

## Intended Use

For the quantitative determination high-density lipoprotein cholesterol in human serum or plasma using the Yumizen C230 and Yumizen C240 analyzers.

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.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup> Low HDL-C levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease.<sup>4-9</sup> 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.<sup>9</sup>

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.<sup>10</sup> This method is considered too time consuming and labor intensive for use in routine analysis.<sup>11</sup> 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.<sup>10</sup> 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  $\pm$  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  $\pm$  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).<sup>10</sup>

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

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.<sup>10</sup>

## Interferences

All interference studies were conducted according to the procedures recommended in NCCLS guideline NO. EP7-P for interference testing in clinical chemistry.<sup>12</sup> 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.<sup>13</sup>

## Materials Provided

Liquid autoHDL™ Cholesterol Reagent Set

**Catalog No. 12-H7545-162**

Reagent 1 3 x 40 ml

Reagent 2 3 x 14 ml

## Materials Required but not Provided

1. Pointe autoHDL/LDL Cholesterol Calibrator, catalog number H7545-CAL
2. Pointe HDL cholesterol controls
3. Yumizen C230 / Yumizen C240 Analyzer
4. Yumizen C230 / Yumizen C240 Operation manual

## 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.<sup>10</sup> For assistance with applications on automated analyzers, please contact the Technical Service Department.

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

# Pointe autoHDL™ Cholesterol Reagent Set

## Test Parameters

Test:	HDL	Chemistry:	Auto HDL Cholesterol
Chemistry No.:	218	Print Name:	HDL Cholesterol
Reaction Type:	Fixed Time	Reaction Direction:	Positive
Pri. Wave:	578 nm	Sec. Wave:	670 nm
Decimal.:	0	Samp. Type:	Serum
Blank Time:		Reaction Time:	2 18
Unit:	mg/dL	Incubation Time:	18

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard;	3	uL	uL	uL 180	uL
Decreased;		uL	uL	uL 60	uL
Increased;		uL	uL		

Linearity Range (Standard):	2-150	Linearity Limit:	
Linearity Range (Decreased):		Substrate Depletion:	
Linearity Range (Increased):		Mixed Blank Abs.:	- 40000 40000
R1 Blank Abs.:	- 40000 40000	On-board Stability:	30 Day (s)
Blank Response	- 40000 40000	Reagent Alarm Limit:	5
Twin Chemistry:			

Prozone Check:			
Q1:	Q2:	Q3:	
Q4:	PC:	ABS:	

Use Qualitative Result:	
Range:	Flag:

Slope Offset:			
Slope	Offset	Unit	
1	0	mg/dL	

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:			
Sample Type:	Gender:	Age Range:	Ref. Range: Critical Range: Unit:

## Calibration Setup Parameters

Chem:	HDL		
Calibration Setting			
Math Model:	Two-Point Linear		
Factor:	Replicates: 2		
Acceptance Limits			
Cal Time:	336 hr.		
Slope Diff:	SD:		
Sensitivity:	Repeatability:		* User Defined
Deter Coeff:			
Auto Calib.			
	<input type="checkbox"/> Cal Time		

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
HDL/LDL Cal	*	*	

## 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 for 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.<sup>10</sup> 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<sup>14</sup>:

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.<sup>9</sup>

## Performance Characteristics

**Assay Range:** 2-150 mg/dl

**Accuracy:** Studies comparing the autoHDL™ Cholesterol method used on the Yumizen 200 series analyzers and a similar analyzer yielded the following results:

Method	autoHDL™ Cholesterol
N	36
Mean HDL Cholesterol	61
Range (mg/dl)	30-124
Standard Deviation (mg/dl)	22
Regression Analysis	Y=0.958x +2.6
Correlation Coefficient	R=0.982

**Precision:**

With-in Day precision for the Liquid autoHDL™ Cholesterol Reagent was determined following a modification of NCCLS document EP5-T2.<sup>15</sup> using the Yumizen 200 series analyzers. Within Day precision studies produced the following results:

Sample	LOW	HIGH
N	20	20
Mean HDL Cholesterol (mg/dl)	28	108
Standard Deviation (mg/dl)	0.5	0.7
Coefficient of Variation (%)	1.8	0.7

Day-to-Day precision was also determined following a modification of NCCLS document EP5-T2.<sup>17</sup> Day-to-Day precision studies run on the Yumizen 200 series analyzers produced the following results:

Sample	LOW	HIGH
N	20	20
Mean HDL Cholesterol (mg/dl)	25	44
Standard Deviation (mg/dl)	1.0	2.1
Coefficient of Variation (%)	3.9	3.9

**Sensitivity:** 2SD limit of detection (95% conf) = 0.365 mg/dl.

**References**

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15. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices" 2<sup>nd</sup> Ed. 1992.

**Symbol Key**

Use by (YYYY-MM-DD)	Lot and batch code
Catalog number	Manufacturer
In vitro diagnostic medical device	Temperature limitation
Consult instructions for use	<b>Rx Only:</b> Prescription Use Only
CE mark	Authorized representative in the European Community

12-H7545-162 Manufactured by HORIBA Instruments Incorporated - Pointe Brand 5449 Research Drive Canton, MI 48188

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**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.

Rev. 11/23 P803-H7545-MIN