

Development of novel homogeneous assay for small dense LDL cholesterol

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OBJECTIVES

Small, dense LDL (sd LDL) is one of such sub-fractions of low-density lipoprotein (LDL) with a smaller particle size and higher density. It is known as an atherogenic lipoprotein, and many epidemiological and pathological studies have recently suggested the relationship between sd LDL-cholesterol (sd LDL-C) level and CHD occurrence. Several different methods have been employed for the determination of LDL particle size such as based on ultracentrifugation, electrophoresis, and nuclear magnetic resonance (NMR). All these methods either require special equipment or very long assay time, making them too laborious for general clinical use. A few years ago we developed a more convenient method for sd LDL-C quantification consisting of 2 steps, the 1st step removing unintended lipoproteins by polyanion and a filter and the 2nd step measuring the cholesterol in sd LDL on an automated, routine chemistry analyzer. That method was much more convenient than the conventional methods, however, it required an off-line sample pretreatment process. We recently developed a fully automated, homogeneous assay for sd LDL-C quantification. We describe the design of the new assay and report the verification results of the assay performance.

METHODS

Our new assay for sd LDL-C quantification employs two liquid, ready-to-use reagents and consists of two steps; the 1st step to decompose non-sd LDL lipoproteins, and the 2nd step to measure sd LDL-C (Fig.1). The reagent for the 1st step of the assay contains 3 key ingredients; () a polyoxyethylene benzylphenyl ether derivative that selectively decomposes chylomicron, VLDL, HDL (Fig.2), () a polyoxyethylene distyrenelphenyl ether derivative that selectively binds to sd LDL to protect sd LDL from the action of enzymes, and () a sphingomyelinase that has much more affinity to Large LDL particle than sd LDL. These ingredients work to decompose the other lipoproteins than sd LDL. Cholesterol released from such non-sd LDL lipoproteins are degraded to water and oxygen by the action of cholesterol esterase, cholesterol oxidase and catalase. At the 2nd step, cholesterol is released from sd LDL by the action of another detergent and lead to color development. Fig.3 shows the specificity of the new assay against LDL subfractions separated by ultracentrifugation. Our new assay can be applied to both serum and plasma samples and is completed in 10 minutes in a completely automated manner on routine chemistry analyzers without any off-line process.

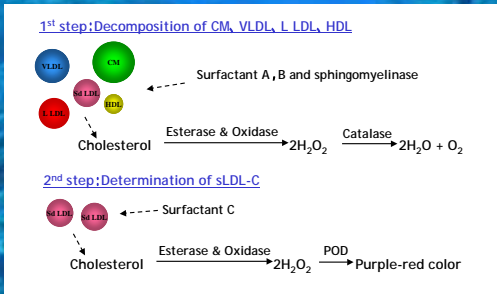


Fig.1. Principle of the homogeneous assay for sd LDL-C.

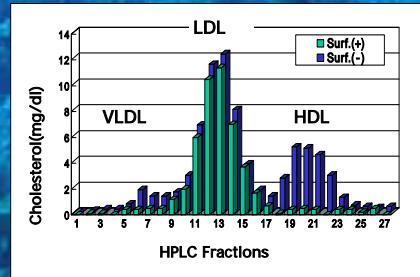


Fig.2. Specificity of the polyoxyethylene benzylphenyl ether derivative.

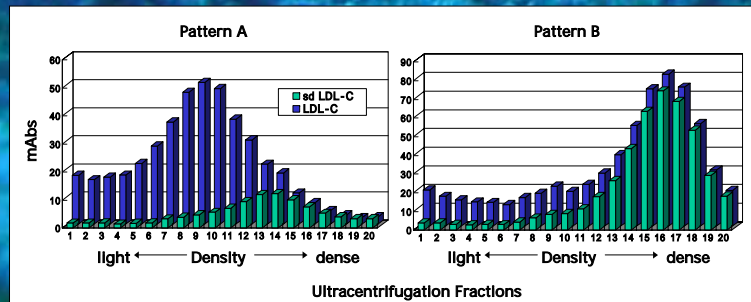


Fig.3. Specificity of the new assay to various LDL subfractions.

