BioSystems

COD 11557 1 x 80 mL
STORE AT 2-8ºC
Reagents for measurement of HDL cholesterol concentration Only for *in vitro* use in the clinical laboratory

CHOLESTEROL HDL DIRECT

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DIRECT
DETERGENT

PRINCIPLE OF THE METHOD

The cholesterol from low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons, is broken down by the cholesterol oxidase in an enzymatic accelerated non-color forming reaction. The detergent present in the reagent B, solubilizes cholesterol from high density lipoproteins (HDL) in the sample. The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below[1].

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{chol.esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{chol.oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \text{4-Aminoantipyrine} + \text{DSBmT} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS AND COMPOSITION

A. Reagent. 1 x 60 mL. Good´s buffer, cholesterol oxidase < 1 U/mL, peroxidase < 1 U/mL, N,N-bis(4-sulfobutyl)-m-toluidine (DSBmT) 1 mmol/L, accelerator 1 mmol/L.

B. Reagent. 1 x 20 mL. Good´s buffer, cholesterol esterase < 1.5 U/mL, 4-aminoantipyrine 1 mmol/L , ascorbate oxidase < 3.0 KU/L, detergent.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration: Presence of particulate material, turbidity.

AUXILIARY REAGENTS

Biochemistry Calibrator Human (BioSystems cod. 18044) or Cholesterol HDL/LDL Calibrator (BioSystems cod. 11693).

S. Cholesterol HDL/LDL calibrator (cod. 11693). Store at 2-8ºC. Human serum. Concentration is given on the values sheet. The concentration value is traceable to the CDC Reference Measurement Procedure (Centers for Disease Control and Prevention). Reconstitute with 1.0 mL of distilled water. Stable for 1 week at 2-8ºC or for 2 months at –18ºC when frozen in aliquots. Avoid repeated freeze-thaw cycles.

Components from human origin have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for Hbs antigen. However, they should be handled cautiously as potentially infectious.

REAGENT PREPARATION

Reagents are provided ready to use.

ADDITIONAL EQUIPMENT

– Thermostatic water bath at 37ºC.

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at (main wavelength) 600 ± 20 nm and(sub-wavelength) 700 nm ± 20 nm.

SAMPLES

Serum collected by standard procedures.

HDL cholesterol in serum or plasma is stable for 7 days at 2-8ºC. EDTA, lithium or sodium heparin may be used as anticoagulants.

PROCEDURE

1. Bring the Reagents and the photometer to 37ºC.
2. Pipette into a cuvette: (Notes 1 and 2)

Reagent A	750 µL
Serum/Calibrator	7 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch. After 5 minutes, read the absorbance (A_1) at 600/700 nm against distilled water.
4. Pipette into a cuvette:

Reagent B	250 µL

Mix.

5. After 5 minutes, read the absorbance (A_2) at 600/700 nm.

CALCULATIONS

The cholesterol HDL concentration is calculated using the following general formula:

$$\frac{(A_2\text{-}A_1)_{\text{Sample}}}{(A_2\text{-}A_1)_{\text{Calibrator}}} \times C_{\text{Calibrator}} = C_{\text{Sample}}$$

REFERENCE VALUES

HDL cholesterol concentrations vary considerably with age and sex. The following cut-off point has been recommended for identifiying individuals at high risk of coronary artery disease[2].

Up to 35 mg/dL = 0.91 mmol/L	High risk
> 60 mg/dL = > 1.56 mmol/L	Low risk

QUALITY CONTROL

It is recommended to use the Lipid Control Serum level I (cod. 18040) and II (cod. 18041) or the Biochemistry Control Serum Human level I (cod. 18042) and II (cod. 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 0.5 mg/dL = 0.01 mmol/L.

– Linearity limit: 200 mg/dL = 5.18 mmol/L.

– Repeatibility (within run):

Mean Concentration	CV	n
32.9 mg/dL = 0.85 mmol/L	0.8 %	20
50.6 mg/dL = 1.31 mmol/L	0.5 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
32.8 mg/dL = 0.85 mmol/L	1.3 %	40
50.0 mg/dL = 1.30 mmol/L	1.5 %	40

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Hemoglobin (10 g/L), lipemia (triglycerides 18 g/L) and bilirubin (20 mg/dL) do not interfere. Other drugs and substances may interfere.

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

HDL play an important part in the removal of cholesterol from tissues and its transportation to the liver for removal as bile acids.

Decreased plasma HDL-cholesterol concentrations are positively correlated with the incidence of atherosclerosic diseases, basis of myocardial infarction and cerebrovascular accidents[4,5].

There are several disease states or environmental influences associated with reduced levels of HDL: acute or chronic hepatocellular diseases, intravenous hyperalimentation, severe malnutrition, diabetes, chronic anemia, myeloproliferative disorders, Tangier disease, analphalipopro-teinemia, acute stress, some drugs and smoking[4,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Sample and Reagent volumes may be varied as long as the same ratio is maintained.
2. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Warnick GR Nauck M, , Rifai N. Evolution of methods for measurement of HDL-cholesterol: from ultracentrigutaion to homogeneous assays. *Clin Chem* 2001; 47: 1579-96.
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3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
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