# Reactivos GPL

**Barcelona, España**

CE

**- HDLc-D-**

# CHOLESTEROL -HDL

Direct. Liquid. Enzymatic

*Store at: +2+8ºC.*

*Presentation:*
*Cod. SU014LQ* CONT: *R1 1 x 30 mL.. + R2 1 x 10 mL. + Cal.*

## Procedure

***Quantitative determination of HDL Cholesterol.***

**Only for *in vitro use in clinical laboratory* (IVD)**

***TEST SUMMARY***

Directly determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample.
The method depends on the properties of a detergent which solubilizes only the HDL so that the HDL-c is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoproteins LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to absorption of the detergents on their surfaces.
The intensity of the color formed is proportional to the HDLc concentration in the sample.

***REAGENTS COMPOSITION***

| | | |
|---|---|---|
| **R.1** | GOOD pH 7.0<br>Cholesterol oxidase<br>Peroxidase<br>DSBmT | <br>< 1000 U/L<br><1300 U/L<br>< 1 mM |
| **R.2** | GOOD pH 7.0<br>Cholesterol esterase<br>4 – Aminoantipyrine (4-AP)<br>Detergent<br>Ascorbic oxidase | <br>< 1500 U/L<br>< 1 mM<br>< 2%<br>< 3000 U/L |
| **HDLc Cal/ LDLc Cal** | Lyophilized human serum. | |

***PRECAUTIONS***

**HDLc/ LDLc CAL**

Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

***REAGENT PREPARATION AND STABILITY***

**R 1 and R 2**: Are ready to use.
**HDLc/ LDLc CAL:** Dissolve the contents with 1 mL of distilled water. Cap vial and mix gently to dissolve contents.
All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use. Do not freeze the reagents.
Do not use reagents over the expiration date.
**R 1 and R 2**: Once opened is stable 8 weeks at 2-8ºC.
**HDLc/ LDLc CAL:** Once reconstitute 1 week at 2-8ºC or 5 weeks at -20ºC.
Do not use reagents over the expiration date.
**Signs of reagent deterioration**:
- Presence of particles and turbidity.

***All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8ºC,. Do not use reagents over the expiration date.***

***SPECIMEN***

Serum or heparinized plasma, free of hemolysis[1]: Anticoagulants containing citrate should not be use.
Removed from the blood clot as soon as possible
Stability of the sample: 7 days at 2-8ºC.

***MATERIAL REQUIRED BUT NOT PROVIDED***

- Spectrophotometer or colorimeter measuring at 600 nm.

***General laboratory equipment.***

***TEST PROCEDURE***

1. Assay Conditions

- Wavelenght : ..... 600 - 700 nm.
- Cuvette: ..... 1 cm light path.
- Temperature .....37ºC.

2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

| | Blank | Standard | Sample |
|---|---|---|---|
| R 1 (µL) | 300 | 300 | 300 |
| Calibrator (µL) | -- | 3 | -- |
| Sample (µL) | -- | -- | 3 |

4. Mix and incubate for 5 min at 37ºC.
5. Read the absorbance ($A_1$) of the samples and calibrator.
6. Add:

| | Blank | Calibrator | Sample |
|---|---|---|---|
| R 2 (µL) | 100 | 100 | 100 |

7. Mix and incubate for 5 min. at 37ºC.
8. Read the absorbance ($A_2$) of the samples and calibrator, against the Blank.
9. Calculate the increase of the absorbance $\Delta A = A_2 - A_1$

***CALCULATIONS***

$$\text{HDL Cholesterol (mg/dL.)} = \frac{(A)\text{Sample}}{(A)\text{Standard}} \times \text{Calibrator conc.}$$

Conversion Factor. mg/dL. x 0.0259 = mmol/L.

***QUALITY CONTROL***

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

***Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.***

***REFERENCE VALUES***

| | Men | Women |
|---|---|---|
| Low risk | > 50 mg/dL | > 60 mg/dL |
| Normal risk | 35-50 mg/dL | 45-60 mg/dL |
| High risk | < 35 mg/dL | < 45 mg/dL |

(These values are for orientation purpose).

***It is suggested that each laboratory establish its own reference range.***

***CLINICAL SIGNIFICANCE***

HDL particles serve to transport lipoproteins in the blood-stream.
HDL is known as "good cholesterol" because high levels are thought to lower the risk of heart disease and coronary artery disease.
A low HDL cholesterol levels, is considered a greater heart disease risk[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

***REAGENT PERFORMANCE***

- *Measuring Range*:
  From detection limit of 2.5 mg/dL. to linearity limit of 200 mg/dL., under the described assay conditions.
  If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply result by 2.
- *Precision*:

| | Intra-assay | | | Inter-assay | | |
|---|---|---|---|---|---|---|
| Mean (mg/dL) | 32.9 | 50.6 | 101.4 | 32.8 | 50.0 | 100.1 |
| SD | 0.3 | 0.2 | 0.7 | 0.4 | 0.7 | 1.1 |
| CV (%) | 0.8 | 0.5 | 0.7 | 1.3 | 1.5 | 1.1 |

- Sensitivity: 1 mg/dL. = 0.0016 A
- Accuracy: Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.
  The results obtained using 50 samples were the following:
  Correlation coefficient (r): 0.996
  Regression Equation: y=0.98x + 3.42 mg/dL.
  The results of the performance characteristics depend on the analyzer used.

***INTERFERING SUBSTANCES***

- No interferes were observed to lipemia up to 1800 mg/dL. bilirubin total and direct up to 60 mg/L and hemoglobin up to 1000 g/L.
- Other substances may interfere. A list of drugs and other substances that could interfere has been reported by Young et. Al[3,4].

***BIBLIOGRAPHY***


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