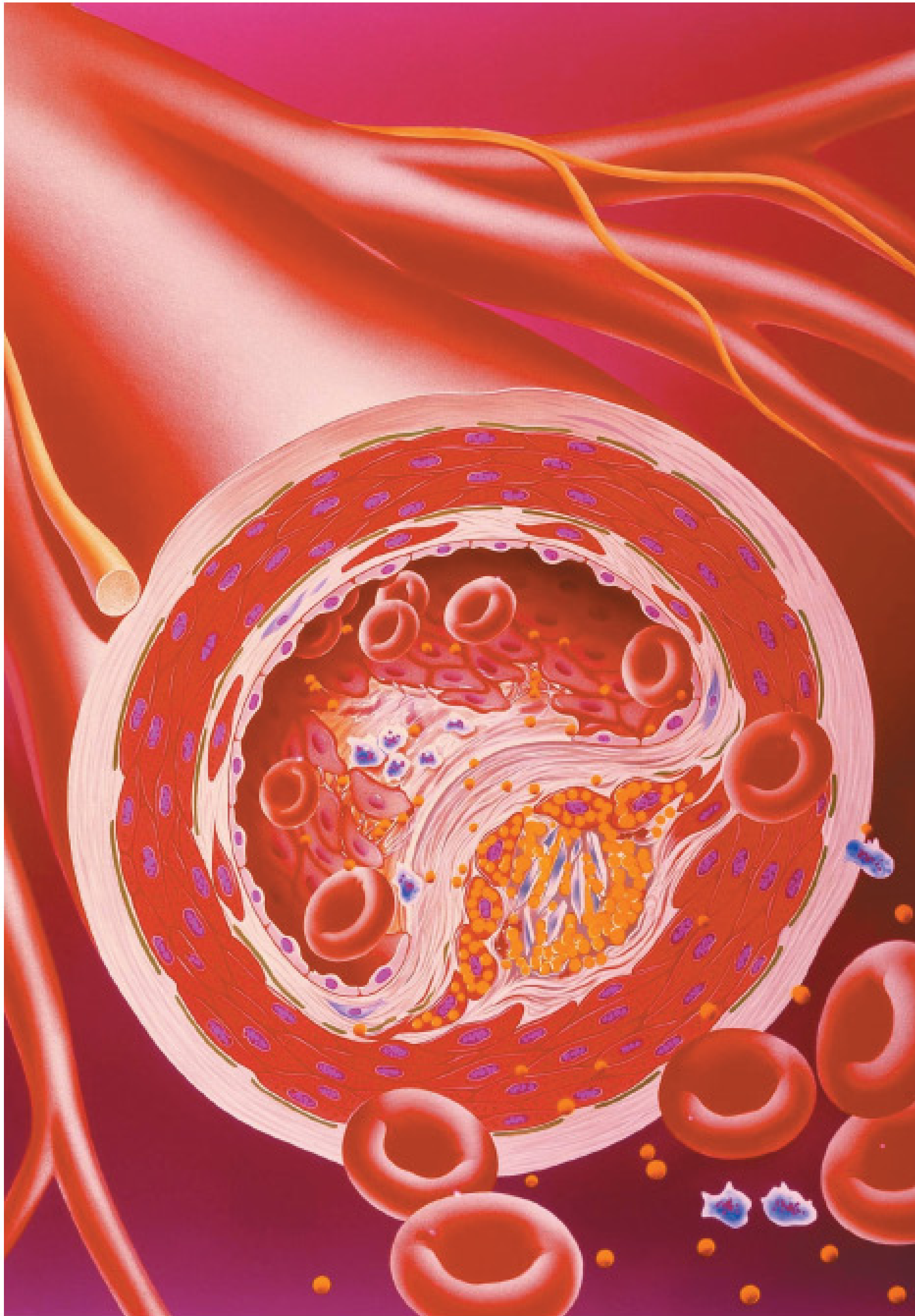
LDL-EX(N)

