesterol and triglyceride were carried out on either a Roche Diagnostics/Hitachi 717, 917 or 911 analyzer.

2.2. Reagents

Reagent kits, calibrators and controls for the determination of cholesterol, triglycerides, free fatty acids, bilirubin, hemoglobin, apolipoprotein B and Lp(a) were obtained from Roche Diagnostics, Mannheim, Germany. The reagents for the determination of LDL by dextran sulfate precipitation (Quantolip® LDL) were obtained from Immuno, Vienna, Austria. Chemicals were obtained from Sigma (Bis–Tris, BSA, $MgCl_2$, SDS), Calbiochem (Zwittergent® 3-14) or Serva Feinbiochemica, Heidelberg, Germany (Celite®545, palmitic acid, stearic acid).

Poly-(2-acrylamido-2-methyl-1-propane sulfonic acid) (PAMPS) was prepared by radical polymerization as follows: 8.412 g of 2-acrylamido-2-methyl-1-propane sulfonic acid were dissolved in 100 ml redistilled water, and the solution was exposed to nitrogen for a period of 30 min. A solution of 22.8 mg ammonium peroxodisulfate in 100 ml redistilled water, which had previously been exposed to nitrogen for a period of 30 min, was added to the reaction mixture while being vigorously stirred, and heated to 50°C. After 30 min, the temperature was raised to 70°C and the reaction mixture reduced to a volume of 20 ml. Using a dialysis tube with an exclusion limit of 12 000–14 000 Da, the reaction mixture was dialyzed against redistilled water for a period of 4 days. The dialysate was evaporated until dryness producing crystalline PAMPS with an average molecular weight of 500 000 Da as determined by gel permeation chromatography.

2.3. Enrichment of serum with free fatty acids

Free fatty acids (FFA) were added to ordinary serum according to Spector and Hoak [28]. The concentration of FFA in the serum sample was quantified using the relevant Roche Diagnostics test kit.

2.4. Quantification of LDL-C

LDL-C was determined using either the new LDL-homogeneous method, dextran sulfate precipitation (LDL-C_{DS}) or an ultracentrifugation/polyanion precipitation method (i.e., β-quantification, LDL-$C_{UC/PhT}$).

For LDL-C_{DS} 1.0 ml of precipitant (Quantolip® LDL) containing dextran sulfate was added to 100 μl of sample. After 25 min at room temperature the reaction mixture was centrifuged at 4000 rpm for 4 min and the cholesterol in the supernatant determined.

For LDL-$C_{UC/PhT}$ 500 μl of serum were overlayed with 500 μl of a solution containing 11.42 g of NaCl/l (solvent density, $d_{20°C}$=1.0063 kg/l) in a thick-walled polycarbonate tube and centrifuged in a TLA 100.3 rotor (Table-Top Ultracentrifuge TL 100, Beckmann Instruments) for 2 h at 100 000 rpm (250 000×g) and 20°C. The supernatant VLDL fraction was removed by aspiration and cholesterol (LDL-C and HDL-C) determined in the infranate. Subsequently, 100 μl of the infranate was mixed with 250 μl of a solution containing phosphotungstate (0.44 mmol/l) and $MgCl_2$ (20 mmol/l), incubated for 10 min at room temperature and finally centrifuged at 11 500 rpm for 5 min in a Eppendorf centrifuge Typ 5413. Cholesterol measured in the supernatant yielded HDL-C. Subtraction of this value from infranate cholesterol resulted in LDL-C.

2.5. LDL-homogeneous

Two hundred and fifty μl of reagent 1 (40 mmol/l Bis–Tris buffer, pH 7.5, containing 24 mmol/l $MgCl_2$, 0.5 g/l bovine serum albumin and 0.06 g/l Zwittergent® 3-14) were added to 5 μl of serum. After a 5-min incubation at 37°C, 50 μl of reagent 2 (40 mmol/l Bis–Tris buffer, pH 7.5, containing 0.15 g/l PAMPS, and 0.06 g/l Zwittergent® 3-14) were added and the resulting turbidity signal measured at 500 nm after a further 5 min at 37°C. To allow comparability with established LDL-C determinations, a calibration curve was generated by the use of two standards of low and high LDL-C concentration as previously determined by ultracentrifugation.

