AutoPure T LDL-C

Third Generation Direct Homogeneous Assay

Introduction

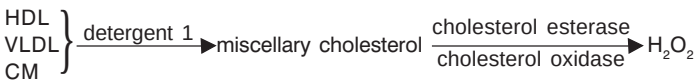
1. AutoPure T LDL-C is a reagent kit for direct quantitative determination of low-density lipoprotein cholesterol (LDL-C) in human serum and plasma on automated clinical chemistry analyzers.
2. AutoPure T LDL-C is a ready-to-use, two liquid reagent system.
3. With AutoPure T LDL-C, the assay is linear upto 450 mg/dl (11.637 mmol/l).

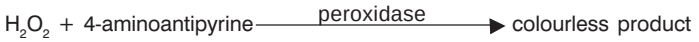
Principle

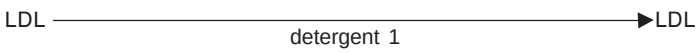
AutoPure T LDL-Cholesterol assay is a homogeneous method for directly measuring LDL-C levels in serum or plasma without the need for any off-line pretreatment or centrifugation steps.

In the first reaction, non LDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase in the presence of 4-AAP to yield a colourless product. The second reagent consists of a detergent capable of solubilizing LDL specifically. Cholesterol esterase and chromogenic coupler react with this solubilized LDL-C to develop colour. The intensity of colour formed is directly proportional to the concentration of LDL-C.

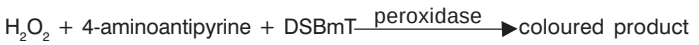
Reaction 1







Reaction 2



Reagent Storage, Stability & Handling

AutoPure T LDL-C is a ready-to-use, two liquid reagent system.

Shelf - Life

Stable till the expiry date indicated on the label, when stored at 2^0 - 8^0C.

On - Board Reagent Stability

R1 : 60 days at 2^0 - 8^0C after opening. Protect the reagent from light and contamination.

R2 : 60 days at 2^0 - 8^0C after opening. Protect the reagent from light and contamination.

Do not freeze the reagent.

Components & Concentration of Working Solution

Component	Concentration
R1	
• Detergent 1	
• 4-aminoantipyrine	0.5 mmol/l
• Cholesterol oxidase	1.2 U/ml
• Cholesterol esterase	
• Peroxidase	
• Good's buffer solution; pH 6.3	
R2	
• Detergent 2	
• N, N-bis(4-sulfobutyl)-m-toluidine disodium salt (DSBmT)	1.0 mmol/l
• Good's buffer solution; pH 6.3	

Specimen Collection & Preservation

Collect sample using standard sampling tube. Serum, EDTA treated or sodium heparinized plasma are the recommended specimens. Patients are not required to fast prior to blood collection.

Serum or plasma should not remain at 15^0- 30^0C for more than 14 hours. If assay is not completed within 14 hours, serum or plasma may be stored at 2^0 - 8^0C upto 1 week. If specimens need to be stored for more than 1 week, they may be preserved at less than -70^0C upto 3 months.

Procedure

AutoPure T LDL-C can be used on various automated analyzers. The procedure described below is for a Hitachi▲▲ 902 auto-analyzer.

Sample : 3 µl R2 : 100µl
R1 : 300 µl

▲▲Hitachi is a registered trademark of Roche Diagnostic, Indianapolis, IN

Calculations

Fully automated systems automatically calculate the LDL-C concentration of each sample.

Result in mmol/l = Result in mg/dl x 0.02586

Application Sheets

Application sheets for the popular fully automated analyzers are provided along with the kit. For additional system applications, contact our local Accurex representative.

Calibration

For calibration, it is recommended to use T LDL-C Calibrator from Accurex. Other commercially available LDL-C calibrators have not been tested with this assay and cannot be supported by Accurex Biomedical Pvt. Ltd. Refer to the T LDL-C Calibrator kit package insert for a description of assignment procedures and instructions. The value of the T LDL-C Calibrator was assigned by procedures traceable to National Reference System for Cholesterol (NRS/CHOL). Calibration material have concentrations around the medical decision level.

Calibration frequency :

Re-calibration is recommended

- Whenever the reagent lot is changed
- As per the requirements of quality control procedures

Quality Control

Each batch of AutoPure T LDL-C is assayed with multiple quality control sera prior to release.

To ensure adequate quality control, it is recommended that the laboratory should use a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both reagent and instrument functions together. The value of these controls should fall within the specified limits. If control values fall outside specified limits, each of the below criteria should be cross-checked and corrected :

- Proper instrument function - wavelength setting, light source and temperature control
- Cleanliness of probes & cuvettes
- Bacterial contamination of wash water used by the instrument
- Expiry date of the reagent kit

Expected Values

Serum/Plasma

The following NCEP cutpoints for patient classification are used for the prevention and management of coronary heart disease :

Desirable	< 130 mg/dl (< 3.36 mmol/l)
Borderline	130 - 159 mg/dl (3.36 - 4.11 mmol/l)
High Risk	≥ 160 mg/dl (4.14 mmol/l)

Note:

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range.

