HDL CHOLESTEROL DIRECT Multi-Purpose (MPR) Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	*Quantity*	*Reagent*	*Storage*
GL602HD	2x45 ml	HDL CHOLESTEROL- 1	2-8°C
	2x15 ml	HDL CHOLESTEROL- 2	
	1X1ml	HDL-C CALIBRATOR	
GL612HD	2x90 ml	HDL CHOLESTEROL- 1	
	2x30 ml	HDL CHOLESTEROL- 2	
	1X1ml	HDL-C CALIBRATOR	
GL622HD	4x90 ml	HDL CHOLESTEROL- 1	
	4x30 ml	HDL CHOLESTEROL- 2	
	1X1ml	HDL-C CALIBRATOR	

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination of High Density Lipoprotein Cholesterol (HDL-C) in serum and plasma on automated and semi-automated analysers.

SUMMARY AND EXPLANATION: [1, 2]

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, high density lipoprotein cholesterol (HDL-C) has become an important tool used to assess an individual's risk of developing CHD since a strong negative relationship between HDL-C concentration and the incidence of CHD was reported [1]. Thus, there has been substantial interest in HDL-C analysis. Selective chemical precipitation techniques are widely used for the determination of HDL-C such as heparin-manganese, dextran-magnesium and phosphotungstate-magnesium. However, these techniques require physical separation via centrifugation, which is not suited to large scale laboratory use.

PRINCIPLE OF THE TEST: [4]

The direct HDL Cholesterol assay is a homogeneous method for directly measuring serum HDL-C levels without the need for any pre-treatment and centrifugation steps.
First step, substances with high affinity to LDL, VLDL and chylomicrons block them involving to enzyme reaction.
Second step, special surfactant that selectively accelerates reaction with the enzyme reagent with HDL cholesterol and determining them

$$\text{HDL-C} \xrightarrow[\text{Cholesterol esterase, oxidase}]{\text{Surfacant}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{HSDA} \xrightarrow{\text{POD}} \text{purple-blue pigment}$$

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Reagent 1: Clear Colourless liquid.
Reagent 2: Clear, pale yellow liquid.
Calibrator: Lyophilised pellet

Any significant changes from the above could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.
Safety Precautions:
Product is not hazardous under EU specification. Material Safety Data Sheet is available upon request.
Handling precautions:

- *Store protected from light.*
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:

Instrument application procedures are available upon request.

COMPONENT COMPOSITION:

	Contents	Conc in test
Reagent 1	TODB	1 mmol/l
	Ascorbate oxidase	3.0 U/ml
	PVS	2 mg/l
	PEGME	0.2%
	$MgCl_2$	2 mmol/l
	Buffer (pH 6.5)	10mmol/l
Reagent 2	Cholesterol esterase	4 U/ml
	Cholesterol oxidase	10 U/ml
	Peroxidase	30 U/ml
	4-aminoantipyrine	2.5 mmol/l
	Detergent	0.5%
	Buffer (pH 6.5)	10mmol/l

REAGENT PREPARATION AND STABILITY:

Reagent 1 and 2 are ready for use.
Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, unopened component is stable until expiry date stated on the label.

Calibrator: Carefully remove the stopper ensuring that no lyophilised material is lost. Reconstitute the calibrator using 1ml distilled water and leave to stand for 30 minutes before use. Calibrator concentrations are lot specific. Please check that the lot number on the vial corresponds to the lot number on the calibrator insert.

TYPE OF SPECIMEN: [5]

Use serum and heparin plasma as specimen.
It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Serum should be separated from cells within 2 hours after collection. Store the specimen at 2-8°C for 6 days. For prolonged storage, specimens should be stored frozen at -70°C for 1 year
EDTA plasma causes decreased values.

TEST PROCEDURE:

Materials required but not supplied:

Description	*Catalog. No.*	*Description*	*Catalog. No.*
Lipid Control Level 1	GL9009	Photometer	N/A
Lipid Control Level 2	GL9019	General Laboratory Equipment	N/A

Assay procedure:
Wavelength: λ: 600 nm
Temperature: 37°C
Optical path: 1 cm light path.

	Blank	Calibrator	Sample
Reagent 1	900 µl	900 µl	900 µl
Sample	----	----	12 µl
Calibrator	----	12 µl	----
Gently mix and Incubate at 37°C Measure the Optical Density A1 after 5 minutes.			
Reagent 2	300 µl	300 µl	300 µl
Gently mix and Incubate at 37°C Measure the Optical Density A2 after 5 minutes			

Calibration:
Using recommended Calibrator, calibrate the assay:

- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:

- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the QC Laboratory Programme.

CALCULATION:

$$\text{Concentration of HDL-Cholesterol} = \frac{OD_{Sample}}{OD_{Calibrator}} \times \text{Concentration of Calibrator}$$

(Conversion Factor: Qty in mmol/l = mg/dl x 0.0258).

EXPECTED VALUES: [3]

	Male (mmol/l)	Male (mg/dl)	Female(mmol/l)	Female(mg/dl)
<60 years	0.78 – 1.63	30 – 63	0.85 – 2.25	33 – 87
>60 years	0.78 – 1.94	30 – 75	0.85 – 2.49	33 – 96

Each laboratory should establish its own reference range. HDL-C results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
Linear up to 2.40 mmol/l
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.

Sensitivity:
The Lowest Detectable Level was estimated at 0.03 mmol/l

Precision:

Within Run N = 20	*Mean (mmol/l)*	*SD*	*% CV*	*Between Run N = 20*	*Mean (mmol/l)*	*SD*	*% CV*
Level 1	1.32	0.006	0.52	Level 1	1.31	0.038	2.94
Level 2	2.46	0.032	1.28	Level 2	2.41	0.095	3.93

Interfering substances

Bilirubin:	Less than 5% interference up to 20 mg/dl Bilirubin.
Intralipid:	Less than 5% interference up to 500 mg/dl Intralipid.
Haemolysis:	Less than 5% interference up to 500 mg/dl Haemoglobin.
Ascorbic acid:	Less than 5% interference up to 50 mg/dl.

Method Comparison:
Using 30 samples, a comparison, between this test (y) and another commercially available test (x), gave the following results:

y = 1.119x + 0.0428	r = 0.9869

BIBLIOGRAPHY:

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2. Recommendation of the second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practise; Eur. Heart J. 1998; 19:1434-1503.
3. Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed. 30-54, 485-487 and 987-988.
4. Shih WJ, Bachorik PS, Haga JA, Myers GL, Stein EA; Clinical Chemistry, 2000; 46:3:351-364
5. Third report of the National Cholesterol education Programme (NCEP) Expert Panel on detection, Evaluation and treatment of High blood cholesterol in adults (Adult treatment Panel III). JAMA Publication, Vol 285, No.19, P2486-2497; 2001

SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	STD	Aqueous Standard
CE	CE Mark - Device complies with the Directives 98/79/EC		
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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