2.6. Investigations of the LDL-PAMPS agglutinate

In order to directly determine the content of cholesterol, Lp(a) and ApoB in the LDL-PAMPS agglutinate the latter was separated from the residual serum matrix by centrifugation as follows:

One ml of reagent 1 was added to 20 μl of human serum and the mixture incubated for 5 min at 37°C. Two hundred μl of reagent 2 were then added. After an additional incubation for 5 min at 37°C the

reaction vial was centrifuged for 10 min at 13 300 rpm in an Eppendorf centrifuge. The supernatant was decanted and used for recovery experiments.

For the quantification of cholesterol the precipitated agglutinate was first dissolved by adding 1 ml of 0.9% NaCl. After the addition of 1 ml Cholesterol HiCo reagent (Roche Diagnostics, art.no. 1730274) the reaction was monitored photometrically.

For the quantification of Lp(a) and apolipoprotein B, the separated agglutinate was washed using 200 μl of a solution containing $MgCl_2$ (24 mmol/l), Zwittergent® 3-14 (0.06 g/l) and PAMPS (0.15 g/l). After carefully decanting the rinsing solution the agglutinate was dissolved with 200 μl of a solution containing Na_2CO_3 (5 g/l), NaCl (9 g/l) and SDS (10 g/l). Despite the large number of manually performed steps the overall reproducibility was good generating CVs of 4.8% for Lp(a) and 3.3% for ApoB ($n=4$, each).

3. Results

The new turbidimetric LDL particle assay (LDL-homogeneous assay) is based on the specific agglutination of LDL particles by the polyanion PAMPS in the presence of Mg^{2+}. Co-agglutination of VLDL particles is avoided by the addition of a zwitterionic detergent (Zwittergent® 3-14). Due to their size the LDL-PAMPS-Mg agglutinates formed cause the scattering of light which can be measured directly turbidimetrically or nephelometrically. The agglutinates are present in a stable form and the turbidity signals highly reproducible. Fig. 1 depicts typical absorption versus time curves obtained with samples of varying LDL-C concentration.

Due to the reaction principle the assay determines the complete, native LDL particle allowing the calculation of the cholesterol fraction of LDL based on the correlation between LDL particles and LDL-cholesterol. This approach is valid for a normal subclass distribution of LDL particles. In order to demonstrate that the turbidity signal generated by the agglutination of LDL particles correlates with the LDL-C content of the serum sample, the LDL-PAMPS agglutinate was separated by centrifugation, and its cholesterol content was determined by enzymatic analysis. PAMPS-induced turbidity can be used as an indirect and quantitative measure of LDL cholesterol as demonstrated in Fig. 2.

Turbidimetric assays can be described on the basis of the Rayleigh-Mie theory of light scattering. To a first approximation turbidity is proportional to the concentration of agglutinates as well as to the square of their radius, i.e., to their cross-sectional area. Thus, in addition to the concentration of particles their size has to be taken into consideration. Based on published data [29] the effect of particle size and cholesterol content of different LDL particle subclasses on the signal of the LDL-homogeneous assay, i.e., the relative turbidity, have been calculated (Table 1 and Fig. 3). As can be seen, identical

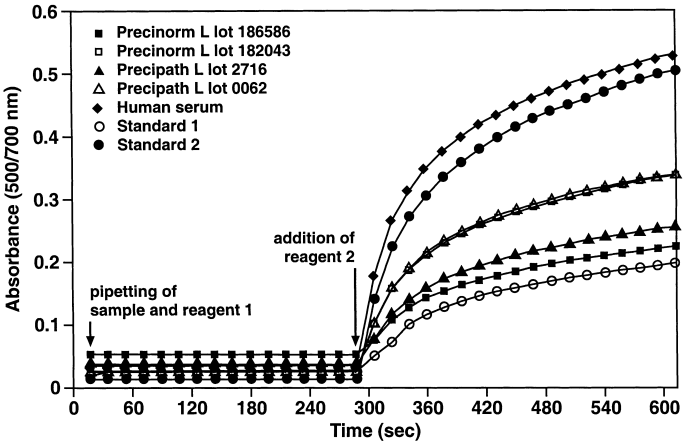

Fig. 1. Turbidity signals of the LDL-homogeneous assay as generated by different samples.

