



Small Dense LDL

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Small LDL Correlates with CVD Risks



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- People with predominance of small, dense LDL (i.e., Pattern B patients) have a 3-fold increased risk of myocardial infarction (Austin M A ,et al, JAMA, 1988).
- Relative risk is 4.5 for CAD and 7 for MI when small, dense LDL > 100mg/dL (Griffin B A ,et al, Atherosclerosis, 1994).

Reports on sd LDL at ISA 2006



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- Small dense LDL are strongly correlated with increased CHD risk especially the proportion and absolute levels. [GGE] (B. Lamarche, Quebec, Canada)
- Small LDL has prolonged plasma residence time compared to large LDL as shown by stable isotope kinetics (B. Lamarche, Quebec, Canada)
- Ashkenazi Jews of exceptional longevity and their offsprings possess larger LDL and HDL particles and increased HDL-C. [NMR] (N. Barzilari, NY, USA)

Reports on sdLDL at ISA 2006



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- In a free-living population with no sign or history of CVD nor on any lipid-lowering therapy, LDL size and HDL-C are the only independent predictors of carotid IMT. Only LDL-C carried in the remnant and sdLDL fraction is strongly correlated with degree of IMT. [UC] (A. Zambon, Milan, Italy)
- In women with angiographically confirmed CAD, both small LDL and HDL phenotypes are predictive of increase in risk. Small HDL alone has greater predictive value. [GGE] (P. Blackburn, Quebec, Canada)

Challenges of sd LDL Interpretation



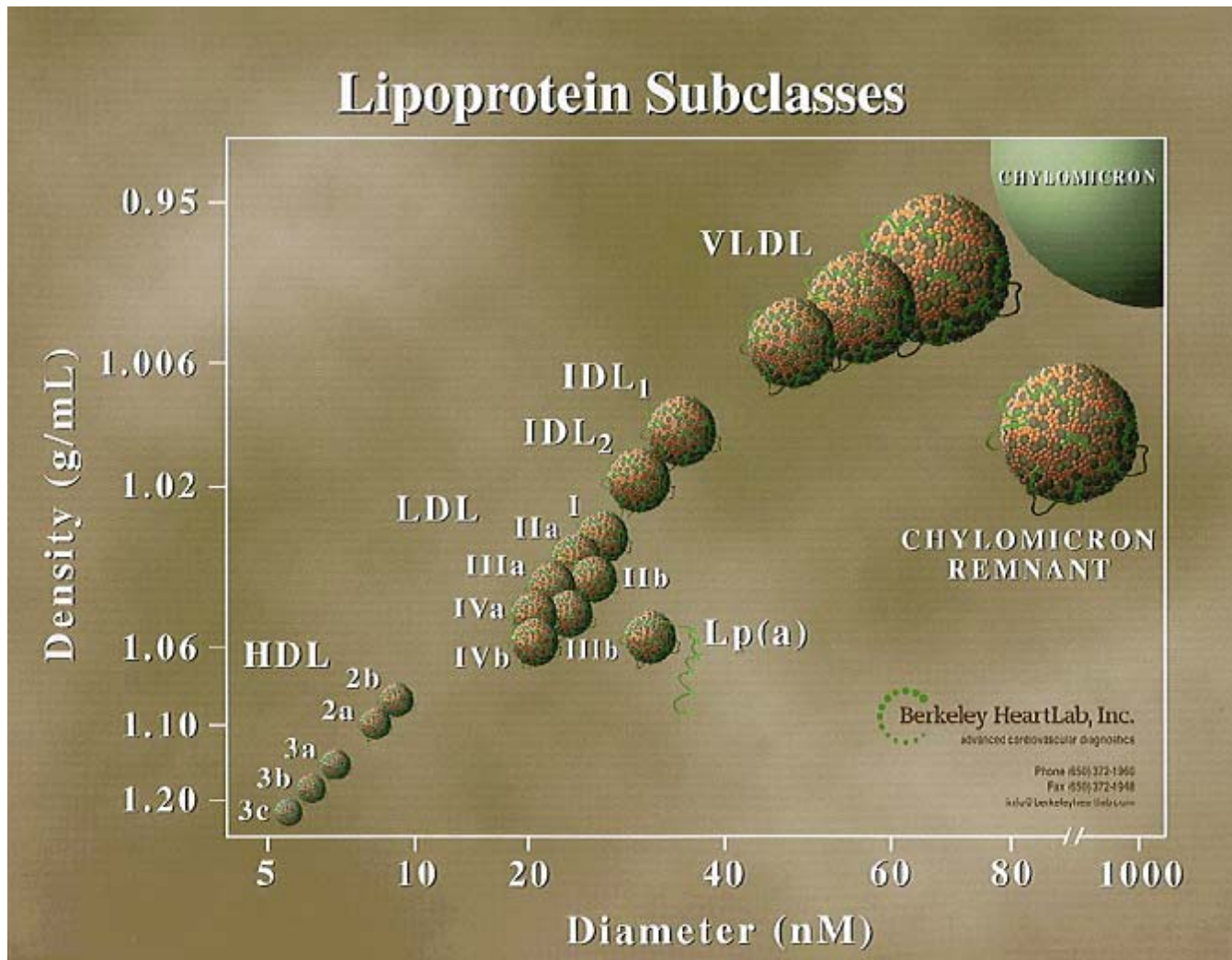
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- Epidemiologic data in support of small LDL being an independent risk factor is still lacking
- Proper interpretation is hampered by the wide variety of LDL subclass measurements used in clinical studies
- Current LDL subclass measurements are based on different principles and are non-standardized among or within technology and non-standardized in reporting convention
- Available methods are often technique demanding, require special equipment and may not be suitable for routine use