Homogenous Assay for Direct Measurement of LDL-C Levels in Serum and Plasma For Use on Chemistry Analyzers



Numerous studies have shown that elevated LDL cholesterol is a major cause of coronary heart disease (CHD). In addition, recent clinical trials have shown that LDL-lowering therapy reduces risk for CHD. For these reasons, the Adult Treatment Panel (ATP) III of the National Cholesterol Education Program (NCEP) identifies elevated LDL as the primary target of cholesterol-lowering therapy.

b-quantification is the most widely accepted reference method for LDL-C determination. However, since b-quantification requires highly specialized ultracentrifugation, it is too labor-intensive and thus impractical for routine testing. Estimation of LDL levels by the Friedewald formula is traditionally used for routine purposes. However, since the Friedewald formula estimates LDL from measurements of total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C), its accuracy and precision have to rely on thsee three different measurements. Moreover, the Friedewald formula is not applicable when triglyceride concentrations are > 400 mg/dL or when chylomicrons or dysbetalipoproteinemia is present. The Denka Seiken LDL-EX Seiken Assay is a homogenous method for direct measurement of LDL-C levels in serum and plasma, and is intended for use on automated chemistry analyzers.

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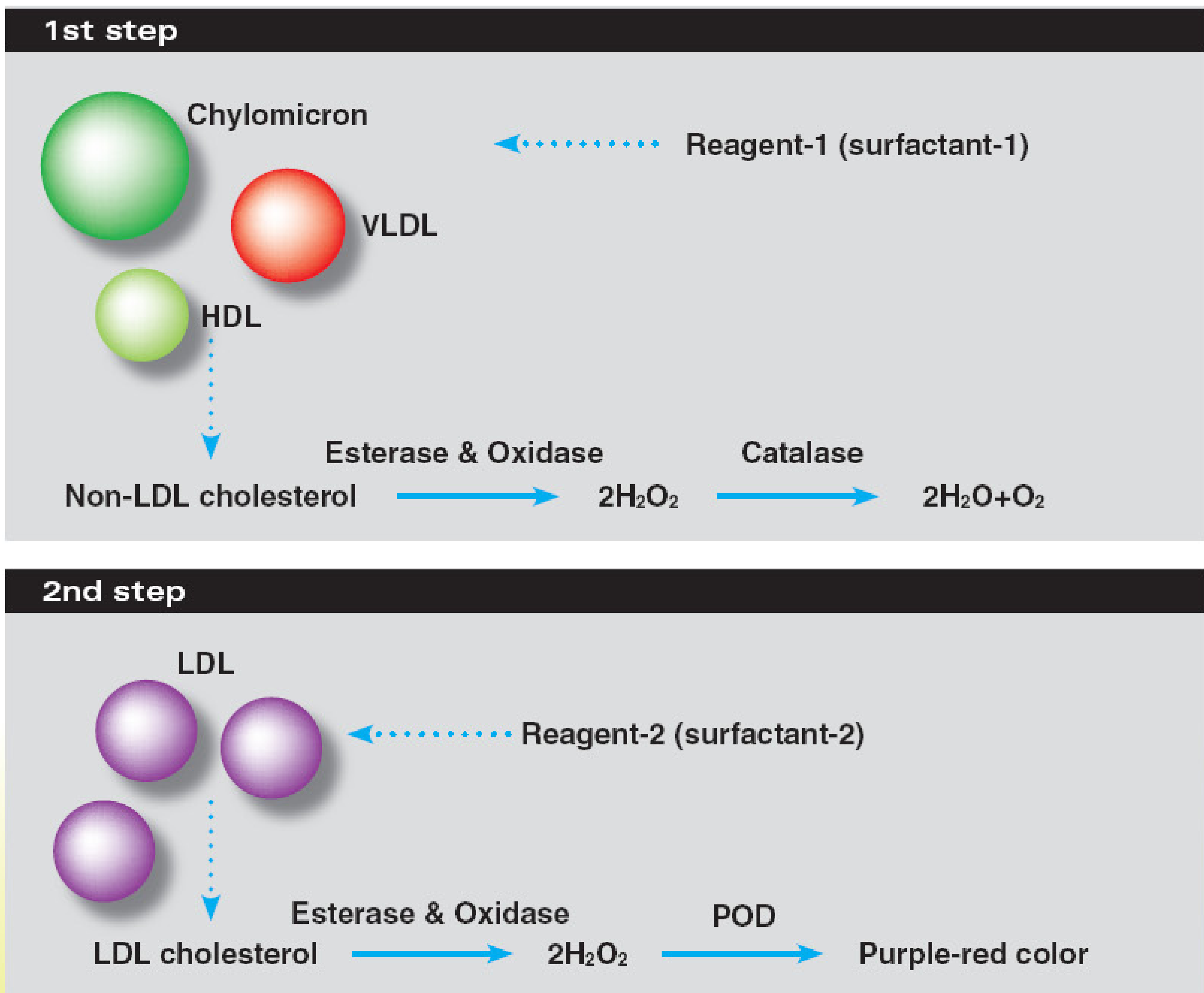
ASSAY PRINCIPLE

