**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 cardiovascular protective mechanism.\(^3\) Low HDL-C levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease.\(^4\) 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.\(^5\)

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.\(^6\) This method is considered too time consuming and labor intensive for use in routine analysis.\(^7\) 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.\(^8\) 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 \(\alpha\)-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: \(\alpha\)-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-aminophenylperoxide 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.

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**Materials Provided**
Liquid autoHDL™ Cholesterol Reagent Set

**Materials Required but not Provided**
1. An autoHDL/LDL Cholesterol Calibrator.
2. HDL cholesterol controls
3. Beckman Coulter AU™ 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.\(^9\) For assistance with applications on automated analyzers, please contact the Technical Service Department.

**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).

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

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.

**Interferences**
All interference studies were conducted according to the procedures recommended in NCCLS guideline NO. E07-P for interference testing in clinical chemistry.\(^10\) Hemoglobin levels up to 100 mg/dl and Bilirubin levels up to 20mg/dl were found to exhibit negligible interference (<20%) 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.\(^11\)

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Sample + Reagent 1 → Reagent 2 → Measurement (Absorb. Difference between 700nm & 600nm)

HDL-C Result

Sample + Reagent 1

4uL 300uL 5min. 100uL 5min.

37°C 37°C

(beckman Coulter AU™400 application)

Specific Test Parameters

Test Number: # Test Name: HDL V Type: Serum V Operational: Yes V
Sample Vol.: 2 DIL. Vol.: 0 Pre-Dilution Rate: 1
Reagents: R1 Volume: 150 Dil. Vol.: 0 Min. OD Max. OD
R2 Volume: 50 Dil. Vol.: 0 L 0.000 H 2.500
Reagent OD Limit:
Wavelength: Pri. 600 V Sec. 700 V First L: -0.100 First H: 0.500
Method: Fixed V Last L: -0.100 Last H: 0.500
Reaction Slope: + V Dynamic Range:
Measuring Point 1: First: 10 Last: 27 L: # H: #
Measuring Point 2: First: Last: Correlation Factor:
Linearity: % A: 1.000 B: 0.000
No Lag Time: NO V On Board Stability Period: #

Specific Test Parameters

VALUE FLAG: # V LEVEL L: # LEVEL H: #
NORMAL RANGES: AGE L AGE H
SEX YEAR MONTH YEAR MONTH L H
○ 1. # V # # # # # #
○ 2. # V # # # # # #
○ 3. # V # # # # # #
○ 4. # V # # # # # #
○ 5. # V # # # # # #
○ 6. # V # # # # # #
7. NONE SELECTED # #
8. OUT OF RANGE L H # #
PANIC VALUE: # # UNIT: mg/dl DECIMAL PLACES: 0

Calibration Specific Parameters

Cal Type: AB V Formula: Y = AX + B V Counts: 2 Process: CONC. V
CAL. NO. OD CONC. FAC/Od-L FAC/Od-H
Point 1. # # -9999999 9999999
Point 2.
Point 3.
Point 4.
Point 5.
Point 6.
Point 7.
1-point Cal. Point: # WITH CONC.0
MB Type Factor: CALIBRATION STABILITY PERIOD: #

# User-Defined

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

Note: For other instrument specific applications please contact Pointe Scientific, Inc.
Technical Service Department at 1-800-445-9853

Limitations
1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Store the reagent 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 the 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. 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
Comparison: A comparison study performed between the Beckman Coulter AU400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.993 and a regression equation of y = 1.079x – 6.7. (n = 30, range 46 – 142 mg/dl)
2. Precision:
Within - day precision study was performed using three levels of material. Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.
3. Sensitivity: The analytical sensitivity of the Liquid autoHDL™ Cholesterol Reagent was determined to be 0.001 absorbance units per 1 mg/dl of HDL Cholesterol when read at 540/660nm.

Precision and Linearity studies were performed following modifications of CLSI Protocols EP-5 and EP6 using a Beckman Coulter AU™400 analyzer.15

References