Lipoprotein Subclasses



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Definitions for small, dense LDL



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Lipoproteins	VLDL	L LDL	sd LDL *	HDL
Diameter (nm)	30 – 80	25.5 - 28.0	22.0 - 25.5	7 – 10
Density (g/mL)	<1.006	1.019 - 1.044	1.044 - 1.063	1.063 - 1.210

* Definition employed at Denka Seiken. The density range of 1.044 – 1.063 g/mL corresponds to a diameter range of 22.0 – 25.5 nm, that is, Pattern B.

Lipoprotein Subclass Measurements



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Method	Principle	Reporting	Availability
GGE	<ul style="list-style-type: none"> •Non-denaturing gradient gel •Separate by size in electrical field 	Size, % fraction	Home brew / proprietary commercial lab
NMR	<ul style="list-style-type: none"> •Proton NMR •Separate by methyl group signal 	Size and particle number; quantification by convoluted algorithm	Proprietary commercial lab
UC	<ul style="list-style-type: none"> •Sequential or density gradient UC •Separate by density •Direct component measurement 	Size, lipid components; subclass quantification by convoluted algorithm in sequential UC	Home brew / proprietary commercial lab
PAGE	<ul style="list-style-type: none"> •Fixed concentration disk PAGE •Separate by charge 	Ave peak size, % fraction	Home brew / Commercially available
HPLC	<ul style="list-style-type: none"> •Size exclusion chromatography •Separate by size •Direct component measurement 	Peak size; lipid components; subclass quantification by convoluted algorithm	Home brew / proprietary commercial lab
Polyanion-cation Precipitation	<ul style="list-style-type: none"> •Heparin-Mg precipitation •Apo content, selective detergent and enzymes •Direct component measurement 	Small dense LDL chol and other components	Commercially available