The assay consists of two steps and is based on the patented technology filed by Denka Seiken*. The method uses a well-characterized and specially selected surfactants that selectively react with certain groups of lipoproteins. In the first step, non-LDL lipoproteins, including chylomicrons, VLDL, IDL and HDL, are decomposed by the action of a surfactant in Reagent-1 that is reactive to those non-LDL lipoproteins. The cholesterol released is then degraded to water and oxygen by the action of enzymes as follows: cholesterol ester is first hydrolyzed by cholesterol esterase (CHE) and then oxidized by cholesterol oxidase (CO). The hydrogen peroxides produced are finally decomposed to water and oxygen by the action of catalase.

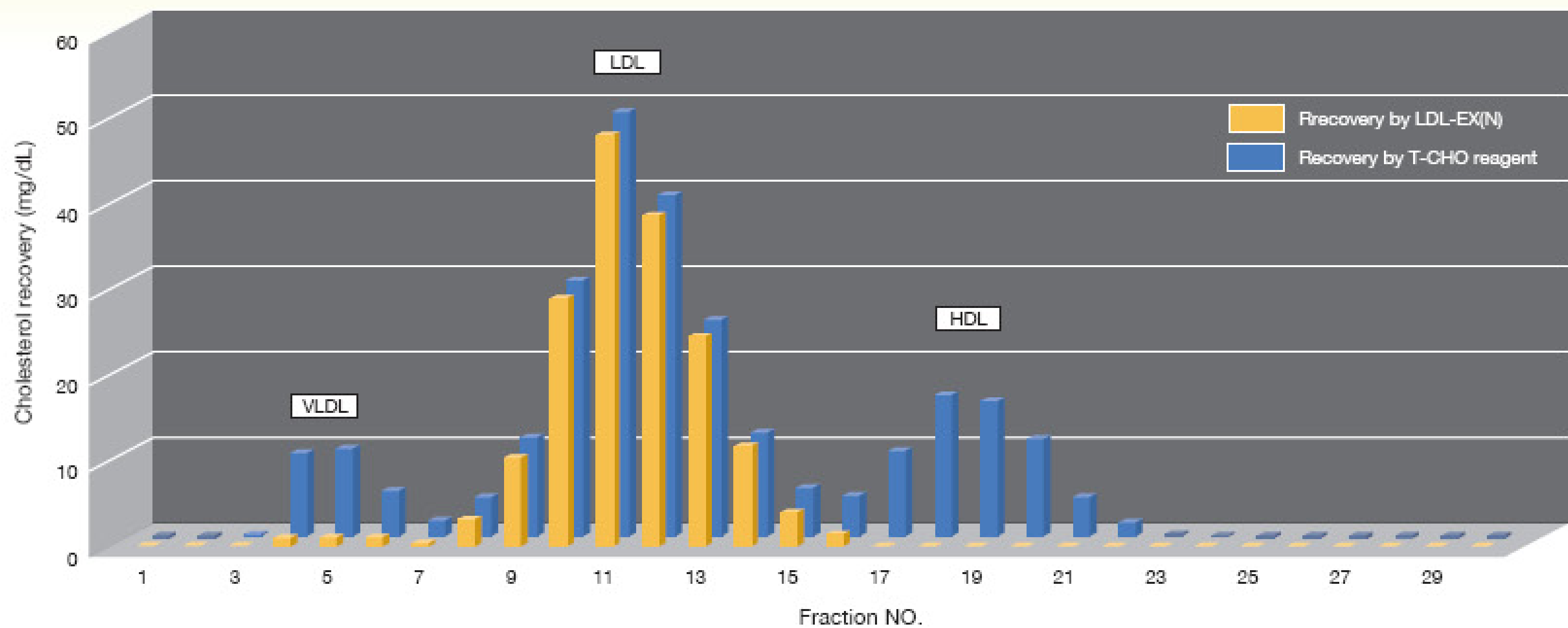
In the second step, another surfactant in Reagent-2 releases cholesterol only from LDL particles and released cholesterol is then subject to the enzymatic reactions as follows: as catalase in the reaction mixture is inhibited by sodium azide in Reagent-2, hydrogen peroxides produced from the reaction with the cholesterol esterase and cholesterol oxidase develop into a purple-red color with the coupler in the presence of peroxidase (POD).



