



HDL cholesterol

Precipitating reagent

For *In-Vitro* and professional use only

Store at 2° to 8°C

PRINCIPLE OF THE METHOD

The very low density (VLDL) and low density (LDL) lipoproteins from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. After removed by centrifugation the clear supernatant containing high density lipoproteins (HDL) is used for the determination of HDL cholesterol^{1,2}.

CLINICAL SIGNIFICANCE

HDL particles carry cholesterol from the cells back to the liver. HDL is known as “good cholesterol” because high levels are thought to lower the risk of heart disease. A low HDL cholesterol levels, is considered a greater heart disease risk^{1,6,7}. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R Precipitating Reagent	Phosphotungstic acid Magnesium chloride	14 mmol/L 2 mmol/L
Optional	Cholesterol	

PREPARATION

The reagent is ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

Signs of reagent deterioration:

– Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

– Spectrophotometer or colorimeter measuring at 505 nm.
– Matched cuvettes 1.0 cm light path.
– General laboratory equipment.

SAMPLES

Serum or plasma¹: Free of hemolysis. Removed from the blood clot as soon as possible.

Stability : HDL Cholesterol is stable for 7 days at 2-8°C .

PROCEDURE

Precipitation

1. Pipette into a centrifuge tube:

R (µL)	100
Sample (mL)	1.0

- Mix well; allow to stand for 10 min at room temperature.
- Centrifuge at 4000 r.p.m. for 20 min or 2 min at 12000 r.p.m..
- Collect the supernatant and test HDLc.

Test

Following the Cholesterol reagent instructions.

CALCULATIONS

- With Calibrator:

(A) Sample x (Calibrator conc.) = mg/dL HDLc in the sample

(A) Standard

- With Factor:

$A_{505\text{ nm}} \text{ Sample} \times 320 = \text{mg/DI HDLc in the sample.}$

$A_{546\text{ nm}} \text{ Sample} \times 475 = \text{mg/DI HDLc in the sample}$

Calculation of LDL-cholesterol

According to the Friedewald Formula:

$$\text{LDL cholesterol} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL cholesterol}$$

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES³

HDL- Cholesterol

	Hombres	Mujeres
Lower risk	> 55 mg/dL	> 65 mg/dL
Standard risk	35-55 mg/dL	45-65 mg/dL
Increased risk	< 35 mg/dL	< 45 mg/dL

LDL- Cholesterol

Suspected above	:	150 mg/dL
Increased above	:	190 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 1.57 mg/dL to linearity limit of 275 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	75.8	33.9	95.2	182
SD	0.89	0.85	2.59	3.04
CV (%)	1.18	2.51	2.72	1.68

Sensitivity: 1 mg/dL = 0.0015 A.

Accuracy: Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.99.

Regression equation: $y = 0,9944x - 1,2346$.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed with triglycerides up to 4 g/L¹.

A list of drugs and other interfering substances with HDL cholesterol determination has been reported by Young et. al^{4,5}.

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