POINTE SCIENTIFIC, INC.
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Liquid autoHDL™ Cholesterol Reagent Set

Intended Use
For the quantitative determination high-density lipoprotein cholesterol in human serum or plasma. For *in vitro* diagnostic use only.

Summary
Lipoproteins are spherical-shaped particles that contain varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids and proteins make up the outer surface of the lipoprotein particle, while the core consists mostly of cholesterol in the esterified form and triglycerides. The purpose of the lipoprotein particles is to transport cholesterol and triglyceride through the bloodstream.

The relative amounts of the protein and lipid constituents determine the density of the lipoprotein particles and provide a basis for their classification.[1] These classes are: chylomicron, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). There have been many clinical studies that have shown that these lipoprotein particles have very distinct and varied effects on the risk of coronary heart disease.[2] The role of HDL particles in lipid metabolism is primarily the uptake and transport of cholesterol from peripheral tissue to the liver. This process is known as reverse cholesterol transport and has been proposed as a cardio protective mechanism.[3] Low HDL-C levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease.[4-9] Thus, the determination of serum HDL cholesterol has been recognized as a useful tool in identifying high-risk patients. The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that all adults 20 years of age and over should have their total cholesterol and HDL cholesterol levels measured at least every 5 years to screen for risk of coronary heart disease.[9]

The CDC reference method for HDL cholesterol uses ultracentrifugation followed by chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement using a modified Abell-Kendall assay.[10] This method is considered too time consuming and labor intensive for use in routine analysis.[11] Historically, most laboratories have used one of several methods for the selective precipitation and removal of LDL and VLDL, followed by the enzymatic measurement of HDL-C in the supernatant fraction.[10] Since almost all of these methods required manual separation steps, HDL cholesterol determinations could not be fully automated. Also, the dilution of the sample resulted in an enzymatic determination of cholesterol with low sensitivity. As a result, the routine determination of HDL cholesterol has suffered from both long turnaround times and poor reproducibility.

Principle
The Liquid autoHDL™ Cholesterol assay is a homogeneous method for directly measuring serum HDL-C levels without the need for any off-line pretreatment or centrifugation steps. The method is in a two-reagent format. The first reagent contains α-cyclodextrin and dextran sulfate to stabilize LDL, VLDL, and chylomicrons. The second reagent contains PEG modified enzymes that selectively react with the cholesterol present in the HDL particles. Consequently, only the HDL cholesterol is subject to cholesterol measurement.

Reagents
R1: α-cyclodextrin 0.5 mM, dextran sulfate 0.5g/L, magnesium chloride 2.0mM, HSDA 0.3 g/L, buffer, pH 7.0 ± 0.1, preservative.
R2: POD>15,000 U/L, PEG-CO>5,000U/L, PEG-CE>800 U/L, 4-aminoantipyrene 0.5 g/L, buffer, pH 7.0 ± 0.1, surfactant, preservative.

HSDA = Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline.
PEG-CO = Cholesterol Oxidase from Nocardia sp.
PEG-CE = Cholesterol Esterase from Pseudomonas
POD = Peroxidase from Horseradish

Reagent Preparation
Reagent 1: Reagent 1 is ready to use.
Reagent 2: Reagent 2 is ready to use.

Reagent Storage and Stability
All reagents are stable until the expiration date on the kit label when stored at 2-8°C.

Precautions
1. For *in vitro* diagnostic use.
2. Do not pipette by mouth.
3. All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing.
4. Do not use the reagent after the expiration date printed on the kit.

Specimen Collection and Preparation
Serum, EDTA-treated or heparinized plasma are the recommended specimens.

Serum: Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection. (within 3 hours).[10]

Plasma: Specimens may be collected in EDTA or heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).[10]

If not analyzed promptly, specimens may be stored at 2-8°C for up to 1 week. If specimens need to be stored for more than 1 week, they may be preserved at less than -20°C for up to 1 month. For storage periods of 1 month to 2 years, samples should be preserved at -70°C.[10]

Interferences
All interference studies were conducted according to the procedures recommended in NCCLS guideline NO. EP7-P for interference testing in clinical chemistry.[12] Hemoglobin levels up to 100 mg/dl and Bilirubin levels up to 20mg/dl were found to exhibit negligible interference (<5%) on this method. Samples with levels of interfering substances higher than the upper limits should be diluted with physiological saline before assaying. Refer to the work of Young for a review of drug effects on serum HDL cholesterol levels.[13]

Materials Provided
Liquid autoHDL™ Cholesterol Reagent Set

Catalog No.	**H7545-40**	**H7545-80**	**H7545-320**
Reagent 1	30ml	60ml	240ml
Reagent 2	10ml	20ml	80ml

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Materials Required but not Provided

1. An autoHDL/LDL Cholesterol Calibrator.
2. HDL cholesterol controls
3. Automated clinical chemistry analyzer capable of accommodating two-reagent assays.