RESULTS

The new assay exhibited CVs of 0.39–.65% in within-run precision studies. The detection limit was found as 1 mg/dL and the assay linearity was observed up to 100 mg/dL. No significant interference was observed with 20 mg/dL bilirubin, 500 mg/dL hemoglobin, 5% Intralipid, 50 mg/dL ascorbic acid and in all the cases the recoveries of sd LDL-C were in the range of 100% +/- 5%. The new assay was compared to the traditional ultracentrifugation method using 60 samples and the linear regression analysis showed; $y = 0.99x - 3.4$, $r = 0.954$. The comparison study with our previous method using polyanion and a filter also showed excellent correlation ($y = 0.99x - 1.5$, $r = 0.904$). Test samples were stable for the new assay at least for 7 days in a refrigerator. Consistent test results were also obtained when test samples were subject to repeated freezing and thawing at least for 5 cycles. In a case-control study employing CAD (n=130) and non-CAD (n=221) people, sd LDL-C measured by this new assay was significantly higher in CAD group compared to non-CAD group ($p < 0.005$).

Table1. Within-run precision of the new assay.

	Cont I	Cont II	Ser-Low	Ser-High
Mean	16.5	50.6	21.8	52.8
SD	0.11	0.23	0.12	0.21
CV	0.65%	0.44%	0.57%	0.39%

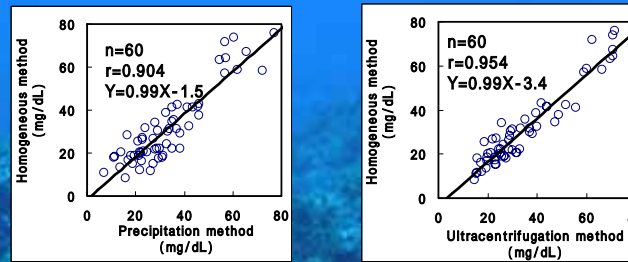


Fig.6. Method comparison with the precipitation method and the ultracentrifugation method.

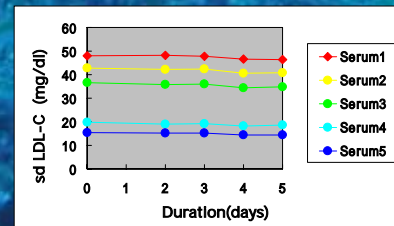


Fig.7. Sample stability (In a refrigerator).

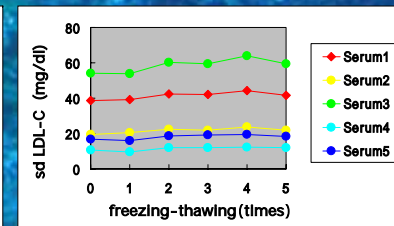


Fig.8. Sample stability (freezing-thawing).

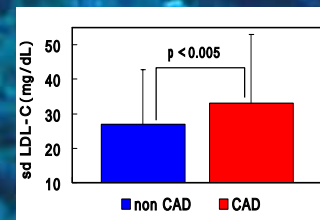


Fig.9. Comparison between non-CAD and CAD subjects.

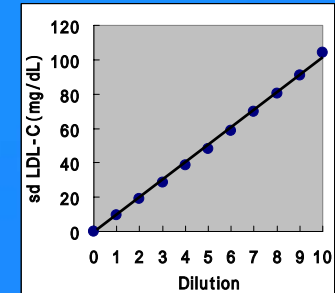


Fig.4. Linearity of the new assay.

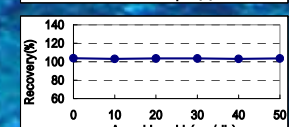
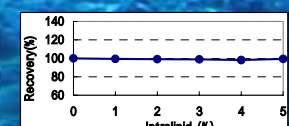
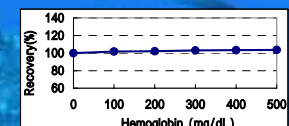
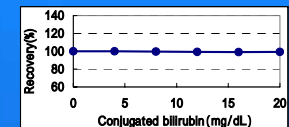
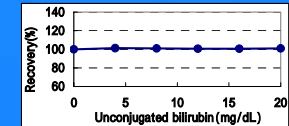


Fig.5. Interference of endogenous substances.

CONCLUSION

Several studies were published using our previous method for sd LDL-C quantification using polyanion and a filter in terms of the risk assessment of CHD, the diagnosis of the familial combined hyperlipidemia, the assessment of the metabolic syndrome, etc. Our new assay showed the excellent correlation with that previous method and was verified to possess robust and valid assay performance. The new assay does not require any off-line sample pretreatment process and thus much simpler and quicker than the previous method. This new assay may contribute to the wide spread use of sd LDL-C.