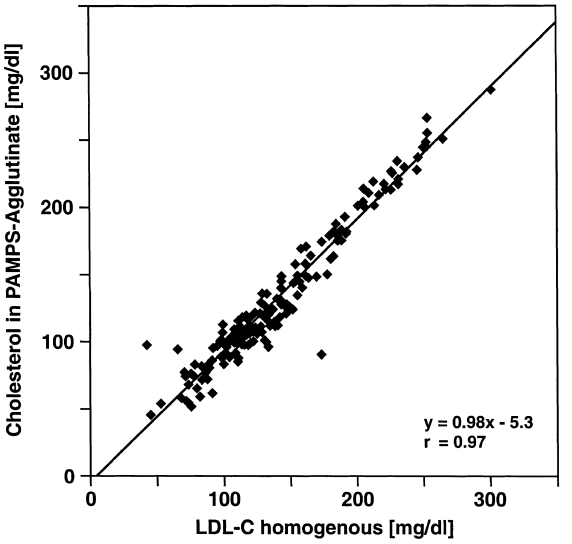

Fig. 2. Comparison of cholesterol concentration in serum samples determined indirectly using the LDL-homogeneous assay and directly in the separated agglutinate using a conventional enzymatic cholesterol assay.

concentrations of LDL cholesterol correspond in the case of LDL-1 (large, low density particles) to a 7% decrease but in the case of LDL-6 (small, high density particles) to a 52% increase in particle number, respectively, when compared to the most common subclass, LDL-3 (nomenclature according to Krauss and Burke [30]). This effect of particle number on turbidity is partially compensated for by the reverse effect of particle size. Combining these two opposite contributions, turbidity varies from 97% for LDL-1 to 124% for LDL-6, when compared to LDL-3.

Since the polyanion PAMPS primarily interacts with clusters of positively charged residues, i.e., the heparin binding site of apolipoprotein B, it was obvious that interference by VLDL was likely to occur. However, co-agglutination of VLDL could be suppressed by the addition of a zwitterionic detergent. A number of zwitterionic detergents were evaluated for their efficiency in masking VLDL-ApoB, and the lipophilic Zwittergent® 3-14 found to be the most suitable.

Due to the interactions occurring in the agglutination reaction, particularly between LDL, PAMPS and $MgCl_2$ as well as between Zwittergent® 3-14 and VLDL, it was necessary to optimize the concentrations of these components. This was done by the use of a multifactorial experimental design study [31]. The potential of Zwittergent® 3-14 to eliminate the interference by VLDL is demonstrated in Table 2. Optimal concentrations of 0.15 g/l PAMPS, 24 mmol/l $MgCl_2$ and 0.06 g/l Zwittergent® 3-14 were determined.

As can be seen in Fig. 3, an increase in triglycerides is parallelled by an increase in relative turbidity, which could be erroneously attributed to an interference of the LDL homogeneous assay by triglycerides or VLDL. In fact, however, the increased relative turbidity reflects the increased contribution of small, dense LDL particles, which

Table 1
Theoretical effect of LDL particle size and cholesterol content on recovery in the homogeneous LDL test[a]

Data for men	LDL 1	LDL 2	LDL 3	LDL 4	LDL 5	LDL 6
Mean diameter (nm)[b]	26.8	26.3	26.3	25.0	24.4	23.8
Normalized square of radius[c] {1} (%)	104	100	100	90	86	82
LDL-C[b] (mg/dl)	115	130	139	140	136	116
ApoB[b] (mg/dl)	81	94	105	116	127	133
ApoB/LDL-C[c]	0.70	0.72	0.76	0.83	0.93	1.15
Normalized number of particles per cholesterol[c] {2} (%)	93	96	100	110	124	152
Normalized turbidity[c] ={1}×{2} (%)	97	96	100	99	107	124

[a] LDL 3, which is the most frequent isoform of LDL in normal population, was used as reference for the calculations.
[b] Data from Campos et al. [9].
[c] Calculated.

