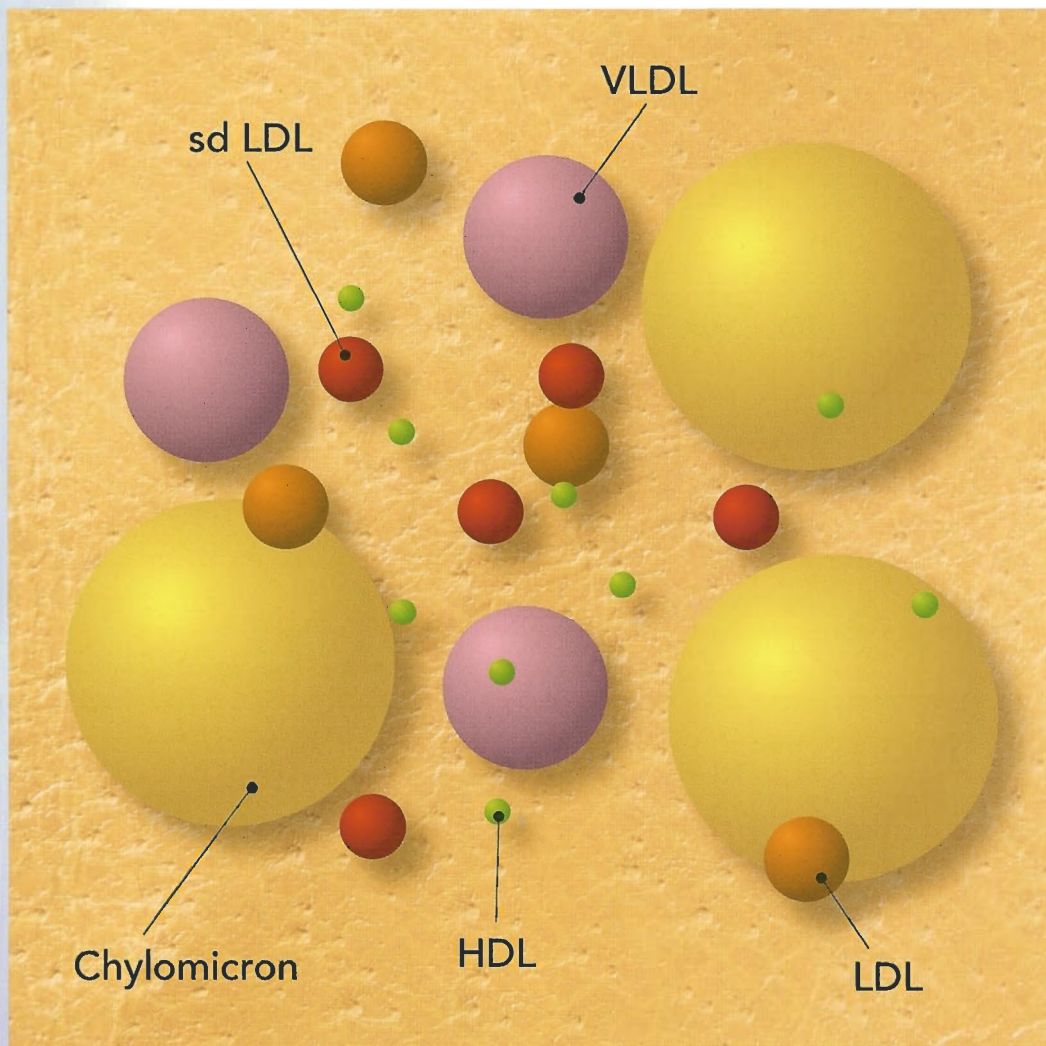


World First

sd LDL-C "SEIKEN"