Procedure

Below is a general example of the autoHDL™ test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations.[10] For assistance with applications on automated analyzers, please contact the Technical Service Department.

Sample	+	Reagent 1	37°C ----➢	Reagent 2	37°C ----➢	Measurement (Absorb. Difference between 700nm & 600nm)
4ul		300ul	5min.	100ul	5min.	

I
I
I
V
HDL-C Result

Limitations

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Store the reagents as per instructions.
4. Samples with values greater than 150 mg/dl must be diluted 1:1 with saline and re-assayed. Multiply the result by two.

Calibration

The autoHDL/LDL™ Cholesterol Calibrator is required for calibration. The value of the autoHDL/LDL™ calibrator was assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Refer to autoHDL/LDL™ Cholesterol Calibrator kit package insert for instructions. If control results are found to be out of range, the procedure should be recalibrated.

Quality Control

Reliability of test results should be routinely monitored with control materials that reasonably emulate performance of patient specimens.[10] Quality control materials are intended for use only as monitors of accuracy and precision. The National Cholesterol Education Program (NCEP) Lipid Standardization Panel (LSP) recommends two levels of controls, one in the normal range (35-65 mg/dl) and one near the concentrations for decision making (<35mg/dl). An acceptable range of HDL cholesterol values should be established for the controls by repeat analysis. The recovery of control values within the appropriate range should be the criteria used in evaluation of future assay performance. Quality control materials are intended for use only as monitors of accuracy and precision. Controls should be run with every working shift in which HDL-C assays are performed. It is recommended that each laboratory establish their own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Results

To convert from conventional units to SI Units, multiply the conventional units by 0.02586.

Mg/dl x 0.02586 = mmol/L HDL cholesterol

Expected Values

The expected values for serum HDL cholesterol are as follows:[14]

Males:	30-70 mg/dl
Females:	30-85 mg/dl

Each laboratory must establish its own range of expected values.

According to the NCEP, HDL values greater than or equal to 35 mg/dl are considered desirable, and values greater than or equal to 60 mg/dl are considered to offer some protection against coronary heart disease. Values below 35 mg/dl are considered to be a significant independent risk factor for coronary heart disease.[9]

Performance

1. Assay Range: 2-150 mg/dl
2. Accuracy: Accuracy of the Liquid autoHDL™ Cholesterol Reagent method was verified by comparison to the Designated Comparison Method (ultracentrifugation, chemical precipitation and enzymatic cholesterol analysis which has been standardized to the Abell-Kendall method)[10] and another manufacturer's lyophilized autoHDL™ Cholesterol Reagent method. Studies comparing the autoHDL™ Cholesterol method to the Designated Comparison Method produced the following results:

Method	Liquid autoHDL™ Cholesterol	Designated Comparison Method
N	30	30
Mean HDL Cholesterol	55	55
Range (mg/dl)	26-94	24-101
Standard Deviation (mg/dl)	18	19
Regression Analysis	y = 0.93x + 3.85mg/dl	
Correlation Coefficient	r = 0.989	

Studies comparing the Liquid autoHDL™ Cholesterol method to the lyophilized autoHDL™ Cholesterol method produced the following results:

Method	Liquid autoHDL™ Cholesterol	Lyo. autoHDL™ Cholesterol
N	95	95
Mean HDL Cholesterol	59	58
Range (mg/dl)	27-109	24-118
Standard Deviation (mg/dl)	20	21
Regression Analysis	y = 0.91x + 6.08mg/dl	
Correlation Coefficient	r = 0.979	

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3. Precision: Within Day precision for the Liquid autoHDL™ Cholesterol Reagent was determined following a modification of NCCLS document EP5-T2.[15] Within Day precision studies produced the following results:

	LOW	MID	HIGH
n	20	20	20
Mean HDL Cholesterol (mg/dl)	38	68	85
Standard Deviation (mg/dl)	0.9	1.0	1.2
Coefficient of Variation (%)	2.2	1.5	1.4

Day to Day precision for the Liquid autoHDL™ Cholesterol Reagent was also determined following a modification of NCCLS document EP5-T2.[15] Day to Day precision studies produced the following results:

	LOW	MID	HIGH
n	20	20	20
Mean HDL Cholesterol (mg/dl)	37	66	84
Standard Deviation (mg/dl)	0.8	1.5	1.6
Coefficient of Variation (%)	2.2	2.3	1.9

4. Sensitivity: The analytical sensitivity of the Liquid autoHDL™ Cholesterol Reagent was determined to be 0.002 absorbance units per 1 mg/dl of HDL Cholesterol.

References

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