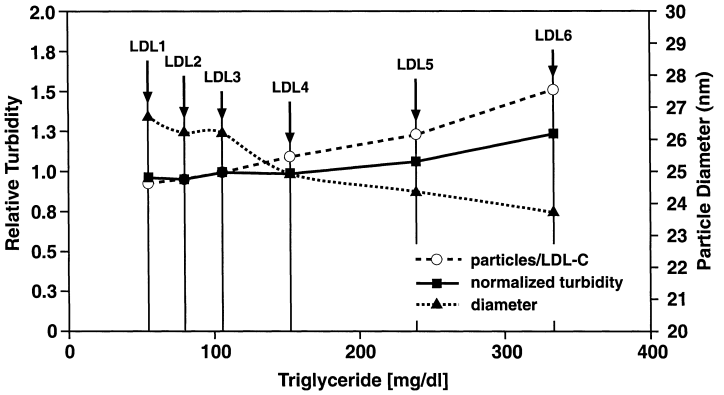

Fig. 3. Size and calculated normalized turbidity of LDL subfractions. Effect of particle number and particle size on LDL-C recovery for LDL subclass when plotted versus TG, which itself correlates to LDL subclass. LDL 3, which is the most frequent isoform of LDL in normal population, was used as reference.

Table 2
Investigation of the effectiveness of Zwittergent® 3-14 in reducing co-agglutination of VLDL particles[a]

	Sample					
	1	2	3	4	5	6
LDL-C (mg/dl)	122	153	178	237	191	182
VLDL-C (mg/dl)	20	35	46	55	65	134
TG (mg/dl)	102	175	229	274	324	672
Zwittergent® 3-14	Recovery of LDL-C (%)					
0.01 g/l	116	112	116	111	128	170
0.05 g/l	106	105	101	100	99	102

[a] The recovery in native serum samples containing known concentrations of LDL-C and VLDL-C — as measured by ultracentrifugation — was determined at different concentrations of the detergent investigated.

predominantly are present at elevated triglyceride levels.

Interference by free fatty acids (FFAs) which are known to interfere in precipitation methods for the quantification of LDL-C [32], was reduced by the addition of BSA to reagent 1. The amount of BSA was optimized by the measurement of a fresh serum pool spiked with FFA. As demonstrated in Fig. 4, a concentration of 0.5 g/l BSA was found to eliminate the FFA interference up to levels of approximately 3

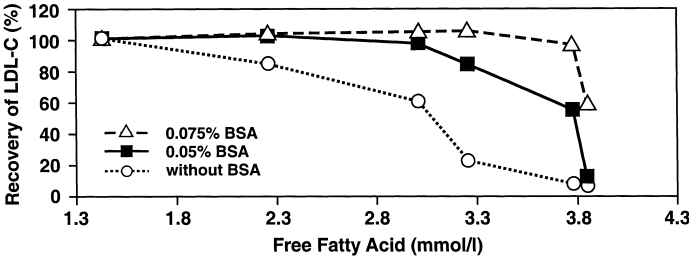

Fig. 4. Effect of the addition of BSA to the LDL-homogeneous reaction mixture. The recovery of LDL-C in a pooled serum sample spiked with FFAs was measured without BSA (♦) and in the presence of 0.5 g/l (■) and 0.75 g/l BSA (▲) in reagent 1.

mmol/l. The addition of higher concentrations of BSA resulted in the elimination of higher levels of FFA interference but produced non-specific effects resulting in an overall decrease in the accuracy of measurement.

As shown in Figs. 5 and 6, the values of LDL-C measured using the LDL-homogeneous assay correlate well with those found by either the reference method β-quantification or a conventional precipitation method. In order to verify the calculated influence of LDL particle size and number (see Table 1 and Fig. 3), the ratio of LDL (cholesterol) values obtained by the new LDL assay and by the indirect LDL cholesterol measurement (dextran sulfate precipitation) was plotted versus triglycerides (note: LDL particle size decreases with increasing serum triglyceride concentration). As demonstrated in Fig. 7 the calculated normalized turbidity per LDL-cholesterol fits reasonably well in fact. This indicates that small LDL particles appear to be overestimated by the new LDL assay when compared to methods, which determine only the comparatively low cholesterol content of this type of LDL particles.

