# Reactivos GPL

CE

Barcelona, España - Cholesterol -

# CHOLESTEROL

## CHOD-POD

Store at: +2+8º C. *Presentation:*

## Procedure

***Quantitative determination of Cholesterol.***

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

***TEST SUMMARY***

The cholesterol present in the sample originates a coloured complex, according to the following reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{4-Cholestenona} + H_2O_2$$

$$2H_2O_2 + \text{Phenol+4-Aminophenazone} \xrightarrow{POD} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the cholesterol concentration in the sample[1,2].

***REAGENTS COMPOSITION***

| | | |
|---|---|---|
| **R.1 (Buffer)** | PIPES PH 6.9 | 90 mmol/L. |
| | Phenol | 26 mmol/L. |
| **R.2 (Enzymes)** | Cholesterol estearase (CHE) | 300 U/L. |
| | Cholesterol oxydase (CHOD) | 300 U/L. |
| | Peroxidase (POD) | 1250 U/L. |
| | 4-Aminophenazone (4-AP) | 0.4 mmol/L. |
| **Cholesterol Cal** | Cholesterol aqueous primary Calibrator | 200 mg/dL. |

***REAGENT PREPARATION AND STABILITY***

Working Reagent (WR): Dissolve ( → ) the contents of one vial R.2 (Enzymes) in one bottle R.1 (Buffer). Cap and mix gently to dissolve contents. (WR) is stable: 4 months at 2-8ºC or 40 days at 15-25ºC. Avoid direct sunlight.

All the components of the kit are stable until the expiration date on the label when stored at 2-8º C, protected from light and contamination prevented during their use.

Do not use reagents over the expiration date.

**Signs of Reagent deterioration**:

- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm. ≥ 0.10

***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 plasma[1,2]: Stability of the sample for 7 days at 2-8º C or freezing at –20º C will keep samples stable for 3 months.

***MATERIAL REQUIRED BUT NOT PROVIDED***

- Spectrophotometer or colorimeter measuring at 505 nm.
- Matched cuvettes 1.0 cm. light path.

***General laboratory equipment.***

***TEST PROCEDURE***

1. Assay Conditions
   - Wavelenght : ..... 505 nm. (500-550).
   - Cuvette: ..... 1 cm light path.
   - Temperature .....37º C. 15-25º C.
2. Adjust the instrument to zero with Blank of reagent.
3. Pipette into a cuvette:

| | Blank | Standard | Sample |
|---|---|---|---|
| R (mL.) | 1.0 | 1.0 | 1.0 |
| Calibrator(note1-2) (µL.) | -- | 10 | -- |
| Sample (µL.) | -- | -- | 10 |

4. Mix and incubate for 5 minutes at 37º C or 10 minutes at room temperature (15-25º C).
5. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable at least 60 minutes.

***CALCULATIONS***

$$\text{Cholesterol (mg/dL.)} = \frac{(A)\text{Sample}}{(A)\text{Standard}} \times 200 \text{ (Calibrator conc.)}$$

**Conversion Factor.** mg/dL. x 0.0258 = mmol/L.

***QUALITY CONTROL***

Control sera are recommended to monitor the performance of the procedure, Normal (Ref. QC001) and Pathological. (Ref. QC002)

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***

Risk evaluation[5,6]:

| | |
|---|---|
| Less than 200 mg/dL | Normal |
| 200-239 mg/dL | Borderline |
| 240 mg/dL and above | High |

(These values are for orientation purpose).

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

***CLINICAL SIGNIFICANCE***

Cholesterol is a fat-like substance that is found in all body cells. The liver makes all of the cholesterol the body needs to form cell membranes and to make certain hormones.

The determination of serum cholesterol is one of the important tools in the diagnosis an classification of lipemia. High blood cholesterol is one of the major risk factors for heart disease[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 0.6 mg/dL. to linearity limit of 600 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 n= 20 | | Inter-assay n= 20 | |
|---|---|---|---|---|
| Mean (mg/dL) | 90.1 | 305 | 90.4 | 301 |
| SD | 0.64 | 3.30 | 1.12 | 2.30 |
| CV | 0.71 | 1.08 | 1.24 | 0.76 |

- Sensitivity: 1 mg/dL. = 0.002 A
- Accuracy: Results obtained LABKIT reagents did not show systematic differences when compared with other commercial reagents.
  The results obtained using 50 samples were the following:
  Correlation coefficient (r): 0.995
  Regression Equation: y=1.004x - 0.931
  The results of the performance characteristics depend on the analyzer used.

***INTERFERING SUBSTANCES***

- No interferences were observed to bilirubin up to 10 mg/L, hemoglobin up to 5 g/L[1,2].
- Other substances may interfere. A list of drugs and other substances that could interfere has been reported by Young et. al[3,4].

***NOTES***

1. CHOLESTEROL CAL: Proceed carefully with this product because due its nature it can get contamined easily.
2. LDF (Lipid Clearing Factor) is integrated in the reagent.

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3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
4. Use clean disposable pipette tips for its dispensation.

***BIBLIOGRAPHY***


1. Naito H.K. Cholesterol. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1194-11206 and 437.
2. Meiattini F. et al. The 4-hydroxybenzoate/4-aminophenazone Chromogenic System. Clin Chem 1978; 24 (12): 2161-2165.
3. Young DS. Effects of drugs on Clin Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.



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