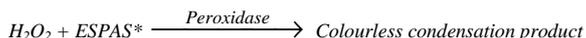
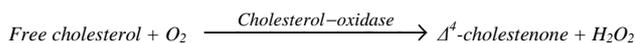
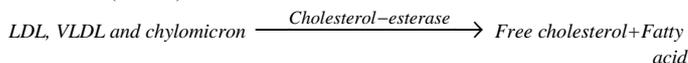


**A direct enzymatic method for the quantitative determination of high density lipoprotein cholesterol (HDL-C) in serum.**

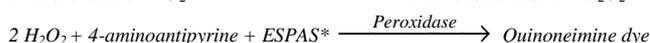
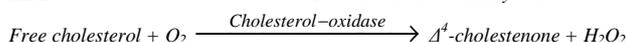
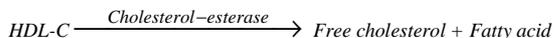
**Principle**

First step.  
Cholesterol in LDL, VLDL and chylomicron (CM) in the specimen are catalyzed by Cholesterol esterase and Cholesterol oxidase with addition of R1 solution into the specimen. HDL cholesterol are not catalyzed at first step. The generated hydrogen peroxide is consumed by the condensation reaction with N-ethyl-N-(3-sulfo-propyl)-m-anisidine (ESPAS) in existence of Peroxidase



Second step.

Subsequently, HDL cholesterol in the specimen is catalyzed by Cholesterol esterase and Cholesterol oxidase with addition of R2 solution into the specimen. ESPAS and 4-aminoantipyrine (4-AA) are condensed by the generated hydrogen peroxide and Peroxidase to generate a quinone dye. The absorbance of the generated quinone dye is analyzed to obtain the HDL cholesterol concentration in the specimen with reference of a standard solution.



**Reference values**

**Adult male: 0.92-2.07 mmol/l (35.3-79.5 mg/dl)**

**Adult female: 1.09-2.29 mmol/l (42.0-88.0 mg/dl)**

It is recommended that each laboratory should assign its own normal range.

**Reagents**

**R1**

Cholesterol Esterase

Cholesterol Oxidase

Peroxidase

ESPAS

**R2**

Cholesterol Esterase

4-aminoantipyrine

**3. HDL cholesterol standard**

Ready for use. For details please check the insert.

Available only in Cat. No.: 42661S and 42662S

**Onboard stability:** 60 days without calibration

**Samples**

Use serum as a specimen. It is recommended to measure HDL-C immediately after collection.

**PROCEDURE**

**Reagents 1 and 2 are ready for use.**

**Assay conditions**

Main wavelength: 600 nm

Sub wavelength: 800 nm

Light path: 1 cm

Temperature: 37 °C

Method: endpoint (increasing)

**Pipette into cuvette**

	Reagent blank	Standard	Sample
<b>Reagent R1</b>	225 µl	225 µl	225 µl
<b>Standard</b>		2,4 µl	
<b>Sample</b>			2,4 µl
<b>Distilled water</b>	2,4 µl		
Mix and incubate for 5 minutes and then measure the difference between absorbance at 600 nm and 800 nm against the reference			
<b>Absorbance1</b>	A(bl)1	A(std)1	A(s)1
<b>Reagent R2</b>	75 µl	75 µl	75 µl
Mix and incubate for 5 minutes and then measure the difference between absorbance at 600 nm and 800 nm against the reference			
<b>Absorbance2</b>	A(bl)2	A(std)2	A(s)2

**Calibration**

S1: Distilled water

S2: Sentinel HC calibrator

Wako HC calibrator

Randox HC calibrator

**Calibration frequency**

Two point calibration is recommended:

- after reagent lot change,
- as required following quality control procedures.

**Calculation**

$$\text{HDL - C (mg/dl)} = \frac{(A(s)2 - A(s)1) - (A(bl)2 - A(bl)1)}{(A(std)2 - A(std)1) - (A(bl)2 - A(bl)1)} \times \text{std. conc (mg/dl)}$$

**Quality control**

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits

**PERFORMANCES DATA**

**Linearity**

The test is linear up to 3,90 mmol/l (150 mg/dl).

The reagent is linear between the concentration of 1-150 mg/dl (0.026-3,90 mmol/l).

**Sensitivity**

The absorbance when measuring water is less than 0,05. The absorbance when measuring standard (100 mg/dl) is in the range of 0,05-0,17

**Specificity**

When measuring control serum, the data spread of assay expected value is within 10%

When the sample's triglyceride concentration exceeds up to 1000 mg/dl, dilute the sample with physiological saline and repeat the measurement.

There is minimal interference of measurements with up to 20 mg/dl bilirubin, 50 mg/dl ascorbic acid, 500 mg/dl hemoglobin, 1200 formazine turbidity unit, 200 mg/dl EDTA (Na salt), and 80 U/ml heparin.

**Precision**

Concentration (mg/dl)	Intra-run precision
	CV%
34,8	1,07
97,6	0,90

**Correlation**

A comparative study has been performed between our reagent and other commercial HDL-cholesterol reagent. The parameters of linear regression are as follows:  
y=0,9895x+2,8556 (mg/dl), r=0,9952, N=73

**Note**

Do not use the pretreatment reagent, which was frozen by mistake.

**For in vitro diagnostic use only.**

**The following symbols are used on labels**

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

**Bibliography**

National Cholesterol Education Program : Executive summary of third report of the National Cholesterol Education Program expert panel on detection, evaluation and treatment of high blood cholesterol in adults (JAMA,285:2486-2497,2001)