HORIBA Instruments Incorporated
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Pointe autoLDL™ Cholesterol Reagent Set

Intended Use

For the direct quantitative determination of low density lipoprotein cholesterol (LDL-C) in human serum or plasma using the Yumizen C230 and Yumizen C240 analyzer. For *in vitro* diagnostic use only. **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

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.

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.

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 1500 mg/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. 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]

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Materials Provided

autoLDL™ Cholesterol Reagent Set	
Catalog No.	12-L7574-162
Reagent 1	3 x 40mL
Reagent 2	3 x 14mL

Materials Required but not Provided

1. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Pointe autoHDL/LDL™ Calibrator, Cat. No. H7545-CAL
4. Pointe Lipid controls, catalog number L7580-18

Procedure

Below is general example of the autoLDL™ 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 HORIBA Medical's Technical Service Department at (800)445-9853.

Sample	+	Reagent 1	37°C →	Reagent 2	37°C →	Measurement (Absorb. Difference between 660nm & 546nm)
3ul		300ul	5min.	100ul	5min.	

↓

LDL-C Result

Test Parameters

Test:	LDL	Chemistry: auto LDL Cholesterol	
Chemistry No.:	224	Print Name: LDL Cholesterol	
Reaction Type:	Endpoint	Reaction Direction: Positive	
Pri. Wave:	546 nm	Sec. Wave: 670 nm	
Decimal.:	0	Samp. Type: Serum	
Blank Time:		Reaction Time: 16	18
Unit:	mg/dL	Incubation Time: 18	

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

Linearity Range (Standard); 0-370		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	g/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: LDL

Calibration Setting

Math Model: Two-Point Linear

Factor: Replicates: 2

Acceptance Limits

Cal Time: 336 hr.

Slope Diff: SD:

Sensitivity: Repeatability:

Deter Coeff:

Auto Calib.

☐ Cal Time

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

* User Defined

Limitations

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Samples with values greater than 370 mg/dl on the Yumizen 200 series analyzers 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 procedure should be re-calibrated.

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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Performance Characteristics

Assay Range: 0-370 mg/dl

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

Method	autoLDL™ Cholesterol
N	30
Mean LDL Cholesterol	106
Range (mg/dl)	50-159
Standard Deviation (mg/dl)	28
Regression Analysis	Y=1.017x + 1.7
Correlation Coefficient	R=0.990

Precision:
Within-Day precision for the autoLDL™ Cholesterol Reagent was determined following a modification of NCCLS document EP5-T2[17] using the Yumizen 200 series analyzers. Within-Day precision studies produced the following results:

Sample	LOW	HIGH
N	20	20
Mean LDL Cholesterol (mg/dl)	28	146
Standard Deviation (mg/dl)	0.5	2.5
Coefficient of Variation (%)	1.7	1.7

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

Sample	LOW	HIGH
N	20	20
Mean LDL Cholesterol (mg/dl)	26	147
Standard Deviation (mg/dl)	0.9	2.9
Coefficient of Variation (%)	3.3	2.0

Sensitivity: Sensitivity: 2SD limit of detection (95% Conf) = 0.627 mg/dl.

References

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Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-L7574-162 Manufactured by HORIBA Instruments Incorporated - Pointe Brand 5449 Research Drive Canton, MI 48188 IVD

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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.

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