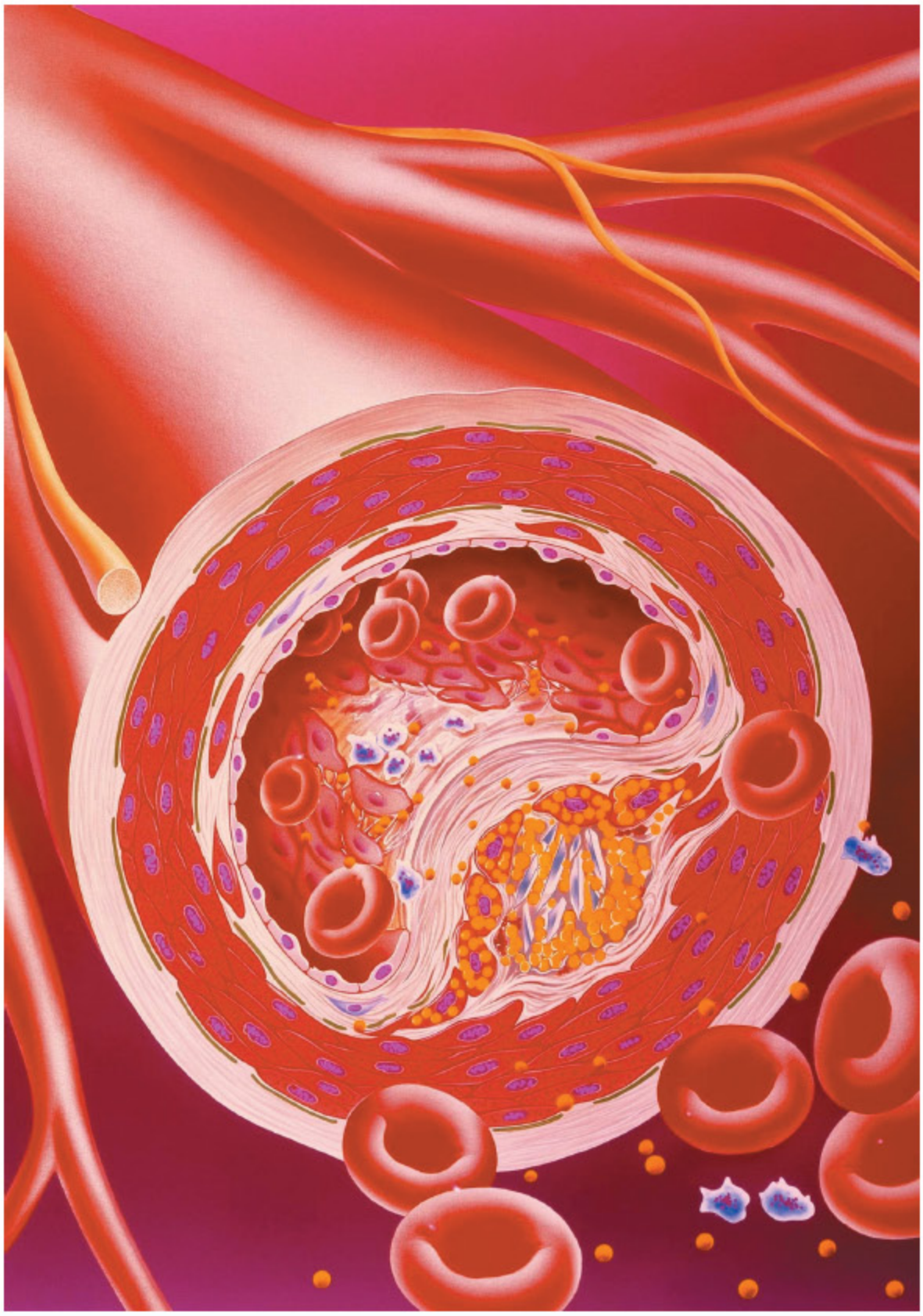
HDL-EX(N)

