HORIBA Instruments Incorporated
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Pointe autoHDL™ Cholesterol Reagent Set

Intended Use
For the quantitative determination high-density lipoprotein cholesterol in human serum or plasma using the Yumizen C560 analyzer. For *in vitro* diagnostic use only. **Rx 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. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Precautions and Hazards
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.

Hazards:
R1 and R2: Hazard Classifications: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture.
Refer to the Safety Data Sheet for this product (SDS-H7545) available by calling 1-734-487-8300.

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

Materials Required but not Provided

1. An autoHDL/LDL Cholesterol Calibrator
2. HDL cholesterol controls
3. Yumizen C560 Analyzer
4. Yumizen C560 Operation manual

Procedure

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.

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 test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

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 its 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]

Specific Performance Characteristics

1. Assay Range: 2-150 mg/dl
2. Accuracy: Studies comparing the autoHDL™ Cholesterol method used on the Yumizen C560 and a similar analyzer yielded the following results:

Method	HDL
N	81
Mean HDL (mg/dL)	51.2
Range (mg/dL)	5-140
Standard Deviation	29.0
Regression Analysis	y = 0.982x + 0.4
Correlation Coefficient	0.9975

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3. Precision: Within Run and Total Precision for the Liquid autoHDL™ Cholesterol Reagent was determined following a modification of NCCLS document EP5-T2.[15] using the Yumizen C560.

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	32.6	67.7	135.6
Standard Deviation	0.6	0.7	0.8
Coefficient of Variation (%)	1.8%	1.1%	0.6%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	32.8	67.4	132.6
Standard Deviation	6.6	0.6	1.2
Coefficient of Variation (%)	1.9%	1.8%	5.0%

4. Sensitivity: 2SD limit of detection (95% conf) = 0 mg/dl.

References

1. Gotto A.M., Lipoprotein metabolism and the etiology of hyperlipidemia, Hospital practice, 23:Suppl.1,4 (1988).
2. Crouse J.R. et al, Studies of low density lipoprotein molecular weight in human beings with coronary artery disease, J. Lipid Res., 26:566 (1985).
3. Badimon J.J., Badimon L., Fuester V., Regression of Atherosclerotic Lesions by High-Density Lipoprotein Plasma Fraction in the Cholesterol-Fed Rabbit, Journal of Clinical Investigation, 1990; 85:1234-41.
4. Castelli, W.P., et al, Cholesterol and other lipids in coronary heart disease, Circulation, 55:767 (1977).
5. Barr, D.P., Russ E.M., Eder H.A., Protein-lipid relationships in human plasma, Am. J. Med. 11:480 (1951).
6. Gordon, T., et al, High density lipoprotein as a protective factor against coronary heart disease, Am. J. Med., 62:707 (1977).
7. Williams, P., Robinson D., Baily A., High density lipoprotein and coronary risk factor, Lancet, 1:72 (1979).
8. Kannel W.B., Castelli W.P, Gordon, T., Cholesterol in the prediction of atheriosclerotic disease; New perspectives based on the Framingham study, Am. Intern. Med., 90:85 (1979).
9. National Institute on Health publication No. 93-3095, September 1993.
10. Warnick G. Russell, Wood Peter D., National Cholesterol Education Program Recommendations for Measurement of High-Density Lipoprotein Cholesterol; Executive Summary Clinical Chemistry, Vol.41, No. 10 (1995).
11. Grundy, S.M., et al, Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II), JAMA 1993, 269: 23, 3015-3023.
12. National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol Number 7, Vol.4, No. 8, June 1984.
13. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd. Ed., AACC Press, Washington DC, 1990, 3-104 thru 3-106.
14. Tietz, N.W., Clinical Guide to Laboratory Tests, W.B. Saunders Co., Philadelphia, 1986, p.256.
15. NCCLS document “Evaluation of Precision Performance of Clinical Chemistry Devices” 2nd Ed. 1992.

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CHEMISTRY PARAMETERS

Chem:	HDL	No.:	218	Sample Type:	Serum
Chemistry:	autoHDL Cholesterol			Print Name:	HDL
Reaction Type:	Fixed Time			Reaction Direction:	Positive
Pri Wave:	605			Sec Wave:	660
Unit:	mg/dL			Decimal	0
Blank Time:	47 49			Reaction Time:	51 82

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	2.0 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: 40 ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	2	150	Linearity Limit:
Linearity Range (Decreased)	____	____	Substrate Depletion:
Linearity Range (Increased)	____	____	Mixed Blank Abs:
R1 Blank Abs:	____	____	Uncapping Time
Blank Response:	____	____	Reagent Alarm Limit:
Twin Chemistry:			☐ Enzyme Linear Extension

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator: * Lot No.: *

Exp Date: *

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	HDL	0	mg/dL
autoHDL/LDL Calibrator	*	*	*	HDL	*	mg/dL

Calibration Setup

Chem: HDL

Calibration Settings

Math Model: Two-Point Linear

Factor: Replicates: 2

Acceptance Limits

Cal Time: 336 Hour

Slope Diff: --- SD: ---

Sensitivity : --- Repeatability: ---

Deter Coeff: ---

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF 14-H7545-300

Manufactured for HORIBA Instruments Incorporated – Pointe Brand
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2°C 8°C IVD

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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CE

Symbol Key

Use by (YYYY-MM-DD) | LOT Lot and batch code | REF Catalog number | Manufacturer | Temperature limitation | Consult instructions for use | IVD *In vitro* diagnostic medical device | **Rx Only:** Prescription Use Only

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