Validation data with respect to the precision of the LDL-homogeneous method are given in Table 3.

The linearity of the LDL homogeneous method

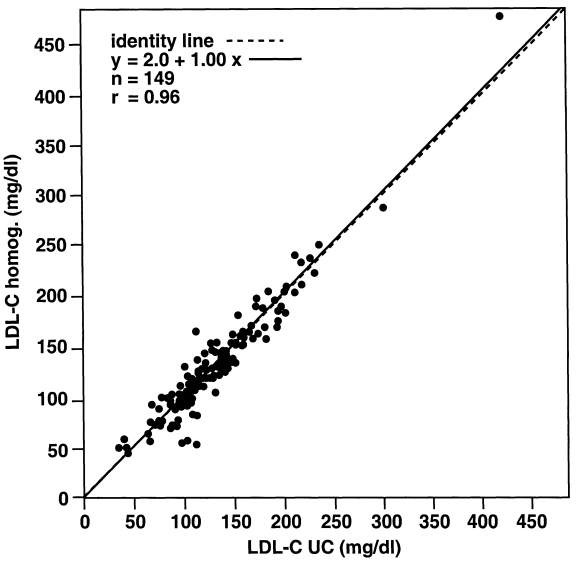

Fig. 5. Comparison of the fully automated LDL-homogeneous assay with the reference ultracentrifugation procedure (LDL-$C_{UC/PhT}$).

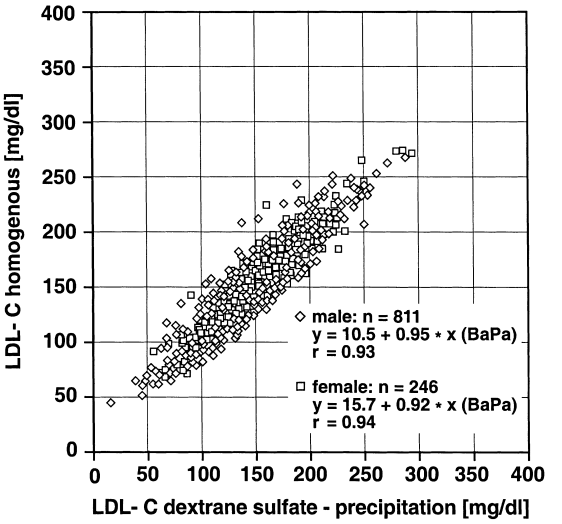

Fig. 6. Comparison of the fully automated LDL-homogeneous assay with a manual dextran sulfate precipitation method (Quantolip® LDL, Immuno, Vienna, Austria).

was measured by dilution of a high LDL-C sample with lipid stripped serum. The assay was found to be linear between 40 and 400 mg LDL-C/dl.

Since PAMPS binds to ApoB it was expected that the LDL-homogeneous method would also measure Lp(a). In order to determine the degree of co-agglutination, Lp(a) was determined directly in the isolated agglutinate. A linear relationship was found between Lp(a) measured in the native serum sample and in the corresponding agglutinate ($Y=0.94X+8.05$; $n=21$; $r=0.92$). From the slope of the regression line it is obvious that Lp(a) particles are co-agglutinated with LDL particles in a quantitative manner.

4. Discussion

The well established role of LDL-C as the primary risk factor for the development of CHD requires that the measurement of LDL-C must be carried out both reliably and easily. When discussing measurements of LDL-C, it must be kept in mind that LDL-cholesterol is actually the cholesterol content of a heterogeneous population of particles. The β-quantification method isolates a population of particles having a hydrated density between 1.006 and 1.063