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



High-density lipoproteins (HDL) transport endogenous cholesterol from the peripheral tissues to the liver, where cholesterol is disposed of as bile acids. Monitoring HDL levels is clinically important since an inverse relationship exists between its level and the risk of atherosclerotic disease. The Adult Treatment Panel (ATP) III of the National Cholesterol Education Program (NCEP) defines a low HDL level as one being less than 40 mg/dL. In a previous recommendation, this was designated as less than 35 mg/dL. This change reflects new findings showing the significance of low HDL levels and the strong link with increased risk of heart disease. For the measurements of HDL, traditionally, selective chemical precipitation techniques have been used, such as heparin-manganese, dextran sulfate-magnesium, phosphotungstate-magnesium, etc. However, these technique require a manual, off-line separation step using centrifugation, which is time-consuming and not fully automatable. HDL-EX(N) from Denka Seiken is a homogenous method for the direct measurement of HDL-C levels in serum and plasma, and is intended to be used on automated chemistry analyzers.

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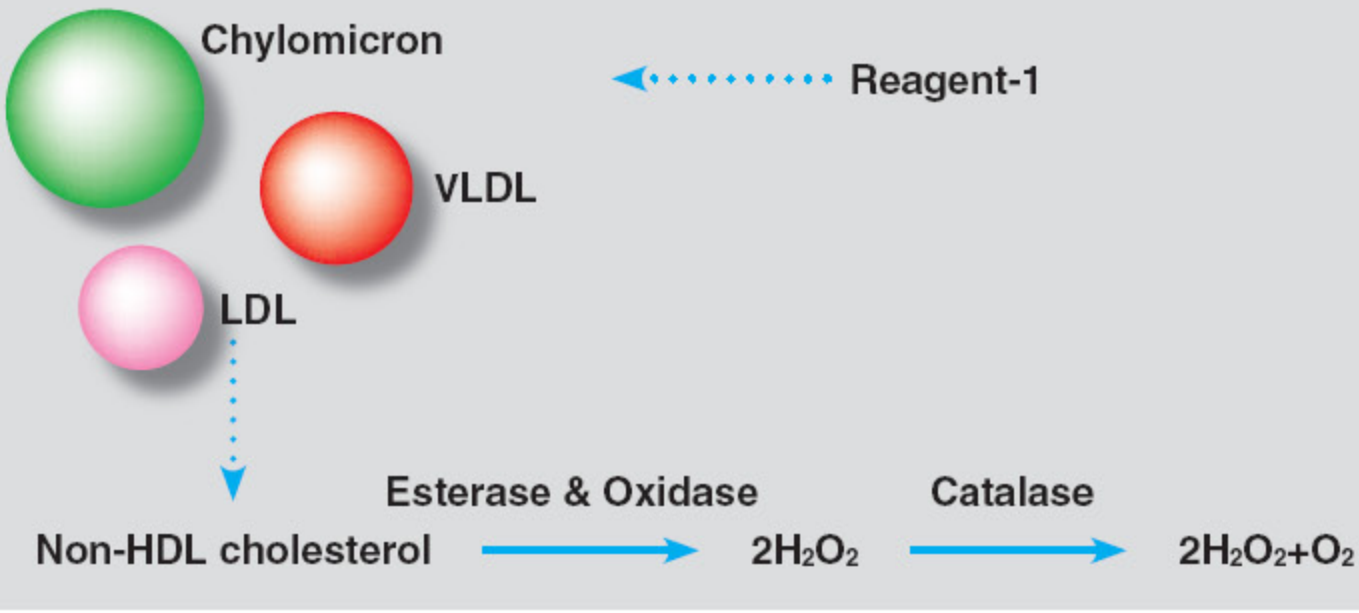
ASSAY PRINCIPLE