* U.S. Patent No. 6194164B1

SPECIFICITY

Specificity of LDL-EX(N) was verified against gel filtration. LDL-EX(N) only reacted with the LDL fractions separated by the gel filtration.

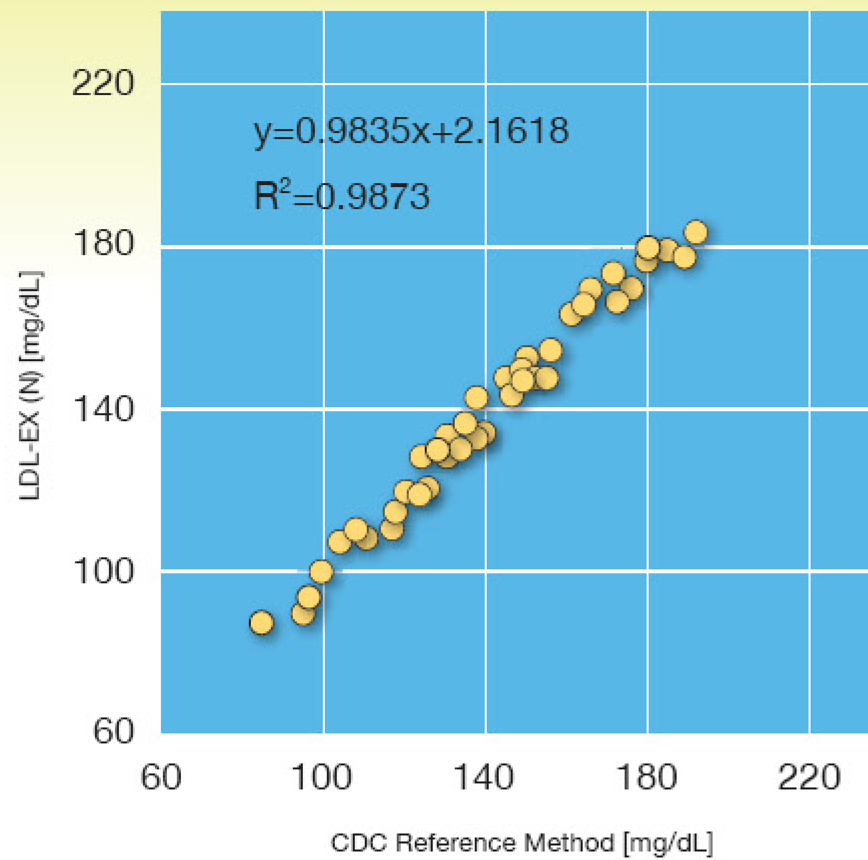


ACCURACY

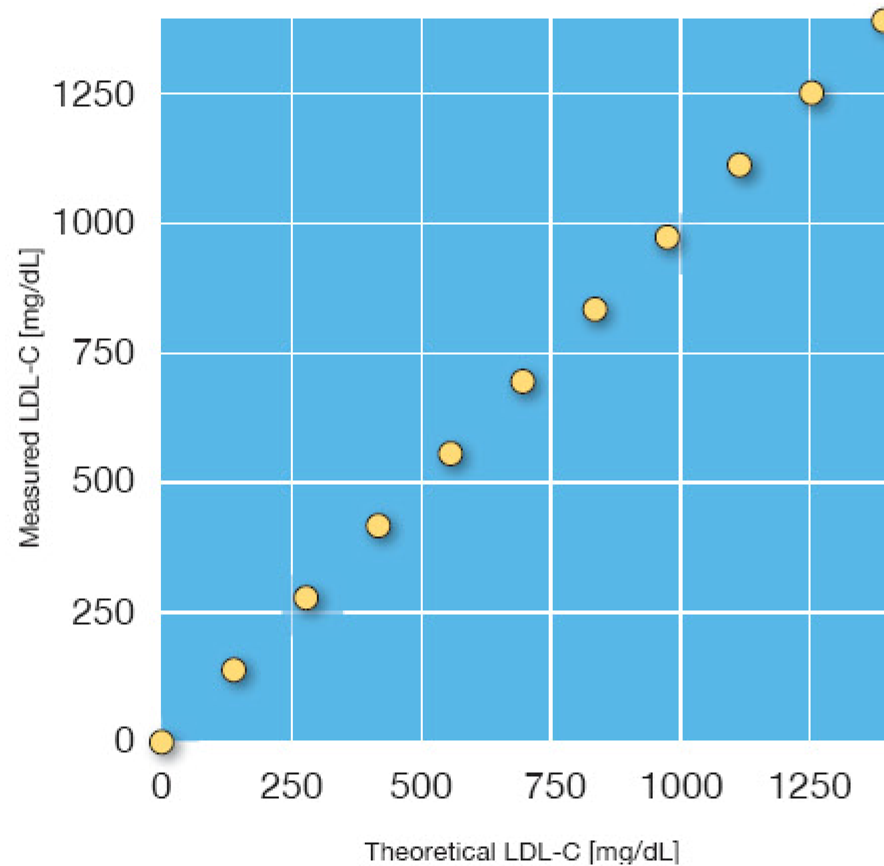
Accuracy of the LDL-EX (N) was verified by comparison to the reference method (RM) for LDL cholesterol (b-quantification method). The following summarizes the results of a study conducted using a Hitachi 917 analyzer and 45 samples that had ranging LDL-C ranging in values from 84.2 mg/dL to 246.8 mg/dL.

	NCEP' s criteria	LDL-EX (N)
Average % Bias	≤ 4%	0.0%
Average Absolute % Bias	≤ 4%	2.2%
Among-run CV	≤ 4%	0.9% (20 days, mean 152.9 mg/dL)
Among-run total error	≤ 12%	1.8%

Correlation with the CDC Reference Method



LINEARITY & PRECISION



Within-run precision			
	Low	Medium	High
N	20	20	20
Mean	70.6	123.6	172.2
SD	0.50	0.66	1.00
CV	0.70%	0.53%	0.58%

Between-run precision			
	Low	Medium	High
Days	20	20	20
Mean	71.6	122.9	174.2
SD	1.17	1.43	1.70
CV	1.63%	1.17%	0.97%

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