INTENDED USE

Reagent for the quantitative measurement of LDL cholesterol concentration in human serum or plasma for monitoring the lipid metabolism and the risk of cardiovascular disease in the general population. This reagent is for use in the BioSystems A25 and A15 analyzers.

For *in vitro* professional use only in the clinical laboratory

CLINICAL BENEFIT

LDL is the main lipoprotein transporting cholesterol from liver to tissues.

Increased plasma LDL-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 increased levels of LDL-cholesterol: Nephrosis, diabetes, obesity, some drugs and smoking[1,2].

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

A specific detergent solubilizes the cholesterol from high density lipoproteins (HDL), very low density lipoproteins (VLDL) and chylomicrons. The cholesterol esters are broken down by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. The second detergent, present in the reagent B, solubilizes cholesterol from low density lipoproteins (LDL) in the sample. The LDL 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 20 mL. MES buffer 50 mmol/L, cholesterol esterase > 0.2 U/mL, cholesterol oxidase < 1.0 U/mL, 4-aminoantipyrine 0.5 mmol/L, peroxidase > 1.0 U/mL, detergent, preservative, pH 6.6.

WARNING: H317: May cause an allergic skin reaction. P261: Avoid breathing vapours. P280: Wear protective gloves,protective clothing, eye protection, face protection. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P332+P313: If skin irritation occurs: Get medical advice. P362: Take off contaminated clothing and wash before reuse.

B. Reagent: 1 x 20 mL. MES buffer 50 mmol/L, N-ethyl-N-(2hydroxy-3-sulfopropyl)-3-methylaniline (TOOS) 1.0 mmol/L, detergent, preservative, pH 6.6.

WARNING: H317: May cause an allergic skin reaction. P261: Avoid breathing vapours. P280: Wear protective gloves, protective clothing, eye protection, face protection. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P332+P313: If skin irritation occurs: Get medical advice. P362: Take off contaminated clothing and wash before reuse.

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.

SAMPLES

Serum and plasma collected by standard procedures. Heparin and EDTA may be used as anticoagulants.

LDL cholesterol concentration in serum or plasma is stable for 1 day at 20-25ºC, 7 days at 4-8ºC and 3 months at -20ºC[4].

TEST PARAMETERS AND CALCULATIONS

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

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

The following uniform cut-off points have been established by the US National Cholesterol Education Program and have also been adopted in many other countries for the evaluation of coronary artery disease risk[5].

Up to 100 mg/dL = 2.59 mmol/L	Optimal
100-129 mg/dL = 2.59-3.34 mmol/L	Near optimal/above optimal
130-159 mg/dL = 3.37-4.12 mmol/L	Borderline High
160-189 mg/dL = 4.14 –4.90-mmol/L	High
> 190 mg/dL = 4.92 mmol/L	Very High

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)	2.79	2.35
Limit of detection (mmol/L)	0.07	0.06
Measuring Range * (mg/dL)	8.42-700	5.17-700
Measuring Range * (mmol/L)	0.22-18.1	0.13-18.1

*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	104 / 2.69	2.3	4.3	105 / 2.72	1.3	2.7
	143 / 3.70	2.1	5.6	140 / 3.62	0.8	5.1
	202 / 5.24	2.2	4.5	198 / 5.12	2.5	3.2

Method Comparison	Serum	Plasma EDTA	Plasma Heparin
Number of samples (n)	118	87	134
Passing-Bablok regression	y = 2.5 + 0.98x	y = 5.6 + 1.01x	y = 1.8 + 1.06x
Correlation Coefficient (r)	0.996	0.991	0.994
Comparison interval (mg/dL)	5.22-639	4.82-605	5.13-674

LIMITATIONS OF THE PROCEDURE

- Interferences: Hemolysis (hemoglobin up to 500 mg/dL), bilirubin (up to 30 mg/dL - 513 µmol/L), lipemia (triglycerides up to 1691 mg/dL - 19.1 mmol/L) and acetaminophen (up to 20 mg/dL - 1324 µmol/L)do not interfere. Ascorbic acid (18 mg/dL – 1022 µmol/L) interfere. Other drugs and substances may interfere[6].

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