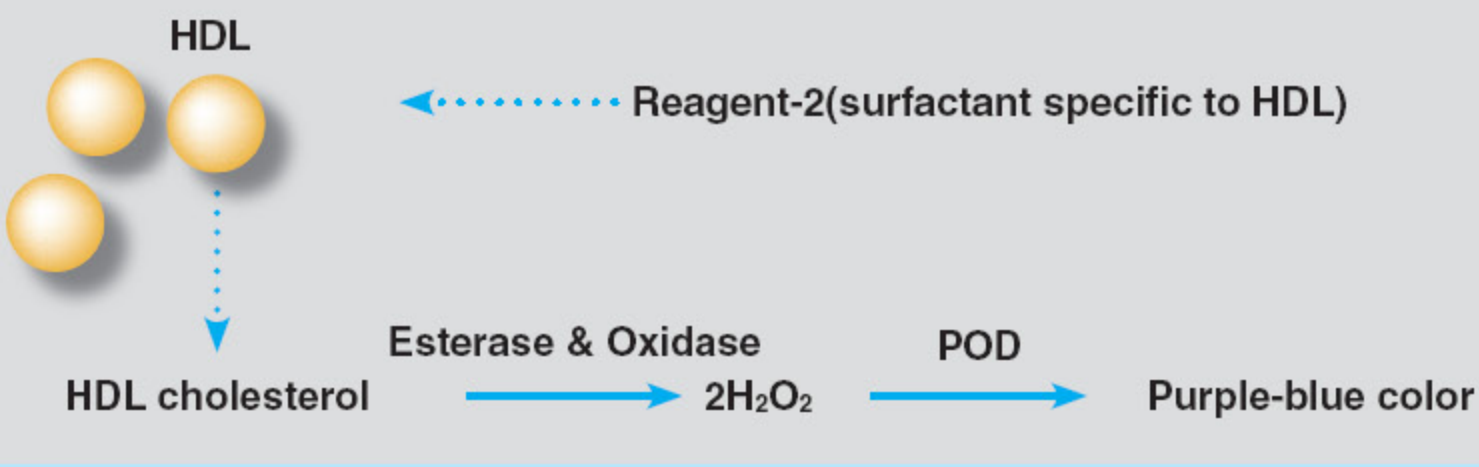
The assay consists of two steps and is based on patented technology filed by Denka Seiken*. The method uses a well-characterized and specially selected surfactant that specifically decomposes HDL particles, but not other lipoproteins. First, non-HDL lipoproteins, including chylomicrons, VLDL, IDL and LDL, are decomposed by the action of the buffer in Reagent-1. The cholesterol released in this step 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, the HDL-specific surfactant in Reagent-2 releases cholesterol only from the HDL particles among the lipoproteins remaining intact after the first step, and the cholesterol released 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-blue color with the coupler in the presence of peroxidase (POD).