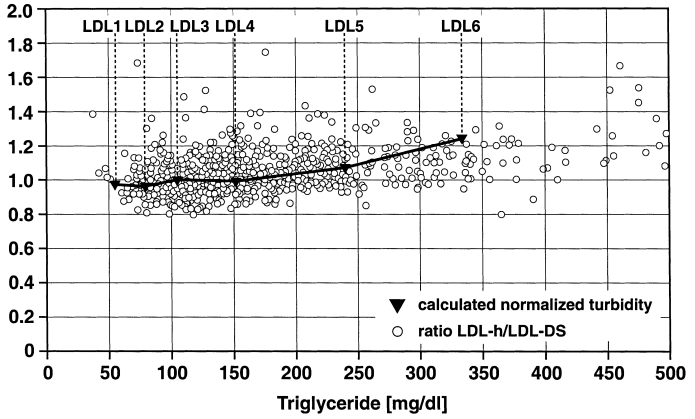

Fig. 7. Ratio of LDL (cholesterol) values obtained by the new LDL-homogeneous assay and by direct LDL cholesterol measurement (dextran sulfate precipitation) versus triglycerides (LDL particle size decreases with increasing triglyceride). Normalized turbidity per LDL cholesterol as shown in Fig. 3.

kg/l. This includes not only LDL but also IDL (1.006–1.019 kg/l) and Lp(a) (1.050–1.080 kg/l).

The most widely used method for the routine determination of LDL-C is currently the calculation by the empirical Friedewald formula. However, this calculation of LDL-C is very problematic under several aspects [21] and it often fails to meet the NCEP performance goals, particularly with respect to the requirement for a precision of ≤4% [33]. Due to the importance of LDL-C as a risk factor for CHD, and since the treatment of hypercholesterolemia may result in a reduction of the LDL-C concentration of less than 10% it is desirable to have a total test variability [34,35] of <5% [30]. Using the Friedewald formula a total test variability of not less than approximately 11% can be achieved [36,37]. In order to reduce the total test variability to an acceptable level, five separate samples of the same patient taken on different days must be analyzed for LDL-C. Due to the postprandial alteration in the distribution of cholesterol between the various lipoprotein fractions, particularly a rise in the levels in the chylomicrons, it is also necessary to obtain samples following a 12–24-h fast in order to calculate LDL-C. This raises obvious problems of noncompliance and errors in the results obtained.

Table 3
Precision of the LDL-homogeneous assay

Sample	PNL[a]	PPL[b]	Human serum 1	Human serum 2
(a) Within run (n=21, Roche/Hitachi 917)				
LDL-C (mg/dl)	108	159	70	172
CV (%)	0.9	0.9	0.6	0.6
(b) Between day (n=21, Roche/Hitachi 917)				
			Human serum	
LDL-C (mg/dl)	128	163	237	
CV (%)	2.1	1.8	1.8	

[a] Precinorm® L.
[b] Precipath® LDL/HDL-C.

The new method described here does not measure the cholesterol content of LDL particles but rather measures the LDL particle itself. This is in contrast to the known procedures for measuring LDL-C. Despite the standardization of the new method against LDL cholesterol determination according to the reference method (β-quantification) it is unlikely, that for all samples an 100% exact correlation to β-quantification will be achieved, since the variable content of cholesterol in different subclasses may cause an LDL subclass specific bias. Nevertheless, a correlation of $r>0.96$ to the reference method β-quantification was found, which allows to claim a good comparability between LDL particle and LDL cholesterol measurements.

We propose the new LDL assay as currently the best approach to routinely assess the atherogenic risk since it takes into account the LDL subclass distribution. The presence of small, dense particles almost certainly plays a role in the level of risk [8], and it has been suggested that the number of LDL particles is more closely related to CHD than the cholesterol content of the LDL fraction [38]. In this context the usefulness of the determination of the cholesterol content of the LDL fraction has been questioned [6] and the determination of total ApoB instead of LDL cholesterol has been proposed as a better alternative. However, total ApoB determinations are biased by the ApoB present in VLDL. The calibration of the new assay according to the LDL-cholesterol reference method (β-quantification) via method comparison allows to stay within the well known range of experience but it nevertheless eliminates the underestimation of atherogenic risk due to the most atherogenic LDL subfraction, i.e., small, dense LDL particles.

The described LDL-homogeneous test shows little interference by triglyceride up to levels of approximately 300–400 mg/dl and, in addition, does not require the use of fasting samples. Moreover, due to its high precision (analytical variability ≤2.5%), the total test variability is lowered to a level which allows the use of at most two serum samples of a patient in order to achieve a reliable diagnosis.