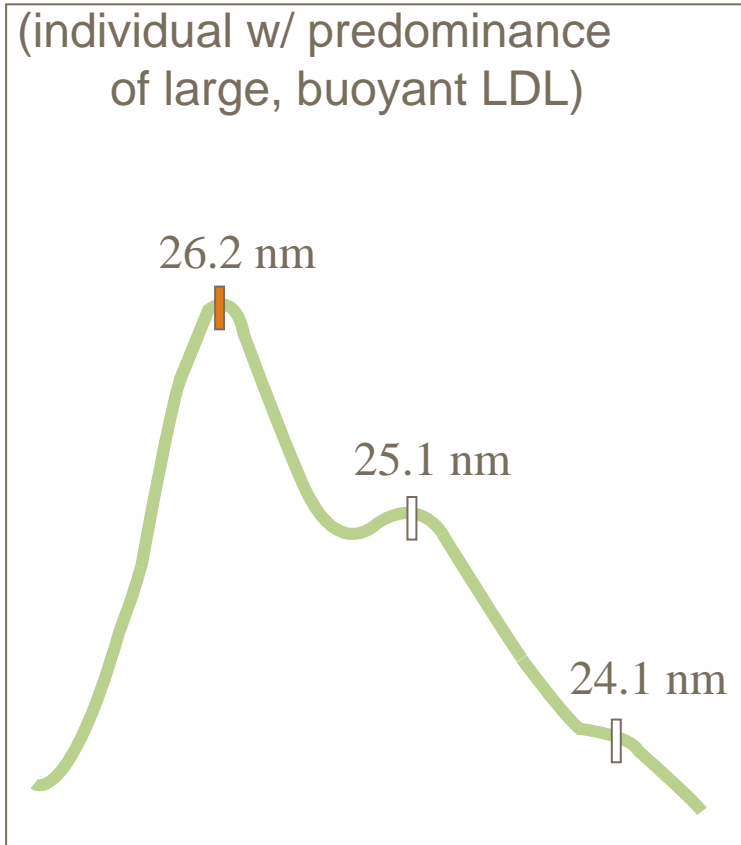
Grouping of patients with major particle size



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Pattern A

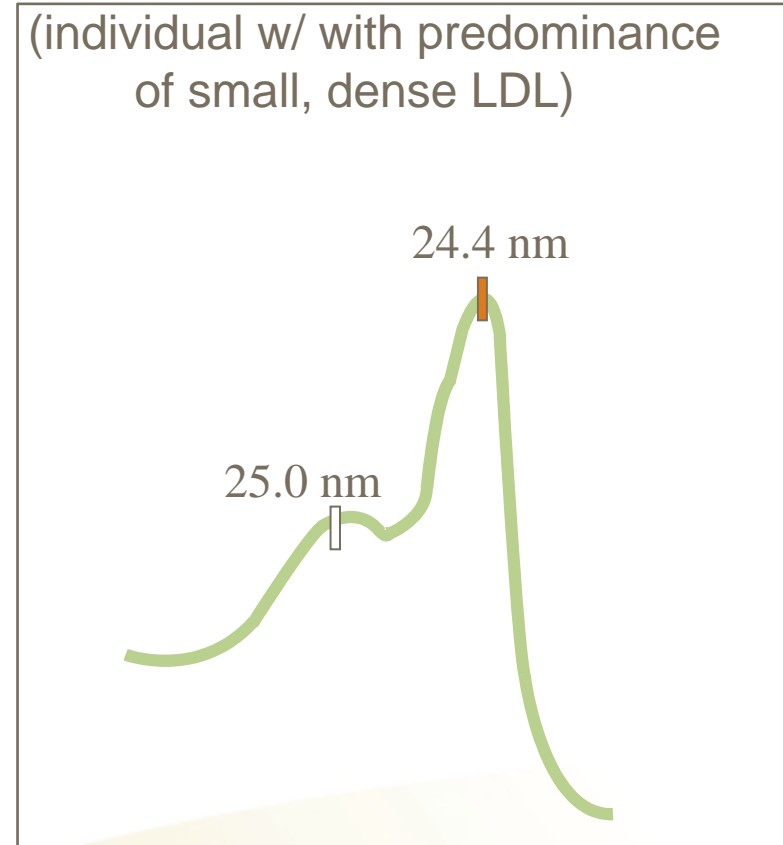
(individual w/ predominance of large, buoyant LDL)



Major peak of particle diameters
At 25.5nm or greater

Pattern B

(individual w/ with predominance of small, dense LDL)



Major peak of particle diameters
At less than 25.5nm

sd LDL-C “SEIKEN” Features



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- Quantitatively measures small, dense LDL, result is reported in mg/dL cholesterol
- Procedure is simple, no special technique is required
- No need for special equipment; only a microcentrifuge, water bath and chemistry analyzer
- Test is completed within 60 min

