HDL CHOLESTEROL

Precipitating Reagent PEG 6000

REF. 0056 **2x100 ml**

CE IVD

Azienda certificata DNV

GIESSE DIAGNOSTICS

INTENDED USE

Quantitative determination of HDL Cholesterol in serum.

PRINCIPLE

The betalipoproteins VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated with a polyanionic reagent (PEG 6000) and on the supernatant, after centrifugation, the alpha fraction, HDL cholesterol (high density lipoproteins) is determined with colorimetric enzymatic method.

SAMPLE

Fresh unhemolyzed serum

HDL in the sample is stable 3 days at 2-8°C and one month at -20°C.

KIT COMPONENTS

Reagent (A) HDL Volume = 2x100 ml	Good Buffer PEG 6000 Sodium Azide	100 mmol/l 100 mmol/l 14 mmol/l
Standard CHOL Volume = 1x5 ml	Cholesterol Sodium azide	50 mg/dl (1.295 mmol/l) 14 mmol/l

The reagents are stable until the expiration date indicated on the label if stored at 2-8°C and protected from light. Do not freeze. Once opened reagents are stable for 2 months at 2-8°C if contamination is avoided.

Keep bottles closed when not in use.

REAGENTS PREPARATION

Liquid Reagents, bring to room temperature (15-25°C) before use.

PRECAUTIONS AND WARNINGS

Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Use the normal precautions required in the laboratory.

Dispose of waste according to local laws.

PROCEDURE

Wavelength:	510 nm (500 – 520)
Lightpath:	1 cm
Temperature:	37°C
Reading:	against blank reagent
Method:	Increasing End Point

PHASE 1:

Pipette:	
Reagent (A) HDL	500 µl
Sample	500 µl

Mix on vortex and incubate for 5 minutes at room temperature, centrifuge at 2000/3000 r.p.m. for 10 minutes.

Rarely can you have an incomplete precipitation (presence of supernatant fat): we recommend using 3 parts of Reagent (A) and one part of serum. Multiply then the result by 4 (dilution factor).

PHASE 2:

Determine on supernatant the HDL Cholesterol with the enzymatic colorimetric method (REF. 0045) as follows:

Pipette:	Blank	Sample	Standard
Reagent (A)	1000 µl	1000 µl	1000 µl
water	25 µl		
Sample		25 µl	
Standard			25 µl

Mix, incubate at 37°C for 5 minutes, read the absorbance of the sample (Ax) and the standard (As).

Volumes can be proportionally modified.

This method describes the manual procedure to use the kit.

For automated procedure, ask for specific applications.

RESULTS CALCULATION

HDL (mg/dl) = Ax/As x 50 (Standard Value) x 2 (dilution factor)

For the calculation of LDL Cholesterol use the following formula:

LDL (mg/dl) = Total Cholesterol – HDL – (Triglycerides/5)

This formula will be valid if the value of triglycerides is less than 400 mg/dl and the patient does not have a type III hyperlipoproteinemia.

EXPECTED VALUES

HDL:

Women:	42 - 88	mg/dl
Men:	35 - 80	mg/dl

LDL:

Adults:	66 - 178	mg/dl

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL

You must perform the controls at each kit's use and verify that the values obtained are within the reference range reported in the operating instructions. For this purpose we recommend the use of **controls (REF.6011).**

PERFORMANCE

Sensitivity: the sensitivity of the method is: 2 mg/dl.

Linearity: the method is linear up to 700 mg/dl. For higher values, dilute the sample 1:5 and multiply the result by 5.

Precision intra-assay:

	Level 1	Level 2
Mean (mg/dl)	32.1	88.9
DS	0.18	0.61
CV %	0.55	0.68

Precision inter-assay:

	Level 1	Level 2
Mean (mg/dl)	32.8	100.1
DS	0.4	1.1
CV %	1.3	1.1

Interferences: bilirubin does not interfere up to 20 mg/dl. Hemoglobin does not interfere up to 500 mg/dl. Triglycerides do not interfere up to 1000 mg/dl.

Correlation against a reference method: Y = 0.96x + 2.5 r = 0.998

REFERENCES

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Ed. 2012/03 rev. 00