Reagent for the quantitative determination of
small, dense LDL



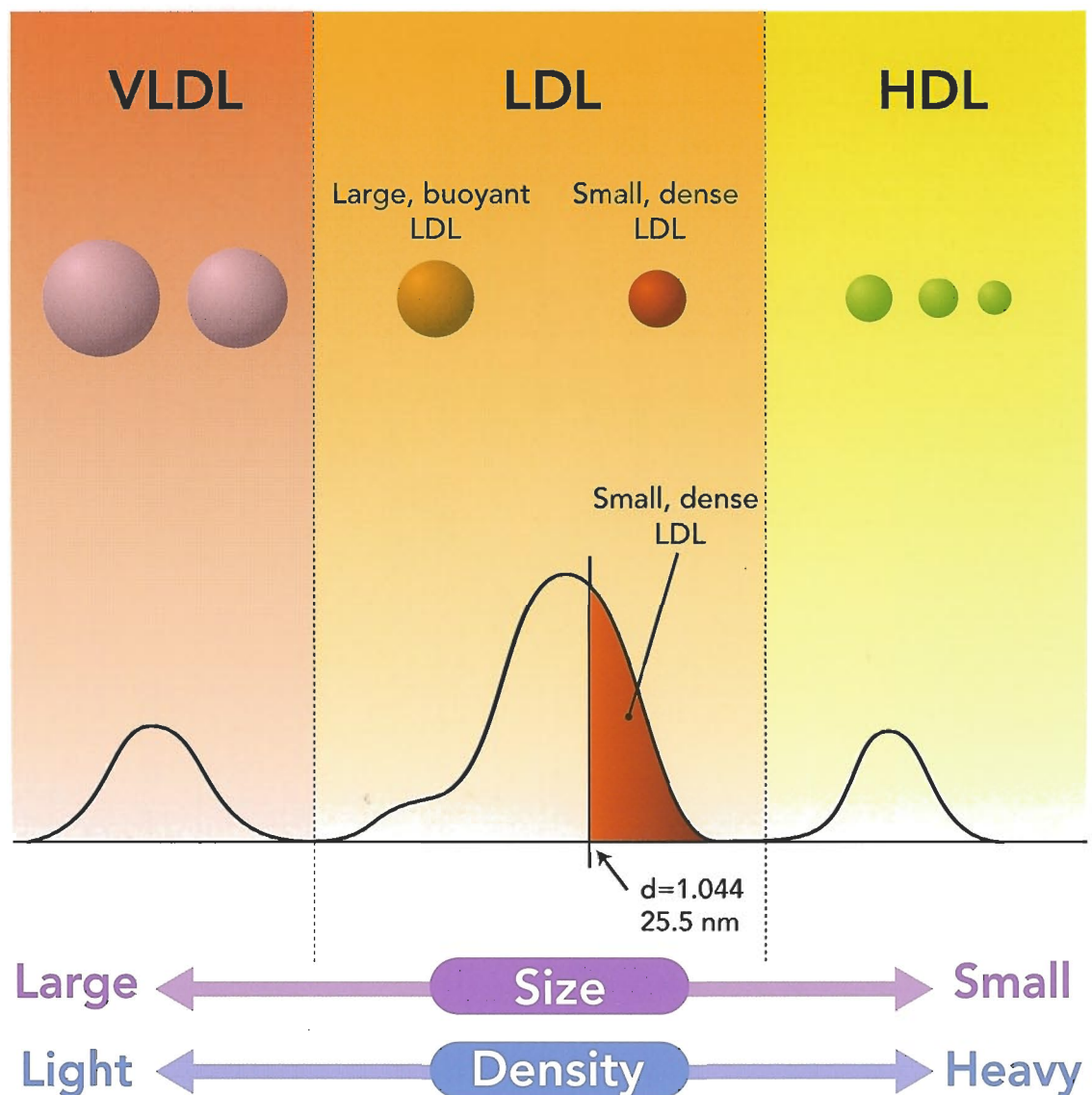
DENKA SEIKEN CO., LTD.

sd LDL-C "SEIK

Applicable on available Chemistry Autoanalyzers, Simple, Easy

What is small, dense LDL?

- > Lipoproteins are different in and classified by their particle size, molecular weight and density.
- > Subclasses exist within each lipoprotein class.

