DIRECT HDL-CHOLESTEROL (HDL)

RX SERIES

INTENDED USE

An HDL-cholesterol test system is a device intended for the quantitative *in vitro* determination of HDL-cholesterol concentration in human serum and plasma. This product is suitable for use on RX **series** instruments which includes the RX **daytona** and the RX **imola**.

Cat. No.

CH 3811	R1. Enzyme Reagent 1	3 x 51 ml
	R2. Enzyme Reagent 2	3 x 20 ml

GTIN: 05055273201291

CLINICAL SIGNIFICANCE

High-density lipoproteins (HDL) are one of the major classes of plasma lipoproteins. They are composed of a number of heterogeneous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serve to remove cholesterol from the peripheral cells to the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL-cholesterol (HDL-C) levels in serum and the incidence/prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognised [1].

Accurate measurement of HDL-C is of vital importance when assessing patient risk from CHD. In this diagnostic test kit a method for direct measurement of HDL-C, without sample pretreatment, is presented. Direct measurement gives improved accuracy and reproducibility when compared to precipitation methods.

PRINCIPLE[2]

The assay consists of 2 distinct reaction steps:

1. Elimination of chylomicron, VLDL-Cholesterol and LDL-Cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase.

$$\text{Cholesterol ester} \xrightarrow{\text{Cholesterolesterase}} \text{cholesterol + fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

2. Specific measurement of HDL-Cholesterol after release of HDL-Cholesterol by detergents in Reagent 2.

$$\text{Cholesterol ester} \xrightarrow{\text{Cholesterolesterase}} \text{cholesterol + fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{HDAOS} \xrightarrow{\text{Peroxidase}} \text{Quinone pigment} + 4H_2O$$

The intensity of the quinone imine dye produced is directly proportional to the cholesterol concentration when measured at 600 nm.

In the second reaction catalase is inhibited by sodium azide in Enzyme Reagent 2.

Key: 4 - AA - 4 - Aminoantipyrine
HDAOS - N - (2 - hydroxy - 3 - sulfopropyl) - 3,5 - dimethoxyaniline.

This assay uses a Rate method and a single point calibration.

SAMPLE COLLECTION AND PREPARATION[3,4]

Sample to be collected from a fasting individual. Samples may be taken from a non-fasting individual but subsequent results should be interpreted with care. Serum, heparinized Plasma or EDTA Plasma are the recommended samples. Serum stable for 6 days at +2 to +8°C. Samples are stable for 1 year when stored at -70°C. If any samples show precipitates, centrifuge before using.

REAGENT COMPOSITION

Contents	Initial Concentration of Solution
R1. Enzyme Reagent 1	
N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid	100 mM, pH 6.6 (25 °C)
N-(2-hydroxy-3-Sulfopropyl)-3,5-dimethoxyaniline, sodium salt (HDAOS)	0.7 mM
Cholesterol Esterase [E.C.3.1.1.13. Microorganism]	≥800 U/l
Cholesterol Oxidase [E.C.1.1.3.6. *Streptomyces* sp]	≥500 U/l
Catalase [E.C.1.11.1.6. Microbial]	≥300 KU/l
Ascorbate oxidase [EC.1.10.3.3. *Acremonium* sp.]	≥3000 U/l
R2. Enzyme Reagent 2	
N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid	100 mM, pH 7.0 (25 °C)
4-Aminoantipyrine	4.0 mM
Peroxidase [E.C.1.11.1.7, Horse Radish, 25°C]	≥3500 U/l
Sodium Azide	0.05 w/v %
Surfactants	1.4 % w/v %

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Enzyme Reagent 2 (R2) contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety Data Sheets are available on request.

Please dispose of all biological and chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Enzyme Reagent 1

Contents ready for use. Stable up to expiry date specified when stored unopened at +2 to +8°C, stored protected from light.