The performance of the assay presents no technical difficulties. All reagents are stable for at least 18 month and, with the exception of the calibrators, are liquid ready-to-use. LDL-C is measured directly from serum, no pretreatment, precipitation or centrifugation of the sample is necessary. The test is rapid with the results being available after 10 min, easy to perform and it is suitable for application on most photometric clinical chemical analyzers using only a low sample volume of approximately 5 μl.

The new turbidimetric LDL assay also measures Lp(a), since the agglutinating reagent PAMPS binds to ApoB present also in Lp(a). The co-measurement of Lp(a) is common to all other routine LDL-C measuring methods as well as the Friedewald method and the reference method, β-quantification. Whether IDL-C is also measured by the LDL-homogeneous method is presently unclear, however, the concentration of IDL-C in most patients is very low and will not have a significant impact on the measurement of LDL-C.

Whether the LDL-homogeneous assay accurately measures samples from patients with Type III hyperlipoproteinemia is presently unknown and remains to be clarified in further clinical trials. These patients have a VLDL that is abnormally rich in cholesterol relative to triglyceride. Measurement of these samples using the Friedewald formula can result in the misclassification of these patients as having Type IIb hyperlipoproteinemia.

In summary, we have described the development and performance of the first direct, nonenzymatic homogeneous assay for the determination of native LDL particles and their cholesterol content. The test is distinguished by its high precision and meets the NCEP performance requirement for an inter-assay precision of ≤4%. In addition only low endogenous interference from triglyceride, VLDL-C, FFAs, bilirubin and hemoglobin are observed. A good correlation to the reference method β-quantification and to the routine method of dextran sulfate precipitation could be demonstrated. Frozen samples and samples from non-fasting patients can be measured. The ease of performance of the assay considerably reduces the laboratory workload and will improve laboratory workflow. Although this test cannot replace β-quantification as the established reference method for the determination of LDL-C, we believe it to be eminently suitable for the initial screening of patient samples. Furthermore it may be used in place of the Friedewald formula or established precipitation methods. Deviant samples should then be further

examined using beta-quantification or other methods such as electrophoresis.

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References

[1] Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins and risk of coronary heart diseases. The Framingham study. Ann Intern Med 1971;74:1–12.
[2] Gordon T, Kannel WB. Castelli, Dawber T.R., Lipoproteins, cardiovascular disease and death: the Framingham study. Arch Intern Med 1981;141:1128–31.
[3] Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial 11: The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. J Am Med Assoc 1984;251:365–74.
[4] Brown BJ, Albers JJ, Fisher LD et al. Regression of coronary artery disease as a result of intensive lipid lowering therapy in men with high levels of apolipoprotein B. New Engl J Med 1990;323:1289–98.
[5] National Cholesterol Education Program. Second Report of the Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults (adult treatment panel 11). National Institutes of Health, National Heart, Lung and Blood Institute. NIH Publication No. 933095, September 1993.
[6] Sniderman AD, Cianflone K. Measurements of apoproteins: time to improve the diagnosis and treatment of the atherogenic dyslipoproteinemias. Clin Chem 1996;42:489–91.
[7] Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. J Am Med Assoc 1988;260:1917–21.
[8] Griffin BA, Freeman DJ, Tait GW, Thomson J, Caslake MJ, Packard CJ, Shepherd J. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. Atherosclerosis 1994;106:241–53.
[9] Campos H, Genest JJ, Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, Wilson PWF, Schaefer EJ. Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 1992;12:187–95.
[10] Coresh J, Kwiterovich PO, Smith HH, Bachorik PS. Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. J Lipid Res 1993;34:1687–97.
[11] Krauss RM. Dense low density lipoproteins and coronary artery disease. Am J Cardiol 1995;75:53B–7B.
[12] Slyper AH. Low-density lipoprotein density and atherosclerosis. Unraveling the connection. J Am Med Assoc 1994;272:305–8.
[13] Lamarche B, Tchernof A, Moojani S, Cantin B, Dagenais GR, Lupien PJ, Després J-P. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Circulation 1997;95:69–75.
[14] Lipid Research Clinics Program. Manual of Laboratory Operations, Vol 1, Lipid and Lipoprotein Analysis. National Institutes of Health, National Heart and Lung Institute. DHEW Publication No. (NIH) 75-628, May 1974.
[15] Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID. Measurement of low-density lipoprotein cholesterol in serum: a status report. Clin Chem 1992;38:150–60.
[16] Armstrong VW, Seidel D. Evaluation of a commercial kit for the determination of LDL cholesterol in serum based on precipitation of LDL with dextran sulfate. Arztl Lab 1985;31:325–30.
[17] Assmann G, Jabs HU, Kohnert U, Nolte W, Schriewer H. LDL cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate. Clin Chim Acta 1985;140:7783.
[18] Kerscher L, Schiefer S, Draeger B, Maier J, Ziegenhorn J. Precipitation methods for the determination of LDL-cholesterol. Clin Biochem 1985;18:118–25.
[19] Wieland H, Seidel D. A simple specific method for precipitation of low density lipoproteins. J Lipid Res 1983;24:904–9.
[20] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[21] Aufenanger J, Zawta B. LDL cholesterol: don't guess. Measure it. Clin Lab 1999;45:617–22.
[22] McNamara JR, Cohn JS, Wilson PWF, Schaefer EJ. Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. Clin Chem 1990;36:36–42.
[23] Wilson PWF, Zeck LA, Gregg RE, Schaefer EJ, Hoeg JM, Sprecher DL, Brewer HB. Estimation of VLDL cholesterol in hyperlipidemia. Clin Chim Acta 1985;151:285–91.
[24] McNamara JR, Cole TG, Contois JH, Ferguson CA, Ordovas JM, Schaefer EJ. Immunoseparation method for measuring low density lipoprotein cholesterol directly from serum evaluated. Clin Chem 1995;41:232–40.