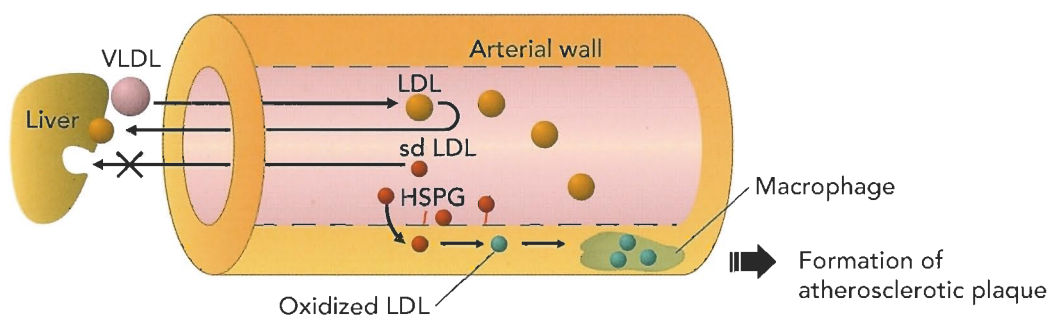


EN^{II}

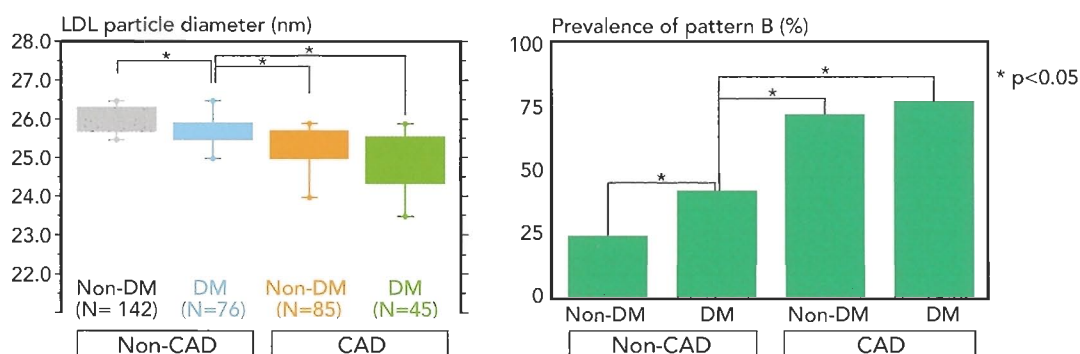
to operate, Results in 30 minutes, Excellent precision <5%

Why small, dense LDL is clinically important?

Potential atherogenic mechanisms

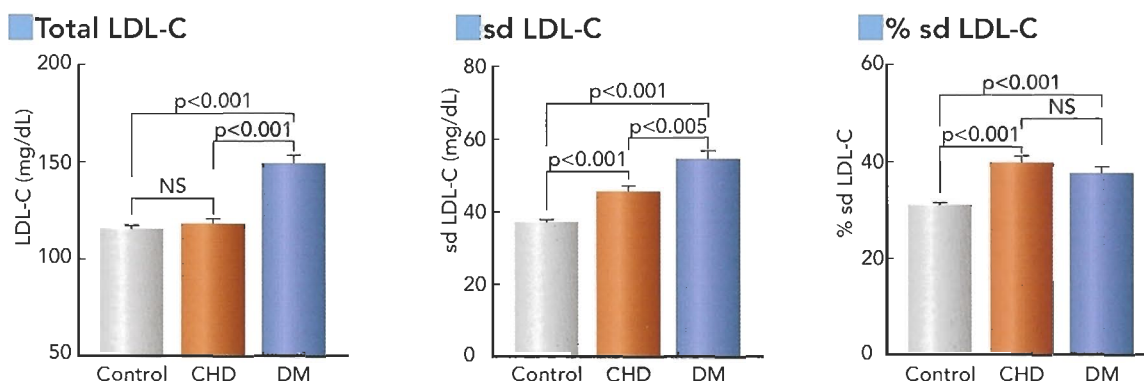


LDL size is inversely proportional to the severity of the disease
 Pattern B is significantly prevalent in CAD patients compared with the control group



(Koba S, Hirano T, et al: Atherosclerosis 160:249-256, 2002)

LDL-C and sd LDL-C level in patients with CHD and DM



What methods are currently available?

