HDL CHOLESTEROL
Insert

Ref.: 13

Intended use . System for selective precipitation of Low and Very Low Density Lipoproteins (LDL and VLDL) and HDL cholesterol measurement in the supernatant, by an end point reaction.

Professional use.

[For in vitro diagnostic use.]

Test principle . Very Low Density Lipoproteins (VLDL) and Low Density Lipoprotein (LDL) are quantitatively precipitated and after centrifugation, the cholesterol bound to High Density Lipoproteins (HDL cholesterol) is measured in the supernatant.

Summary . In order to select a system for measuring HDL Cholesterol, Labtest Research and Development group decided for the phosphotungstic acid and magnesium chloride that, precipitating selectively and quantitatively VLDL and LDL, allow to obtain results comparable to the reference method.
After centrifugation, HDL Cholesterol is measured in the supernatant by the enzymatic system Cholesterol Liquiform Labtest (Ref.: 76).

The colorimetric measurement system is easily applied to most automatic equipments which are able to measure an end point reaction at 500 nm.

Methodology . Labtest.

Reagents

1. R 1 - Precipitant. Store at 2 - 8 ºC.
Reagent label bears expiration date. Phosphotungstic acid (1.5 mmol/L) and magnesium chloride (54 mmol/L).

2. CAL - Standard - 20 mg/dL. Store at 2 - 30 ºC.
Reagent label bears expiration date. Cholesterol (0.52 mmol/L), and sodium azide (14.6 mmol/L).

Precautions and warnings

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

The Standard contains sodium azide as preservative. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label. In order to avoid evaporation of the Standard, keep the bottle tightly closed.

Deterioration . Microbial or chemical contamination may decrease reagents stability.

Reagent 1 (Cholesterol Liquiform Ref. 76) is not suitable for use if it has an absorbance ≥0.300 at 500 nm when measured versus water as reference, in case of contaminations signs or if it develops turbidity.

Sample

HDL cholesterol is reportedly stable in serum or plasma (lithium heparin and EDTA) for about 14 hours at 15 - 30 ºC. After that, the samples can be stored for about 7 days at 2 - 8 ºC, for about 30 days at -20 ºC and for long term storage at ≤-70 ºC.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Bilirubin up to 5 mg/dL, hemoglobin up to 180 mg/dL and triglycerides up to 750 mg/dL do not interfere significantly.
Bilirubin values over 5 mg/dL yield false decreased results.
Triglycerides values over 750 mg/dL yield false increased results.

HDL cholesterol measured in the same person in different occasions may differ due to the biological and analytical method variations.
Recent diet, alcohol ingestion, physical weight variation, physical exercises, and smoking may strongly affect the HDL cholesterol concentration in blood. Hormones and other drugs also cause variation in the HDL cholesterol concentration.
It is considered that the biological variation is around of 7.5%.
In this way, repeating the measurement in the same person, two thirds of the results would be ranging ± 7.5% of the average. Therefore, biological variation consists in the most important factor of HDL cholesterol total variability. Biological variation effects may be controlled up to some point by the standardization of the conditions of patient preparation and blood collecting process, but HDL cholesterol can not be reliably estimated with one assay in only one sample. Several samples should be obtained and the average of results may be considered as HDL cholesterol usual concentration or, more exactly, may be considered a usual range of results for this person.

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Materials required not provided

1. A constant temperature water bath (37 ºC).
2. Photometer capable of measuring absorbance at 490 - 540 nm.
3. Pipettes to measure reagents and samples.
4. Timer.
5. Centrifuge (centrifugation capacity over 3500 rpm).
6. Reagent for cholesterol determination.

Manual procedure

Precipitation of VLDL and LDL.

In one tube (12 x 75 mm) add:

Serum:	0.25 mL
Precipitant:	0.25 mL

Always keep the proportion Sample:Precipitant equal to 1:1.

Shake vigorously during 30 seconds. It is suggested because is fundamental for obtaining consistent results. Centrifuge at 3500 rpm for at least 15 minutes in order to get a clear supernatant.
After centrifugation, remove the clear supernatant avoiding false increased results.

Lipemic samples and occasionally non-lipemic samples may present a cloudy supernatant. In this case, dilute the sample 1:2 with NaCl (150 mmol/L) and repeat the precipitation. Multiply the final result by 2. In case of the supernatant remains cloudy the sample should not be use for HDL cholesterol measurement.

Some samples, mainly lipemic, may present a clear supernatant with a layer on the surface that should not be pipetted avoiding false increased results.
Avoid mixing the precipitate in order not to get false increased results.

Colorimetric assay . See notes No. 1, 2 and 3.

Use Reagent 1 - Cholesterol Liquiform Labtest (Ref. 76).

Set up three tubes and proceed as follows:

	Blank	Unknown	Standard
Supernatant	----	0.1 mL	----
Standard	----	----	0.1 mL
Reagent 1	1.0 mL	1.0 mL	1.0 mL

Mix and incubate in a water bath at 37 ºC during 10 minutes. Measure the absorbance of the Unknown and Standard against Blank at 500 nm or green filter (490 - 540). The color is stable during 60 minutes.

