AutoPure T HDL-C

Third Generation Direct Homogeneous Assay

Introduction

1. AutoPure T HDL-C is a reagent kit for direct quantitative determination of high-density lipoprotein cholesterol (HDL-C) in human serum and plasma on automated clinical chemistry analyzers.
2. AutoPure T HDL-C is a ready-to-use, two liquid reagent system.
3. With AutoPure T HDL-C, the assay is linear upto 150 mg/dl (3.89 mmol/l).

Principle

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

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

Reaction 1

LDL, VLDL, CM $\xrightarrow{\text{detergent 1}}$ miscellary cholesterol $\xrightarrow[\text{cholesterol oxidase}]{\text{cholesterol esterase}}$ H_2O_2

H_2O_2 + DSBmT $\xrightarrow{\text{peroxidase}}$ colourless product

HDL $\xrightarrow[\text{detergent 1}]{}$ HDL

Reaction 2

HDL $\xrightarrow{\text{detergent 2}}$ miscellary cholesterol $\xrightarrow[\text{cholesterol oxidase}]{\text{cholesterol esterase}}$ H_2O_2

H_2O_2 + 4-aminoantipyrine + DSBmT $\xrightarrow{\text{peroxidase}}$ coloured product

Reagent Storage, Stability & Handling

AutoPure T HDL-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	
• N, N-bis(4-sulfobutyl)-m-toluidine disodium salt (DSBmT)	0.5 mmol/l
• Cholesterol oxidase	1.0 IU/ml
• Peroxidase	
• Detergent 1	
• Good's buffer solution; pH 6.0	
R2	
• 4-aminoantipyrine	1.0 mmol/l
• Cholesterol esterase	
• Detergent 2	
• Good's buffer solution; pH 6.0	

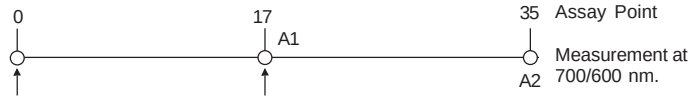
Specimen Collection & Preservation

Collect sample using standard sampling tube. Serum, EDTA treated or sodium heparinized plasma are the recommended specimens. 12-14 hours fasting specimen is required.

Serum or plasma should not remain at 15^0- 30^0C longer 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 HDL-C can be used on various automated analyzers. The procedure described below is for a Hitachi▲▲ 902 auto-analyzer.



Sample : 3 µl R2 : 100 µl

R1 : 300 µl

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

Calculations

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

Result in mmol/l = Result in mg/dl x 0.0259

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 HDL-C Calibrator from Accurex. Other commercially available HDL-C calibrators have not been tested with this assay and cannot be supported by Accurex Biomedical Pvt. Ltd. Refer to the T HDL-C Calibrator kit package insert for a description of assignment procedures and instructions. The value of the T HDL-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 HDL-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 expected values for serum HDL-Cholesterol are as follows :

Men: 30 - 70 mg/dl

Women: 30 - 85 mg/dl

Note:

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range. According to NCEP, HDL-C values greater than or equal to 40 mg/dl are considered desirable and greater than or equal to 60 mg/dl are considered to offer some protection against coronary heart disease. Values below 40 mg/dl are considered to be a significant independent risk factor for coronary heart disease.

Performance Characteristics

Linearity

With AutoPure T HDL-C, the assay is linear upto 150 mg/dl (3.89 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 60 mg/dl of conjugated & unconjugated bilirubin, 1800 mg/dl of triglycerides, 1000 mg/dl of haemoglobin, 100 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 (<40 mg/dl)	32.9	0.3	0.8	32.8	0.4	1.3
Mid (40-59 mg/dl)	50.6	0.2	0.5	50.0	0.7	1.5
High (>60 mg/dl)	101.4	0.7	0.7	100.1	1.1	1.1

Co-Relation Studies

A comparison of HDL-C determination using AutoPure T HDL-C and the Designated Comparison Method (DCM) gave the following co-relation (mg/dl) :

Linear Regression

y = 0.99x + 2.81

r = 0.996

No. of samples measured : 52

References

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9. ***National Institutes of Health Publication*** No. 93-3095, September (1993).
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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 35 AHL-2009-05-00I

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

T HDL-C™

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Direct Homogeneous Assay

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