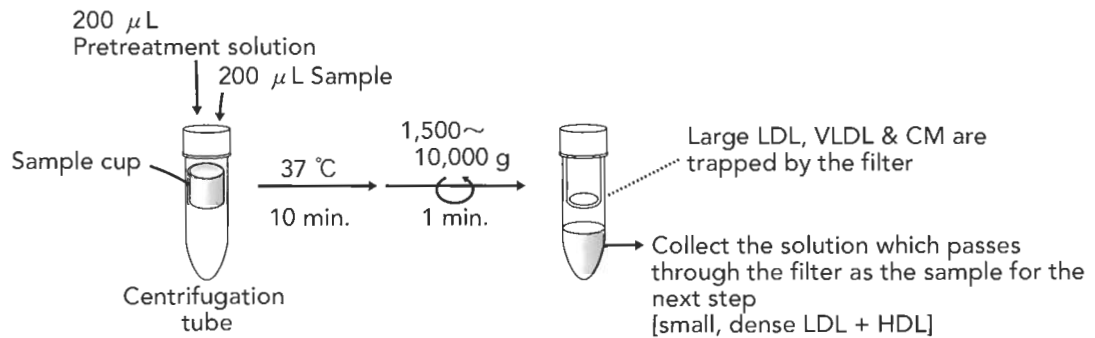
Ultracentrifugation, Electrophoresis and/or NMR-based method
 >>> Time consuming, Expensive, Technically demanding,
 Need special equipment



LDL-C "SEIKEN" Test procedures

Assay Step-1 (pretreatment of sample)

Off-line pre-treatment to remove large, buoyant LDL with polyanion and divalent cations.

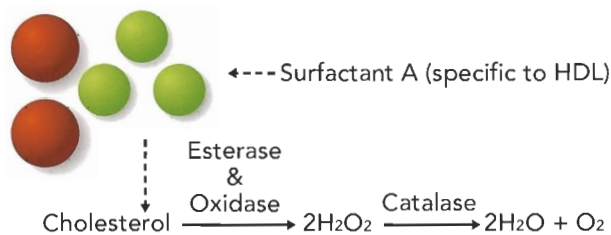


Assay Step-2 (with chemistry analyzer)

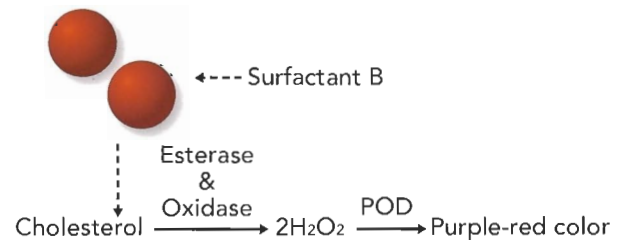
Determination of small, dense LDL-cholesterol with liquid reagents using Chemistry analyzer.

Determination of cholesterol in sd LDL by 2-reagent assay.

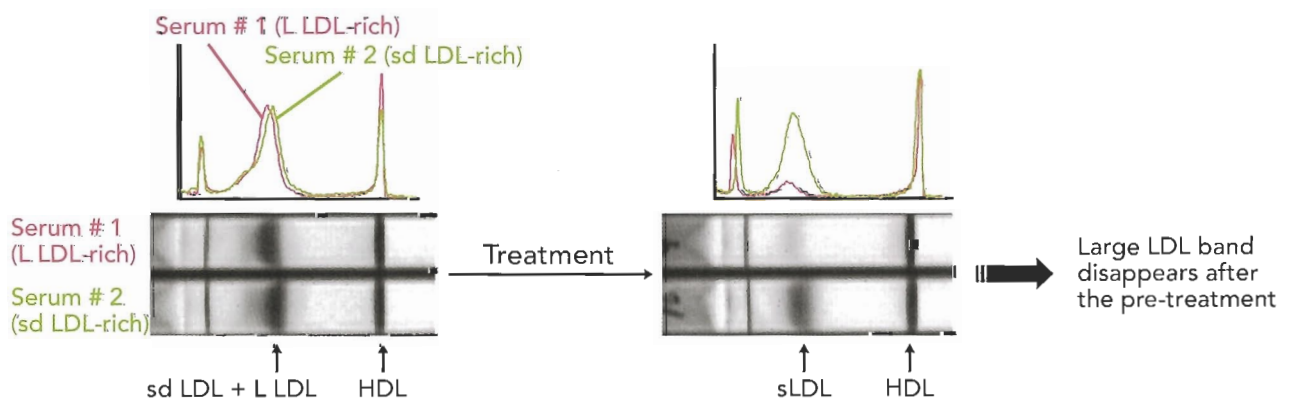
Decomposition of HDL (by R-1)



Determination of sLDL-C (by R-2)