Quality control . For quality control use Qualitrol H Level 1 and Qualitrol H Level 2 or other suitable control material. The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective measures to be taken if values fall outside the control limits.

Calculations . Due the dilution 1:2 applied to the samples during the VLDL and LDL precipitation process, the Standard value must be 40 mg/dL.

$$\text{HDL cholesterol (mg/dL)} = \frac{A_{unknown}}{A_{standard}} \times 40$$

Due the high reproducibility that this methodology offers, the factor method may be applied.

$$\text{Calibration Factor} = \frac{40}{A_{standard}}$$

$$\text{HDL cholesterol (mg/dL)} = A_{Unknown} \times \text{Factor}$$

Measurement/reportable range

Up to 200 mg/dL.
If HDL Cholesterol concentration exceeds 200 mg/dL, the sample must be diluted 1:2 with 0.85% NaCl. Multiply the result by factor 2.

Expected values . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Recommended and expected values substitute the reference values and are determined from epidemiologic data, statistically analyzed, that relate cholesterol levels to the Ischemic Coronary Disease (ICD) prevalence.

Total Cholesterol, LDL and HDL (mg/dL) ATP III Classification:

Children and adolescents[9]

Total cholesterol (mg/dL)

2 - 19 years old	
Expected	<170
Borderline	170 - 199
High	≥200

LDL cholesterol (mg/dL)

2 - 19 years old	
Expected	<110
Borderline	110 - 129
High	≥130

HDL cholesterol (mg/dL)

< 10 years old	Expected	≥40
10 - 19 years old	Expected	≥35

Adults[10]

Total cholesterol (mg/dL)

Expected	<200
Borderline High	200 - 239
High	≥240

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LDL cholesterol (mg/dL)

Optimal	<100
Near Optimal	100 - 129
High Borderline	130 - 159
High	160 - 189
Very High	≥190

HDL cholesterol (mg/dL)

Low	<40
High (expected)	≥60

Performance characteristics[11]

Recovery Studies . In two samples with HDL cholesterol concentrations of 28 and 54 mg/dL were added different quantities of analyte. Subsequent analyses provided recoveries ranging from 95 to 100 %. The mean proportional systematic error at 60 mg/dL decision level was 1.2 mg/dL or 2.0 %.

Method comparison . A group of 80 sera were assayed by the proposed method and a similar technique. Serum HDL cholesterol values ranged from 7 - 86 mg/dL. The comparisons yielded a correlation coefficient of 0.993 and regression equation was y = 0.932x + 1.337. The mean proportional systematic error at 60 mg/dL decision level was 2.74 mg/dL or 4.5 %.

Imprecision - Within run

	N	Mean (mg/dL)	SD (mg/dL)	%CV
Sample 1	20	40	0.60	1.5
Sample 2	20	59	0.49	0.8

Imprecision - Run-to-run

	N	Mean (mg/dL)	SD (mg/dL)	%CV
Sample 1	20	40	0.83	2.0
Sample 2	20	58	0.99	1.7

Analytical sensitivity . Detection limit: 0.40 mg/dL. The detection limit represents the lowest measurable HDL cholesterol concentration that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without HDL cholesterol.

How to calculate VLDL and LDL cholesterol concentration . VLDL and LDL cholesterol concentration can be calculated by the Friedewald equation, that is exact for samples which triglycerides concentration do not exceed 400 mg/dL and are not from patients with Type III lipoproteinemia.

Friedewald Equation
VLDL cholesterol = triglycerides / 5

LDL cholesterol = Total cholesterol - (HDL + VLDL)

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsimes/cm and silicates concentration must be <0.1mg/L.

3. In order to review physiopathological source and drugs interference in results and methodology; it is siggested to consult: <www.fxol.org/>

References

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10. Executive Summary of Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of high Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001; 285:2486-97.
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Presentation

Product	Reference	Contents	
HDL Cholesterol	13-25	R 1	1 X 25 mL
		CAL	1 X 5 mL
	13-50	R 1	1 X 50 mL
		CAL	1 X 5 mL

Application procedures using HDL Cholesterol are available for various automated instruments.

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

 **Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests	 **Risco biológico** Riesgo biológico Biological risk
 **Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)	 **Marca CE** Marcado CE CE Mark
 **Material Calibrador** Material Calibrador Calibrator Material	 **Tóxico** Tóxico Poison
 **Material Calibrador** Material Calibrador Calibrator Material	 **Reagente** Reactivo Reagent
 **Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)	 **Fabricado por** Elaborado por Manufactured by
 **Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	 **Número do lote** Denominación de lote Batch code
 **Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use	 **Controle** Control Control
 **Número do catálogo** Número de catálogo Catalog Number	 **Controle negativo** Control negativo Negative control
 **Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes	 **Controle positivo** Control positivo Positive control
 **Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device	 **Controle** Control Control
 **Liofilizado** Liofilizado Lyophilized	 **Corrosivo** Corrosivo Corrosive
 **Período após abertura** Período post-abertura Period after-opening	 **Uso veterinário** Uso veterinario Veterinary use
 **Instalar até** Instalar hasta Install before	Ref.: 140214

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