CHOLESTEROL Liquiform
Insert

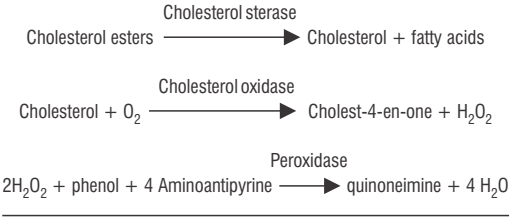
Ref.: 76

Intended use . Enzymatic system for total cholesterol determination in serum samples by end point reaction.

Professional use.

[For in vitro diagnostic use.]

Test principle . Total cholesterol determination involves the following reactions:



Cholesterol esters are hydrolized by cholesterol sterase to yield free cholesterol and fatty acids. The free cholesterol is oxided by cholesterol oxidase to cholest-4-en-one and hydrogen peroxide. Phenol and 4 aminoantipyrine are oxidized yielding quinoneimine which has maximum absorptivity at 500nm.

The red color intensity resulted from the end point reaction is proportional to the cholesterol concentration in the sample.

Summary . The increased cholesterol values, mainly the cholesterol low density lipoprotein-bound, are one of the most important risk factors to the development of coronary arterial disease. The National Cholesterol Education Program (NCEP)[6] recommends that the cholesterol measurement systems present performance characteristics able to reach the accuracy and precision requirements needed so that the results have medical importance.

The data of accuracy, reproducibility and repeatability tests obtained with Cholesterol Liquiform (Labtest) have shown that the method is able to present results that exceed the NCEP expectative, being so much safe for reliable total cholesterol measurements in the most important decision levels. Furthermore, the comparison among the imprecision found on the reproducibility and repeatability tests show that the measurement system is robust in the region of significant concentrations for clinic use, indicating a stable performance in daily life.

The system is composed by only one reagent, ready to use. Its stability guarantees the consistent performance in its original liquid form and the maintenance of the excellent reactions conditions.

Cholesterol Liquiform has a high efficient clarifier system that remove the positive interferences resulted from triglycerides values up to 2600 mg/dL.

The system is easily applied to most automatic and semi-automatic equipments which are able to measure an end point reaction at 500 nm and it can be used to measure HDL cholesterol after the selective precipitation of LDL and VLDL.

Methodology . Enzymatic - Trinder.

Reagents

1. R 1 - Reagent 1 - Store at 2 - 8 ºC.
Contains buffer ≤ 100 mmol / L, pH 7.0, phenol ≤ 24 mmol/L, sodium cholate 0.005 - 0.05%, sodium azide 14.6 mmol/L, 4aminoanthypyrine 300 - 500μmol/L, cholesterol esterase 250 - 1000 U/L, cholesterol oxidase 250 - 1000 U/L and peroxidase 250 - 1000 U/L, cofactor, stabilizers and surfactants.

2. CAL - Standard - 200 mg/dL. Store at 2 - 30 ºC.
Contains cholesterol 200 mg/dL, stabilizer, surfactant and preservative.Reagent label bears expiration date. Sodium azide (15 mmol/L).

Precautions and warnings

In order to preserve the performance, the Reagent 1 may be outside the refrigerator only while pipetting the volume to be used. Avoid the directly sun light exposure.

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

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

The reagents contain 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

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reagents stability.

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

Sample

Total cholesterol is reportedly stable in serum for about 7 days at 2 - 8 ºC and 6 months at -20 ºC[6].

Homogenize the lipemic sample before starting the measurement. Do not use samples with severe hemolysis.

Use serum. Anticoagulants such as citrate, oxalate and EDTA produce false decreased results.

Since the sample volume is small, it must be carefully pipetted in order to minimize the measure system imprecision.

In general, the blood sample may be obtained from fasting or non-fasting individual, but it is strongly recommended to collecting the blood of fasting individual for all lipids measurement in blood, including cholesterol. The advantages of using fasting individuals sample is resulting from the standardization of the blood collecting process because other lipids require fasting what decrease the postprandial lipemia interference that is present in non-fasting individuals sample.

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

The patient posture during blood collecting must be a standard in the laboratory because it may significantly affect the results. If the samples are collected in the seat position, it must be standardized that the patient remains seated during 15 minutes and no more than 30 minutes. Hemoconcentration may be a result of a prolonged time with tourniquet (after one minute), and it may increase the cholesterol values in 5 % after 2 minutes and 10 to 15 % after 5 minutes. Therefore, it is very important to get the blood sample after remove the tourniquet. The blood collecting procedure must be standardized.

The cholesterol Biological Variation, result of the cholesterol transporter lipoprotein variation, is observed when the cholesterol measurement is repeated in the same laboratory in a small period of one week. This is independently of the analytical error and may vary in a range of 1.7 to 11.6 % in an average of 6.1 % mainly due to the LDL Biological Variation, which is the main cholesterol transporter lipoprotein.

Falsely low cholesterol results have been associated with the presence of ascorbic acid in serum due the competition with the chromogenic substance in the peroxidase reaction. However, this effect decrease when samples are allowed to stand 90 minutes before assay.