Effect of sample pre-treatment

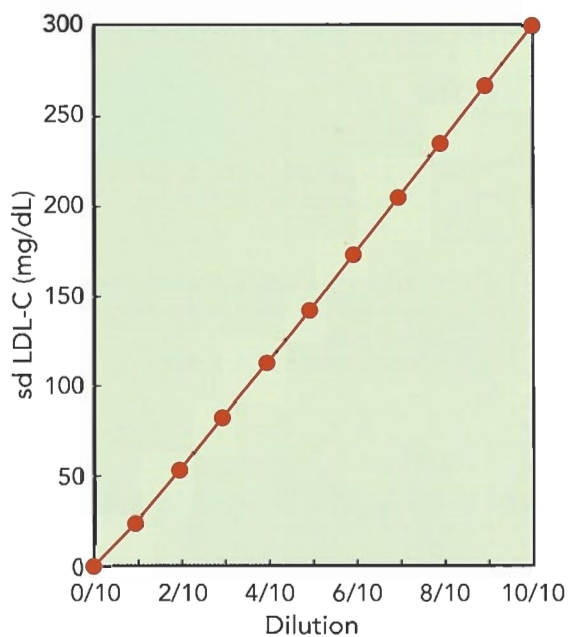


Characteristics "SEIKEN"

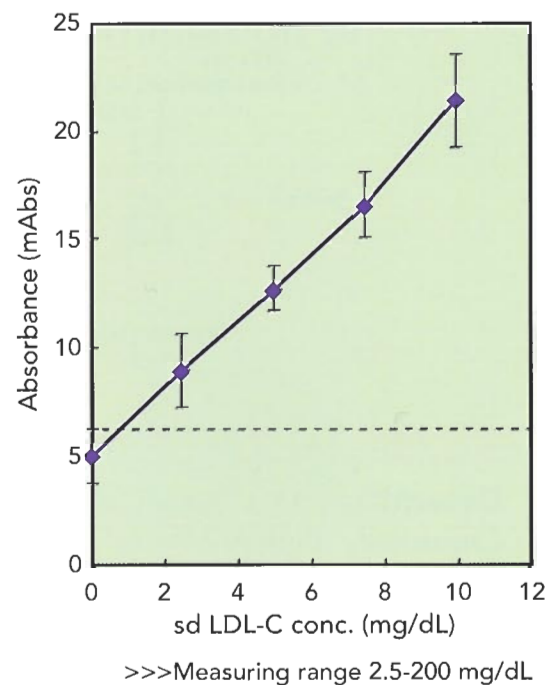
- > Quantitatively measures small, dense LDL
- > Procedure is simple, no special technique is required
- > No need for special equipment
- > Results in 30 minutes
- > Cost effective

Performance "SEIKEN"

Linearity



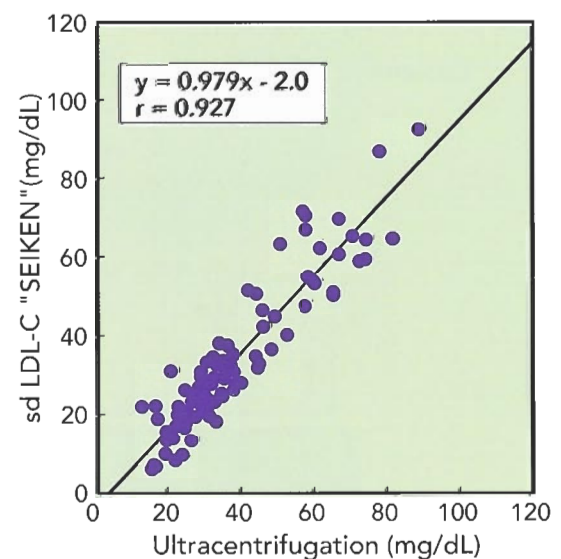
Lower detection limit



Reproducibility

	Control 1	Control 2
1	25.4	70.8
2	26.5	70.3
3	27.0	73.1
4	26.1	71.5
5	27.4	70.5
6	26.4	70.9
7	25.5	72.1
8	25.5	72.0
9	27.3	72.1
10	27.0	72.8
n	10	10
Mean	26.4	71.6
SD	0.76	0.97
CV (%)	2.9	1.3

