Pointe autoLDL™ Cholesterol Reagent Set

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Intended Use

For the direct quantitative determination of low density lipoprotein cholesterol (LDL-C) in human serum or plasma using the Yumizen C560 analyzer. **Rx Only**.

Summary

Plasma lipoproteins are spherical particles that contain varying amounts of cholesterol, triglycerides, phospholipids, and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, the inner core contains mostly esterified cholesterol and triglycerides. These particles serve to solubilize and transport cholesterol and triglycerides in the bloodstream.

The relative proportions of protein and lipid determine the density of these plasma lipoproteins and provide a basis for their classification.[1] The classes are: very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have varied effects.[2-4] The studies all point to LDL cholesterol as the key factor in the pathogenesis of artherosclerosis and coronary artery disease (CAD),[2-8] while HDL cholesterol has often been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated risk for CAD.[4]

Over the years a variety of methods have been employed for the determination, or estimation, of LDL cholesterol. The Friedewald equation, in a variety of forms, has been most frequently used for the estimation of LDL cholesterol. However, its usefulness is limited and its accuracy has been questioned. Determination of LDL cholesterol by beta-quantification is recognized as the reference method, but the procedure is so cumbersome relatively few laboratories use this method. A recent method using immunoseparation has become popular. However, this method is still requires sample pre-treatment prior to cholesterol determination, making it unsuitable for full automation of the procedure. The method presented here offers direct determination of LDL cholesterol in a two part, liquid stable reagent that is easily adapted to most automated chemistry analyzers.

Reagent Composition

Components	Appearance	Ingredients
Reagent 1	Liquid	MES Buffer (pH 6.3)
		Detergent 1, Cholesterol esterase, Cholesterol oxidase, Peroxidase, 4-aminoantipyrine, Ascorbic acid oxidase, Preservative
Components	Appearance	Ingredients
Reagent 2	Liquid	MES Buffer (pH 6.3)
		Detergent 2, N,N-bis (4-sulfhobutyl)-m-Toluidine-disodium, (DSBmT), Preservative

Cholesterol Oxidase from Nocardia sp., Cholesterol Esterase from Pseudomonas sp., Peroxidase from Horseradish, Ascorbic Acid Oxidase from Cucurbita sp.

Principle

The autoLDL™ Cholesterol Reagent is a two-part, liquid stable method for directly measuring LDL-C levels in serum or plasma. The method depends on the properties of a unique detergent which eliminates the need for any off-line pre-treatment or centrifugation steps. This detergent (Reagent 1) solubilizes only the non-LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

HDL, VLDL, Chylomicrons → Solubilized HDL, VLDL, Chylomicrons → Consumed HDL, VLDL, Chylomicrons (No color)

LDL —(Detergent/Rgt1)→ Non-solubilized LDL Cholesterol —(Detergent/Rgt2)→ Solubilized LDL Cholesterol —(Cholesterol Esterase, Cholesterol Oxidase)→ + H_2O_2

H_2O_2 + DSBmT + 4-AA —(Peroxidase)→ Color Development (Measured Bichromatically at 546 & 660nm)

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 label when stored at 2 to 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

1. Reagent is intended for *in vitro* diagnostic use only.
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 reagents beyond the expiration date printed on the kit label.

Hazards:

R1 and R2: Hazard Classifications: Not a hazardous substance or mixture.
Pictogram and 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-L7574) available by calling 1-734-487-8300.

Specimen Collection and Storage

Serum, EDTA-treated or heparinized plasma are the recommended specimens. Patients are not required to fast prior to blood collection.
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 5 days. If specimens must be stored for more than 5 days, they may be frozen at – 80°C.

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 at levels up to 400 mg/dl, Bilirubin at levels up to 20 mg/dl and Triglycerides to 1380 mg/dl were found to exhibit negligible interference (<10%) on this method. Samples with levels of interfering substances higher than the upper limits should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor. For a comprehensive review of drug interference on serum LDL cholesterol levels see Young et al.[13]

Materials Provided

autoLDL R1 Reagent, autoLDL R2 Reagent

Materials Required but not Provided

1. autoHDL/LDL™ Calibrator, Cat. No. H7545-CAL
2. Yumizen C560 Analyzer
3. Yumizen C560 Operation manual
4. Lipid controls, catalog number L7580-18

Procedure

All analyzer applications should be validated in accordance with NCEP and CLIA recommendations.[10] For assistance with applications on automated analyzers, please contact HORIBA Medical Technical Service Department at (800) 445-9853.

Limitations

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Samples with values greater than 650 mg/dl on the Yumizen C560 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 values of the calibrator were assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Refer to autoHDL/LDL™ Cholesterol Calibrator 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 the performance of patient specimens.[10] Quality control materials are intended for use only as monitors of accuracy and precision. The recovery of control values within the appropriate range should be the criteria used in evaluation of future assay performance. Controls should be run with every working shift in which LDL-C assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be determined in conformance with local, state, and/or Federal regulations or accreditation requirements.

Results

To convert from conventional units to S.I. units, multiply the conventional units by 0.02586.
Example: mg/dL x 0.02586 = mmol/L LDL-C

Expected Values

The following NCEP recommendations for patient classifications are suggested for the prevention and management of coronary heart disease:[8]

LDL Cholesterol	Classifications
<130mg/dl (3.36mmol/L)	Desirable
130-159m/dl (3.36-4.11mmol/L)	Borderline High Risk
160mg/dl (4.14mmol/L)	High Risk

It is highly recommended that each laboratory establish its own range of expected values.

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Specific Performance Characteristics

1. Assay Range: 0-650 mg/dL.
2. Correlation: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	LDL
N	80
Mean LDL (mg/dL)	105.4
Range (mg/dL)	8-238
Standard Deviation	53.4
Regression Analysis	y = 1.086x – 5.8
Correlation Coefficient	0.9841

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.[12]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	183.0	251.7	538.6
Standard Deviation	1.4	1.6	9.6
Coefficient of Variation (%)	0.8%	0.6%	1.8%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	188.2	257.6	563.2
Standard Deviation	9.8	12.5	26.0
Coefficient of Variation (%)	5.2%	4.8%	4.6%

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).
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4. Castelli, W.P., et al., Cholesterol and other Lipids in coronary heart disease, Circulation, 55-767 (1977).
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17. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices" 2nd Ed. 1992.

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CHEMISTRY PARAMETERS

Chem:	LDL	No.:	224	Sample Type:	Serum
Chemistry:	autoLDL Cholesterol			Print Name:	LDL
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	546			Sec Wave:	660
Unit:	mg/dL			Decimal	0
Blank Time:	47 48			Reaction Time:	80 82

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent
Standard:	1.5 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)	0	650	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	*	*	LDL	0	mg/dL
autoHDL/LDL Calibrator	*	*	*	LDL	*	mg/dL

Calibration Setup

Chem: LDL

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-L7574-320

Manufactured for
HORIBA Instruments Incorporated - Pointe Brand
5449 Research Drive Canton, MI 48188

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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