1st step



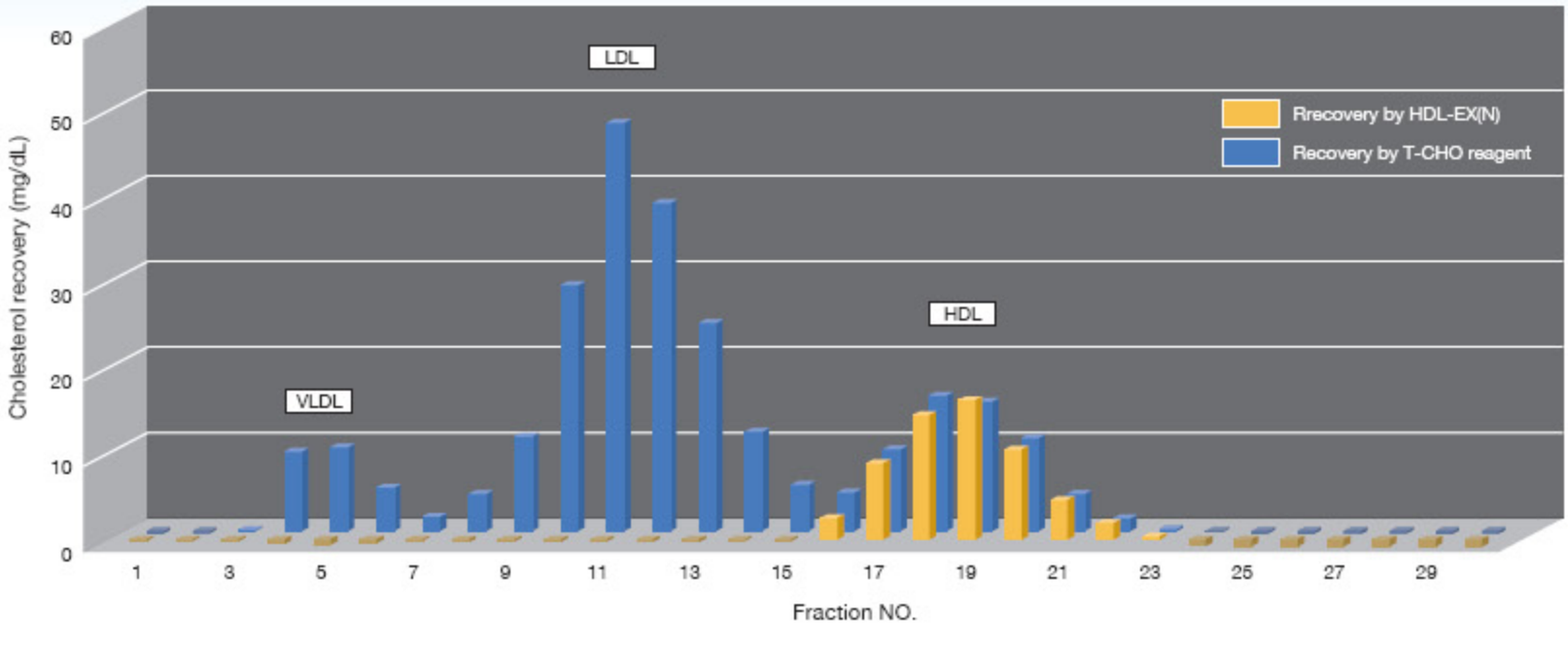
2nd step



* U.S. Patent No. 6479249B2

SPECIFICITY

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

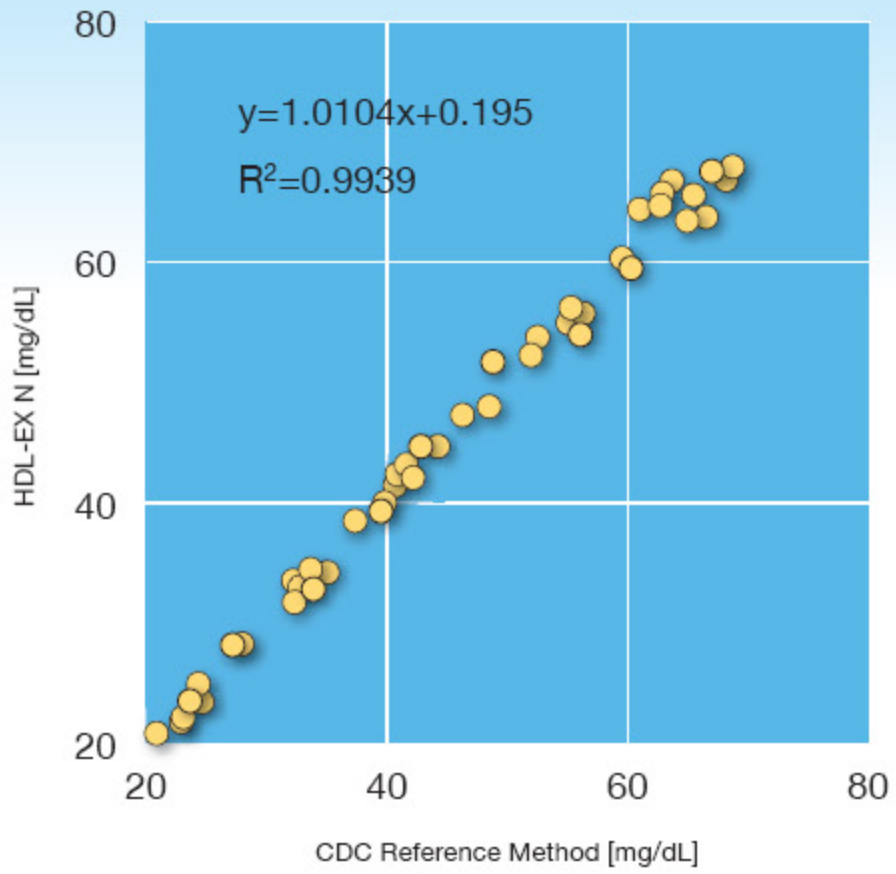


ACCURACY

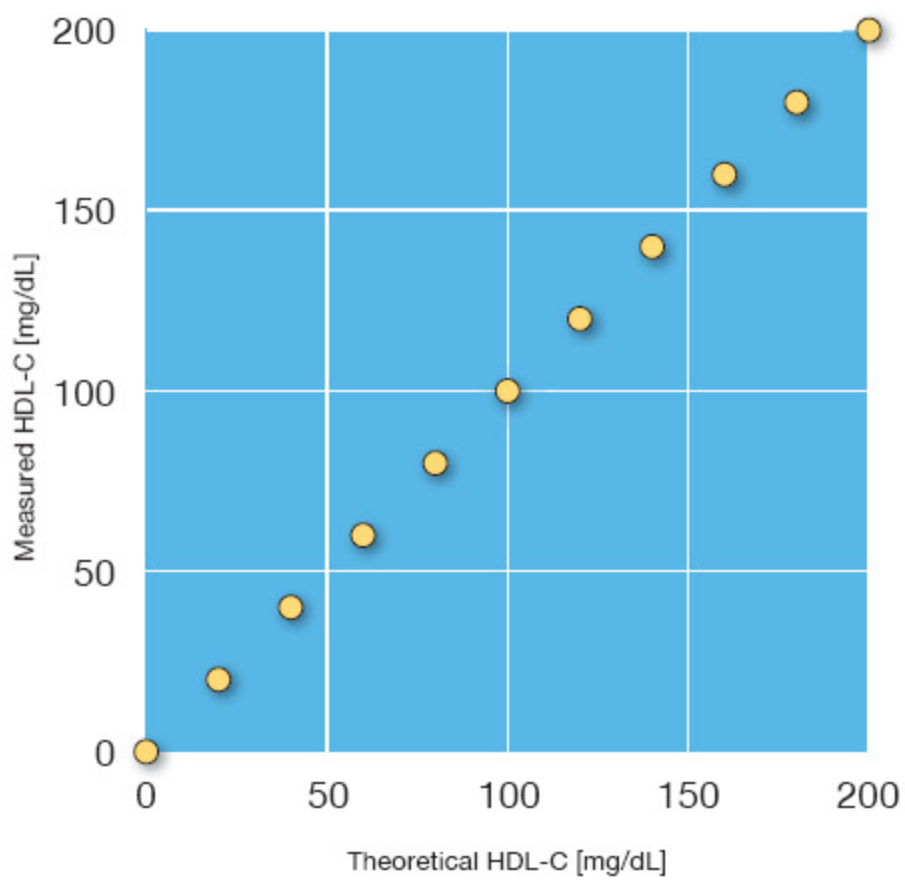
Accuracy of the HDL-EX N was verified by comparison to the CDC reference method for HDL cholesterol (removal of chylomicrons and VLDL by ultracentrifugation, precipitation of apoB containing lipoproteins with heparin plus MnCl2, and then quantification of cholesterol in HDL by Abell-kendall Cholesterol Reference Method). The following summarizes the results of a study conducted using a Hitachi 917 analyzer and 50 samples that had HDL-C ranging in values from 20.5 mg/dL to 68.7 mg/dL.

	NCEP' s criteria	HDL-EX (N)
Average % Bias	≤ 5%	1.4%
Average Absolute % Bias	≤ 5%	2.4%
Among-run CV	≤ 4%	0.7% (20 days, mean 51.6 mg/dL)
Among-run total error	≤ 13%	2.8%

Correlation with the CDC Reference Method



LINEARITY & PRECISION



Within-run precision			
	Low	Medium	High
N	20	20	20
Mean	29.85	56.02	92.14
SD	0.24	0.38	0.39
CV	0.79%	0.67%	0.42%

Between-run precision			
	Low	Medium	High
Days	20	20	20
Mean	29.00	55.30	88.83
SD	0.47	0.89	1.49
CV	1.62%	1.60%	1.68%

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