Performance Characteristics

Linearity

With AutoPure T LDL-C, the assay is linear upto 450 mg/dl (11.637 mmol/l). Determine samples with higher concentrations via the rerun function. On analyzers without rerun function, manually dilute sample with higher concentrations using 0.9% NaCl or distilled / deionized water (e.g. 1 + 4). Multiply the result by the appropriate dilution factor (e.g. 5).

Interference

There is no significant interference in samples containing upto 20 mg/dl of conjugated & unconjugated bilirubin, 1293 mg/dl of triglycerides, 500 mg/dl of haemoglobin, 50 mg/dl ascorbic acid & 5000 mg/dl gamma globulins.

Precision

Reproducibility was determined using three levels of pooled human sera as shown below :

	n=20			n=40		
	Within run			**Between run**		
Serum Pool	Mean mg/dl	SD mg/dl	%CV	Mean mg/dl	SD mg/dl	%CV
Low (<130 mg/dl)	98.1	0.72	0.73	98.1	2.2	2.27
Mid (130-159 mg/dl)	146.5	0.96	0.66	142.7	2.8	1.95
High (>160 mg/dl)	209.8	1.31	0.62	207.3	3.6	1.73

Co-Relation Studies

A comparison of LDL-C determination using AutoPure T LDL-C and the Reference method gave the following co-relation (mg/dl) :

Linear regression

y = 0.95x + 3.02

r = 0.96

No. of samples measured : 54

References

1. ***Centers for Disease Control/National Institutes of Health Manual*** "Biosafety in Microbiological and Biomedical Laboratories", 1988.
2. National Committee for Clinical Laboratory Standards, Preparation and Testing of Reagent Water in the Clinical Laboratory Third edition: ***Approved Guideline NCCLS Document*** C3-A3, 1997.
3. Gotto, A.M. Lipoprotein Metabolism and the Etiology of Hyperlipidemia. ***Hospital Practice*** 1988; 23 Suppl:1 4-13.
4. Crouse, J.R. et al. Studies of Low Density Lipoprotein molecular weight in human beings with coronary artery disease. ***J. Lipid Res*** 1985; 26:566.
5. Badimon, J.J. et al. Regression of Artherosclerotic Lesions by High Density Lipoprotein Plasma Fraction in the cholesterol-Fed Rabbit. ***J. Clin. Invest.*** 1990; 85:1234.
6. Castelli, W.P. et al. HDL Cholesterol and other lipids in Coronary Heart Disease, ***Circulation*** 1977; 55:767.
7. Barr; D.P. et al. Protein-Lipid Relationships in Human Plasma. ***Am. J. Med.*** 1951; 11:480.
8. Gordon, T. et al. High Density Lipoprotein as a Protective Factor Against Coronary Heart Disease. ***Am. J. Med.*** 1977; 62:707.
9. Bachorik, P.S. et al. National Cholesterol Education Program Recommendations for Measurement of Low Density Lipoprotein Cholesterol: Executive Summary. ***Clin. Chem.*** 1995; 41(10):1414.
10. National Committee for Clinical Laboratory Standards, Internal Quality Control Testing: Principles and Definitions, ***Approved Guideline NCCLS Document*** C24-A, Number 6, Vol. 11, 1991.
11. National Committee for Clinical Laboratory Standards, Procedures for the Handling and Processing of Blood Specimens, ***Approved Guideline NCCLS Document*** H18-A, Number 12, Vol. 10, 1990.
12. Tietz, N.W. ***Clinical Guide to Laboratory Tests,*** W.B. Saunders Co., Philadelphia, PA., 256(1986).
13. National Committee for Clinical Laboratory Standards, ***National Evaluation Protocols for Interference Testing, Evaluation Protocol,*** Proposed Guideline NCCLS Document EP7-P Number 7, Vol. 6, No. 13, August 1986.
14. Young, D.S. Effects of Drugs on Clinical Laboratory Tests, 3rd ed. ***AACC Press,*** Washington, D.C., 3-104 (1990).

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

CE European Conformity

AR. No.: I 34 ALL-2009-05-00I

Accurex Biomedical Pvt. Ltd.

Head Office - Mumbai. Tel.: 91(022) 67446744; Fax: 91(022) 67446755

E-mail: accurex@vsnl.com; Website: www.accurex.org

Plant : G-54, MIDC Tarapur, Boisar, Thane - 401 506. INDIA.

T LDL-C™

Third Generation
Direct Homogeneous Assay

ACCUREX

AutoPure