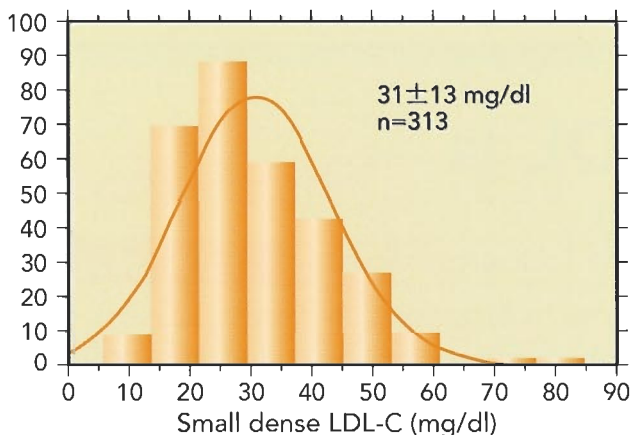
Correlation with ultracentrifugation



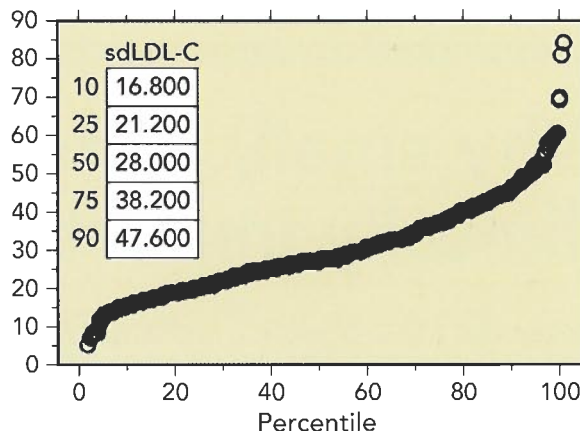
89 samples from healthy people, CAD and Diabetic patients

Normolipidemic controls

Frequency

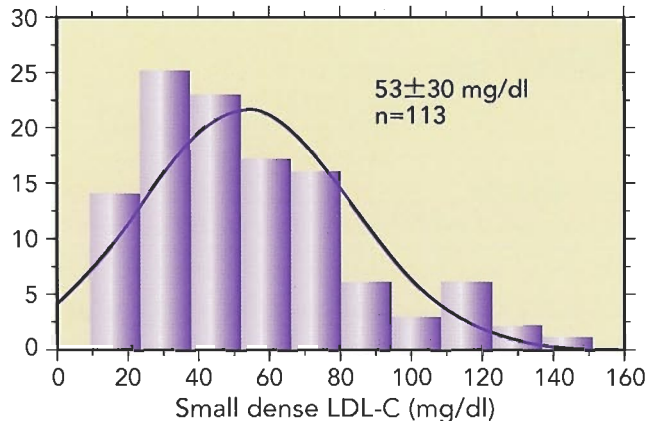


Small dense LDL-C (mg/dl)

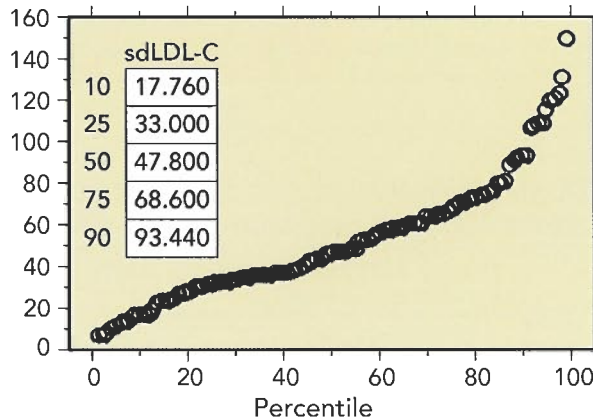


CHD

Frequency



Small dense LDL-C (mg/dl)



Upper panel Histogram and percentiles of small dense LDL-C in Healthy subjects with normolipidemia (TG<150 mg/dL and LDL-C<140 mg/dL).

Lower panel Histogram and percentiles of small dense LDL-C in patients with CHD.

(Hirano T, et al : Arterioscler Thromb Vasc Biol., March, 2004)

References

- 1) Austin MA. et al, Low-density lipoprotein subclass patterns and risk of myocardial infarction, JAMA, 260, 1917 (1988) .
- 2) Griffin BA. et al, 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, 106, 241 (1994) .
- 3) Hirano T. et al, A novel and simple test method for quantification of small, dense LDL, J. Lip. Res., 44, 2193 (2003) .
- 4) Hirano T. et al, Clinical significance of small, dense low-density lipoprotein cholesterol levels determined by the simple precipitation method, Arterioscler. Thromb. Vasc. Biol., 24, 558 (2004) .