Bilirubin up to 5 mg/dL, hemoglobin up to 180 mg/dL and triglycerides up to 2600 mg/dL do not interfere significantly. Bilirubin values between 5 and 38 mg/dL provide false decreased results proportional to bilirubin concentration.

Materials required not provided

1. A constant temperature water bath (37 ºC).
2. Photometer capable of measuring absorbance at 490 - 510 nm.
3. Pipettes to measure reagents and samples.
4. Timer.

Procedure

See notes 1, 2 and 3

Set up three tubes and proceed as follows:

	Blank	Unknown	Standard
Sample	------	0.01 mL	------
Standard	------	------	0.01 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. The water level in the water bath must be higher than the level of reagents in the tubes. Measure the absorbance of the Unknown and Standard against Blank at 500 nm or green filter (490 - 510). The color is stable during 60 minutes.

Calibration

System Traceability
The Standard is traceable to 911 Standard Reference Material (SRM) of the National Institute of Standards and Technology (NIST).

Manual Calibrations
Perform a new calibration after reagent lot change or when the internal quality control indicates.

Automatic Systems
Blank of reagents: water or 0.85 % NaCl;
Standards: Calibra Series (Labtest calibrator for automated systems), which are traceable to 1951 SRM of NIST.

Calibration Frequency
Two or three point calibration after reagent lot change;
Two or three point calibration when the internal quality control indicates.

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

$$\text{Cholesterol (mg/dL)} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard}} \times 200$$

Due the great reproductive results of the assays system, it is possible to use the Calibration Factor method:

$$\text{Calibration Factor} = \frac{200}{\text{Absorbance of Standard}}$$

Cholesterol (mg/mL) = Absorbance of Unknown x Calibration Factor

Measurement/reportable range

Up to 500 mg/dL.

If cholesterol concentration is ≥500 mg/dL, the sample must be diluted with 0.85 % NaCl. Multiply the result by the appropriate dilution factor. Dilute the sample so that the obtained value is around 150 and 300 mg/dL.

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.

Adults[10]

Total Cholesterol (mg/dL)

Desirable	<200
Borderline high	200 - 239
High	≥240

HDL Cholesterol (mg/dL)

Low	<40
High	≥60

LDL Cholesterol (mg/dL)

Optimal	<100
Near optimal/above optimal	100 - 129
Borderline high	130 - 159
High	160 - 189
Very high	≥190

Children and adolescents[9]

Total Cholesterol (mg/dL)

2 to 19 years old	Desirable	<170
	Borderline	170 - 199
	High	200

HDL Cholesterol (mg/dL)

<10 years old	Desirable	40
10 to 19 years old	Desirable	35

LDL Cholesterol (mg/dL)

2 to 19 years old	Desirable	<110
	Borderline	110 - 129
	High	130

Conversion: Conventional unit (mg/dL) x 0.026 = unit IS (mmol/L).

Performance characteristics[11]

Method comparison . The proposed method was compared with a similar method using 20 samples with values between 112 and 357 mg / dL. The comparison resulted in the regression equation: y = 11.93 + 0.984x and a correlation coefficient (r) equal to 0.996. The total systematic error (constant and proportional) verified at the concentration of 250 mg/dL was equal to 3.17%. As the samples were selected randomly in outpatients, it can be inferred that the method has an appropriate methodological specificity.

Imprecision - Within Run

	N	Mean	SD	CV (%)
Sample 1	20	71	1.01	1.4
Sample 2	20	115	2.69	2.3

Imprecision - Run-to-Run

	N	Mean	SD	CV (%)
Sample 1	20	71	1.87	2.6
Sample 2	20	115	3.03	2.6

Methodological sensitivity . A protein sample containing no cholesterol was used to calculate the detection limit of the assay and a value of 1.80 mg/dL was found, equivalent to the average of 10 assays plus three standard deviations.

Effects of matrix dilution . Two samples with values equal to 330 and 400 mg/dL were used to evaluate the system response in the matrix dilutions with NaCl 150 mmol/L (0.85 %). Using dilution factors rangers from 2 to 8 was obtained recoveries between 99 and 113 %.

Notes

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

2. The water in the laboratory to prepare reagents and use in the measurements, must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be <0.1mg/L (Type II reagent water). The water for washing must be Type III, having resistivity ≥0.1 megaohms or conductivity ≤10 microsiemens. For the final washing, use Type II reagent water.

3. It is suggested to consult ''Young DS. Effects of Drugs on Clinical Laboratory Tests, 3rd Edition, Washington: AACC Press, 1990.'' in order to review physiopathological source and drugs interference in results and methodology.

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Presentation

Product	Reference	Contents	
Cholesterol Liquiform	76-2/100	R 1	2 X 100 mL
		CAL	1 X 5 mL
	76-2/250	R 1	2 X 250 mL
		CAL	1 X 5 mL
Cholesterol Liquiform Labmax 560/400	76-4/70	R 1	4 X 70 mL
		CAL	1 X 5 mL

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The number of tests for automatic systems depends on the programming parameters of each equipment.

See availability of applications with Customer Service.

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.

CE

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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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