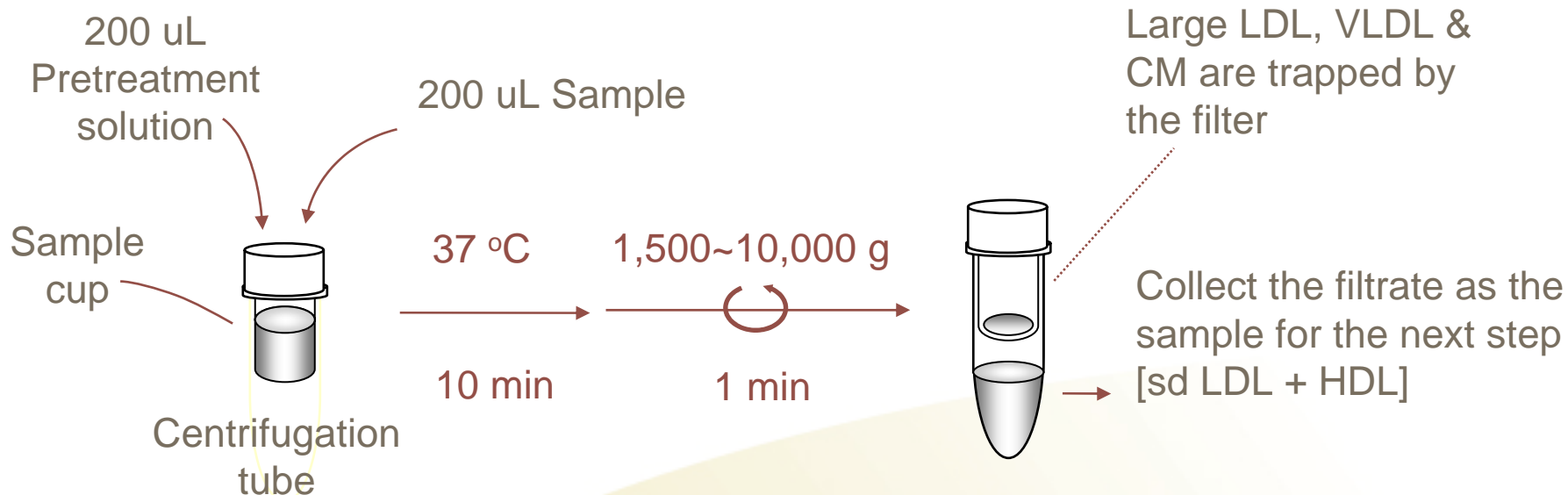
sd LDL-C “SEIKEN” Procedure



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Step 1 : Sample pre-treatment

Separate small, dense LDL from large LDL, VLDL, IDL, CM with heparin and Mg Pretreatment Solution

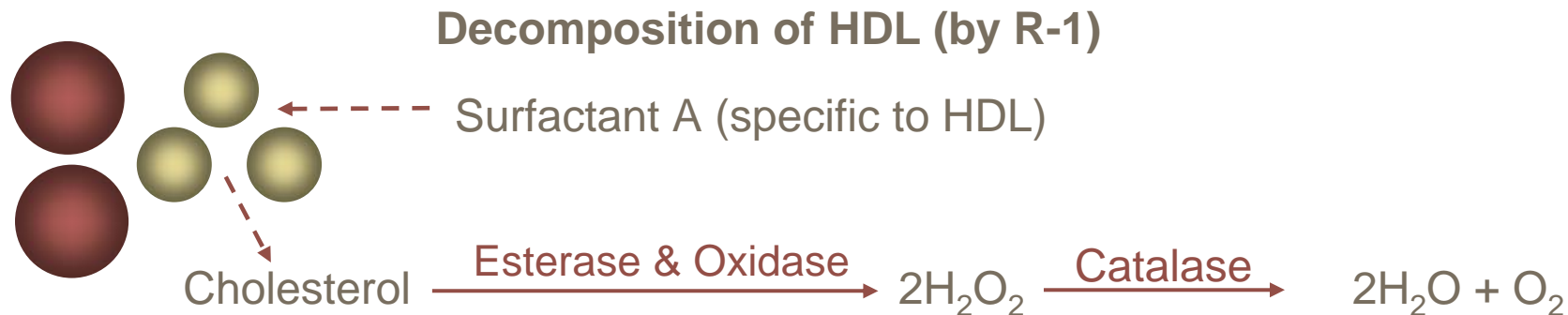


sd LDL-C “SEIKEN” Procedure



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Assay Step-2 : Cholesterol Analysis in Two-reagent Assay



