CHOLESTEROL HDL DIRECT

COD 12757

BioSystems

CHOLESTEROL HDL
DIRECT TOOS

INTENDED USE

Reagent for the quantitative measurement of HDL cholesterol concentration in human serum or plasma for monitoring the lipid metabolism and the risk of cardiovascular disease in the general population.

The reagent can be used in A15-A25 BioSystems analyzers. For *in vitro* professional use only in the clinical laboratory.

CLINICAL BENEFIT

Decreased plasma HDL-cholesterol concentrations positively correlate with the incidence of atherosclerosic diseases, basis of myocardial infarction and cerebrovascular accidents[1,2].

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[1,2].

Based on clinical guidelines and textbooks, and when used in conjunction with other diagnostic technologies and options, this medical information is useful for the assessment of HDL cholesterol variations. Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

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[3].

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

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

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

CONTENTS AND COMPOSITION

A. Reagent. 3 x 20mL: MES buffer at 100 mmol/L, polymers, 4-aminoantipyrine at 0.5 mmol/L, detergent, pH 6.5.

WARNING: H317 - May cause an allergic skin reaction. P333+P313 - If skin irritation or rash occurs: Get medical advice/attention. P362 - Take off contaminated clothing and wash before reuse. P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.

B. Reagent. 1 x 20 mL: MES buffer 50 mmol/L, cholesterol esterase 1.0 U/mL, peroxidase 1.0 U/mL, cholesterol oxidase 0.5 U/mL, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS) 4.5 mmol/L, detergent, pH 5.5.

WARNING: H317 - May cause an allergic skin reaction. P333+P313 - If skin irritation or rash occurs: Get medical advice/attention. P362 - Take off contaminated clothing and wash before reuse. P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.

STORAGE AND STABILITY

Store at 2-8ºC.

Components are stable once opened until the expiry date marked in the label if they are kept at the recomended storage temperature, well closed and care is taken to prevent contamination during their use.

On board stability: The reagents opened and stored in the refrigerated compartment of the analyzer are stable for 3 months.

Indications of deterioration: Absorbance of the blank over the limit indicated in the parameterization of the analyzer.

WARNINGS AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

- Biochemistry Calibrator Human (BioSystems cod. 18044).
- 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.
- Quality control materials (see Quality Control section).

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.

R1: Use Reagent A, R2: Use Reagent B.

TEST PARAMETERS AND CALCULATIONS

Test parameters and calculations are programed in the BioSystems A15-A25 analyzers.

SAMPLES

Serum or plasma collected by standard procedures.

HDL cholesterol in serum or plasma is stable for 2 days at 20-25ºC, 7 days at 4-8ºC and 3 months at -20ºC[4].

CALIBRATION

A reagent blank should be done every day and a calibration at least every 3 months, after reagent lot change or as required by quality control procedures. It is recommended to use the calibrators mentioned in the paragraph on Additional Materials Required.

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 accuracy of the measurement procedure. See the corresponding IFU.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if control results are not within the acceptable limits.

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[5].

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

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

ANALYTICAL AND CLINICAL PERFORMANCE

The metrological characteristics described below have been obtained following the guidelines of the Clinical & Laboratory Standards Institute (CLSI).

Limits and ranges	A15	A25
	Serum / plasma	Serum / plasma
Limit of Detection (mg/dL)	1.90	2.07
Limit of detection (mmol/L)	0.05	0.05
Measuring Range * (mg/dL)	7.66 - 180	6.22 - 180
Measuring Range * (mmol/L)	0.20 – 4.66	0.16 – 4.66

*For samples with higher values, dilute manually or refer to the Test Parameterization for Automatic dilution (note that all these samples will be diluted with the same dilution ratio). Measuring range: from Limit of Quantification to Limit of Linearity.

Precision	A15			A25		
	Mean (mg/dL) / (mmol/L)	Repeatability (CV)%	Within-laboratory (CV)%	Mean (mg/dL) / (mmol/L)	Repeatability (CV)%	Within-laboratory (CV)%
Serum / Plasma	30.7 / 0.80	3.6	5.1	32.5 / 0.85	3.5	5.1
	45.8 / 1.19	2.6	3.5	45.2 / 1.17	1.8	3.8
	77.4 / 2.01	1.5	3.6	75.4 / 1.96	1.2	3.9

Method Comparison	Serum	EDTA Plasma	Heparin plasma
Number of samples (n)	97	86	95
Passing-Bablok regression	y = 0.51 + 1.005x	y = 6.07 + 0.921x	y = 4.99 + 0.893x
Correlation Coefficient (r)	0.997	0.996	0.990
Comparison interval (mg/dL)	14.3 - 140	7.05 - 137	4.1 - 156

LIMITATIONS OF THE PROCEDURE

- Interferences: Bilirubin (up to 18 mg/dL - 308 μmol/L), hemolysis (hemoglobin up to 500 mg/dL), lipemia (triglycerides up to 1681 mg/dL - 19 mmol/L) and acetaminophen (up to 20 mg/dL - 1324 μmol/L) do not interfere. Ascorbic acid interferes. Other drugs and substances may interfere[6].

BIBLIOGRAPHY

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5. National Cholesterol Educarion Program Expert Panel. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda: National Heart, Lung, and Blood Institute; 2001.
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