R2. Enzyme Reagent 2

Contents ready for use. Stable up to expiry date when stored unopened at +2 to +8°C, stored protected from light.

MATERIALS PROVIDED

Direct HDL-C Reagents

MATERIALS REQUIRED BUT NOT PROVIDED

Direct HDL-C/LDL-C Calibrator (CH 2673)
Randox Lipid Control Level 1 (LE 2661), Level 2 (LE 2662) and Level 3 (LE 2663)

PROCEDURE NOTES

The Chemistry parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software using the chemistry parameters importing software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the barcode. If the barcode cannot be read by the analyzer, enter manually the series of numbers given beneath the barcode. If problems continue, contact Randox Laboratories RX Services, Northern Ireland (028) 94451070.

CALIBRATION

0.9% NaCl solution and Randox Direct HDL-C/LDL-C Calibrator are recommended for calibration.

TRACEABILITY

Values are assigned according to the requirements of the "HDL Cholesterol Method Evaluation Protocol for Manufacturers" of the US National Reference System for Cholesterol, CRMLN.

This assay uses a **linear** calculation and a **reagent blank** at calibration. Ensure that on the [Calibration] [Checks (F10)] screen the following are selected for this test:

Sampling Method for Standards
- **Duplicate**

Reagent Blank measurement
- **Enable Reagent Blank - None**

Reagent Blank measurement
- **Reagent Blank (system water)**

QUALITY CONTROL

Randox Lipid Control Sera, Level 1, Level 2 and Level 3 are recommended for daily quality control. Quality control materials are intended for use only to monitor accuracy and precision. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories RX Services, Northern Ireland (028) 94451070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

INTERFERENCES

The analytes below were tested up to the following levels and were found not to interfere:

Haemoglobin	1000 mg/dl
Free Bilirubin	25 mg/dl
Conjugate Bilirubin	25 mg/dl
Intralipid®	800 mg/dl
Triglycerides	1000 mg/dl

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [4]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

EXPECTED VALUES [5,6]

mg/dl	mmol/l	
< 40	<1.04	Low
≥60	≥1.55	High

As HDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should establish its own reference ranges.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were obtained using an RX **daytona**.

LINEARITY

The method is linear up to 3.73 mmol/l (144 mg/dl). In the event of a rerun, the upper limit of the assay range is increased to 5.60 mmol/l (216 mg/dl).

SENSITIVITY

The minimum detectable concentration of HDL Cholesterol with an acceptable level of precision was determined as 0.189 mmol/l (7.30 mg/dl).

PRECISION

Within Run Precision

	Level 1	Level 2	Level 3
Mean(mmol/l)	0.788	1.32	2.00
SD	0.016	0.019	0.062
CV%	1.80	1.45	3.11
n	20	20	20

Between run precision

	Level 1	Level 2	Level 3
Mean (mmol/l)	0.817	1.38	2.01
SD	0.023	0.033	0.055
CV(%)	2.81	2.40	2.73
n	20	20	20

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.05 X - 0.06

and a correlation coefficient of r = 0.99.

40 patient samples were analyzed spanning the range 0.53 to 2.31 mmol/l.

REFERENCES

1. National Institutes of Health Consensus Development Conference Statement : Triglyceride, High Density Lipoprotein and Coronary Heart Disease. Washington D.C. Feb 26-28, 1992.
2. Izawa S., Okada M., Matsui H., and Horita Y. J. Medicine and Pharmaceutical Sci., 1385 - 1388, **37** (1997).
3. Shih WJ, Bachorik PS, Haga JA, Myers GL, Stein EA; Clinical Chemistry, 2000; **46:3**:351 – 364.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.
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.
6. Jacobs, D. *et al*. In Laboratory and Test Handbook; Jacobs, D.S; Kasten, B.L., De Mott, W.R., Wolfson, W.L., Eds; Lexi - Comp Inc: Hudson (Cleveland), 1990; P. 219.

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The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

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