[25] Bartl K, Ziegenhorn J, Streitberger I, Assmann G. Turbidimetric kinetic method for serum low density lipoprotein quantitation. Clin Chim Acta 1983;128:199–208.
[26] Akizawa K, Chiba H. Basic performance and stability to hypertriglyceridemia and increased apoE-rich HDL of a homogeneous LDL cholesterol assay. Clin Chem 1998;44(Suppl):A81.
[27] Sugiuchi H, Irie T, Uji Y, Ueno T, Chaen T, Uekama K, Okabe H. Homogeneous assay for measuring low-density lipoprotein cholesterol in serum with triblock copolymer and a-cyclodextrin sulfate. Clin Chem 1998;44:522–31.
[28] Spector AA, Hoak JC. An improved method for the addition of long-chain free fatty acid to protein solutions. Anal Biochem 1969;32:297–302.
[29] Campos H, Blijlevens E, McNamara JR, Ordovas JM, Posner BM, Wilson PWF, Castelli WP, Schaefer EJ. LDL particle size distribution. Results from the Framingham offspring study. Arterioscler Thromb 1992;12:1410–9.
[30] Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982;23:97–104.
[31] Haaland PD. In: Biotechnology experimental design, New York: Marcel Dekker, 1989.
[32] Siekmeyer R, Mdrz W, Groí W. Insufficient accuracy and specificity of polyanion methods for quantifying low-density lipoproteins. Clin Chem 1990;36:2109–13.
[33] Bachorik PS, Ross JW. National Cholesterol Education Program recommendations for measurement of low-density lipoprotein cholesterol. Clin Chem 1995;41:1414–20.
[34] The Lipid Research Clinics Program. The Lipid Research Clinics Primary Prevention Trial Results 1. Reduction in the incidence of incidence of coronary heart disease. J Am Med Assoc 1984;251:351–64.
[35] Aufenanger J, Zawta B. Pre-analytical aspects of lipoprotein measurement. Clin Lab 1999;45:535–46.
[36] Schectman G, Sasse E. Variability of lipid measurements: relevance for the clinician. Clin Chem 1993;39:1495–503.
[37] Cooper GR, Myers GL, Smith J, Schlant RC. Blood lipid measurements. Variations and practical utility. J Am Med Assoc 1992;267:1652–60.
[38] Brown WV. Lipoproteins: what, when, and how often to measure. Heart Disease and Stroke Jan/Feb 1992:20–6.