sd LDL-C Sample Stability



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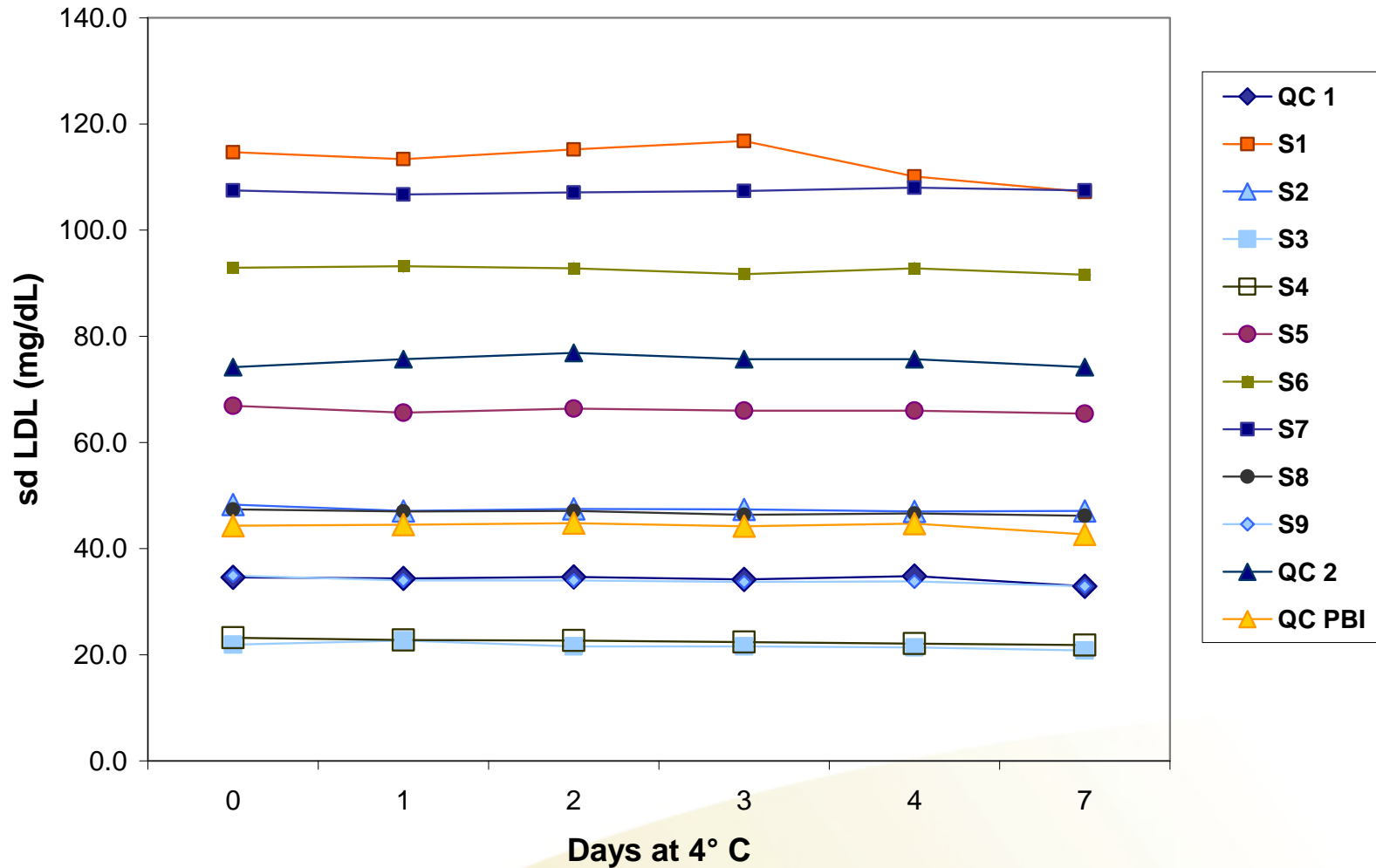
Maximum Stability:

- In whole blood up to 6 h at 8 °C or 25 °C
- In serum or EDTA plasma
 - 2 d at 8 °C
 - 7 d at -20 °C
 - 12 mo at -80 °C
- In filtrate from pretreatment up to 7-15 d

Stability of sd LDL Filtrates at 4°C



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sd LDL-C Precision



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Within Run Precision			
QC Name	PBI L	QC Kit L	QC Kit H
Mean	2.7	33.7	71.4
SD	0.37	0.77	3.28
CV%	13.8	2.3	4.6
n	20	20	20

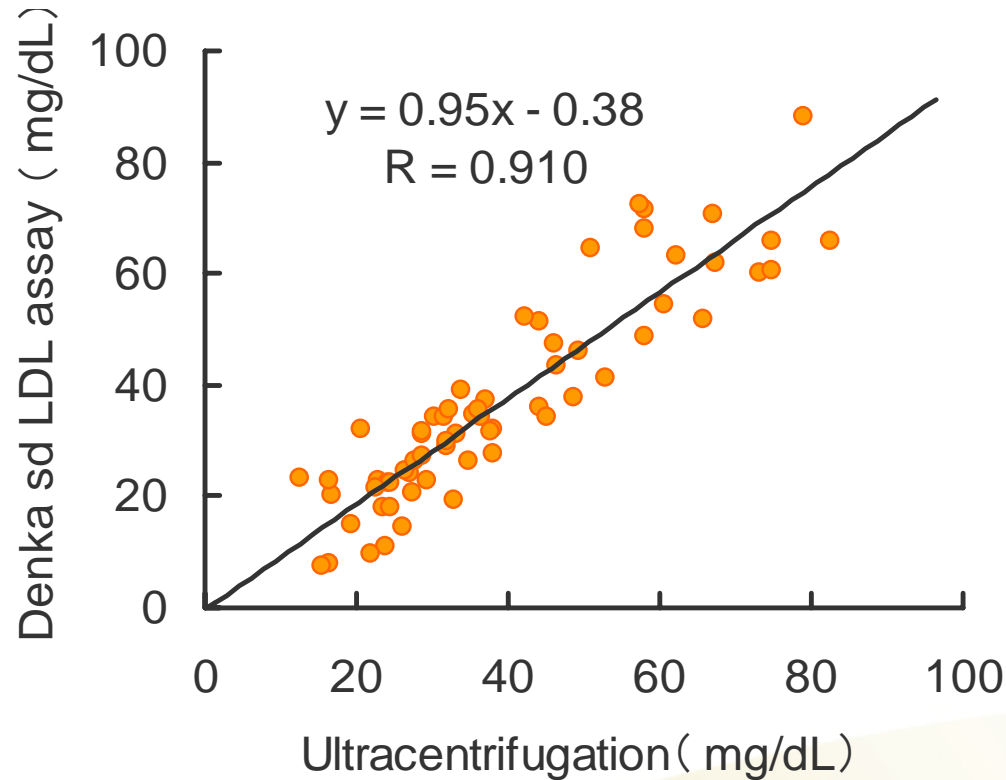
Between Run Precision			
QC Name	PBI L	QC Kit L	QC Kit H
Mean	3.9	32.3	69.8
SD	0.79	1.6	5.7
CV%	20.2	4.9	8.2
n	10	16	15

sd LDL-C “SEIKEN”



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Correlation with ultracentrifugation method



64 Samples from healthy people, CAD and Diabetic patients

Summary



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- Current LDL subfractionation methods are based on a variety of technologies and principles. They are often technically demanding, laborious or proprietary thus not suitable for use in the routine laboratory
- Lack of consistency and standardization of LDL subclass measurements prevents true comparison, limiting opportunity for greater exploration of small LDL as a marker of CVD
- Most representative small dense LDL assay for CVD risk remains to be determined
- Denka sdLDL is a promising new tool suitable for the routine lab that may assist in investigating this important area

Further Considerations of Denka sdLDL Assay



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- Further streamlining of procedure to improve robustness
- Establish long-term reagent lot-to-lot consistency to support clinical diagnostic use
- Evaluate specificity in various phenotypes, clinical samples and in the presence of potential interferences
- Investigate best representative marker of small dense LDL - chol? total or free? apo B? phospholipid?

Acknowledgement



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