# **Cholesterol PAP** – Instructions for use (IFU)

Distributed by: ISE S.r.l. CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

IVD CE

6 x 50 mL • REF A-R0100000501

3 x 18,5 mL • REF R3330000014

**INTENDED USE**

Product for use in the quantitative determination in vitro of the concentration of the Cholesterol in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. For professional use only.

**CLINICAL SIGNIFICANCE**

Cholesterol is an essential component of the cell membrane of all animal cells. Cholesterol forms the basis for the synthesis of steroid hormones such as aldosterone, cortisone and testosterone; it is also essential for development of the embryo. Although part of the cholesterol present in the human body is derived from the diet, it is mostly produced in the liver and used largely for bile production. As cholesterol, like all lipids, is insoluble in the blood, aggregated complexes such as lipoproteins are used for its transportation in circulation. For this reason, when in medicine the term "cholesterol" is used, reference is in fact being made to lipoproteins which circulate in the blood, the concentration of which is known as cholesterolemia.

**PRINCIPLE**

Esterified cholesterol is hydrolyzed into free cholesterol and fatty acid by cholesterol esterase (CE). Cholesterol oxidase (CO) then oxidates the free cholesterol to cholest-4-en-3-one with formation of hydrogen peroxide which, in the presence of peroxidase (POD), reacts with hydroxybenzoate (HBA and 4-aminophenazone, giving rise to a coloured compound, the intensity of which is read at a wavelength of 510 nm; the result is directly proportional to the cholesterol concentration in the sample.

$$\text{Cholesterol Esters} \xrightarrow{CE} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{HBA} + \text{4-Aminophenazone} \xrightarrow{POD} \text{Coloured Compound}$$

**REAGENTS**

A-R0100000501 - 6 x 50 mL

**Reagent:** n° 6 vials x 50,0 mL ready for use

R3330000014 - 3 x 18,5 mL

**Reagent:** n° 3 vials x 14,0 mL ready for use

**Concentrations**

| Reagents | | |
|---|---|---|
| | **Conc.** | **U.M.** |
| PIPES Buffer pH 6.7 | 100 | mmol/L |
| Hydroxybenzoate (HBA) | 10.0 | mmol/L |
| 4-Aminophenazone | 0.50 | mmol/L |
| Cholesterol esterase (CE) | 300 | U/L |
| Cholesterol oxidase (CO) | 100 | U/L |
| Peroxidase (POD) | 200 | U/L |
| Sodium Azide | 14.6 | mmol/L |

**Precautions**

In addition to risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentration of these components is lower than the limits reported in the EC 1272/2008 Regulation and subsequent amendments and additions. However, it is recommended to handle reagents according to the standards of good laboratory practice

**Reports of serious incidents**

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

**Storage and stability**

Store at 2 - 8°C and protect from direct light. When correctly stored, the reagents are stable up the expiry date reported on the label. A slight variation in the composition of the reagent may occur between batches, but this has no effect on the test results. After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protected from contamination, evaporation, direct light and stored at correct temperature.

**Reagent Preparation**

Liquid reagent ready for use.

After opening the reagent is stable for 30 days if closed, stored at 2 - 8°C, and protect from direct light. Do not mix different batches.

**SAMPLE COLLECTION**

**Type of sample and storage**

Serum or Heparinized-plasma samples should be used. Samples can be stored for 3 days at 4 - 8°C and 3 months at - 20°C.

**Precaution**

All human samples must be handled and disposed of as potentially infectious materials.

**PROCEDURE**

**Quality control**

Control sera with known Cholesterol content are commercially available for quality control, including certificates of analysis showing the values and limits of confidence. Normal and pathological control sera "Normal control serum" cod. R0400000006 and "Pathological control serum" code R0400000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:

- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

**Automation**

Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".

All applications that are not explicitly approved cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user.

**Calibration**

For calibration use the kit "Multicalibrator" code R0300000006. For ISE srl instruments, calibration is recommended every 10 days.

**Traceability:**

The Cholesterol value is reported in the package insert supplied with the "Multicalibrator".

**Reaction conditions**

| | |
|---|---|
| Wavelength (primary): | 510 nm |
| Wavelength (secondary): | 620 nm |
| Temperature: | 37°C |

**Technique – Procedure with Reagent B as starter**

Bring the reagents to the reaction temperature.

| | **U.M.** | **Calib. Serum** | **Sample** | **Blanck** |
|---|---|---|---|---|
| **Reagent** | µL | 1000 | 1000 | 1000 |
| **Calib. Serum** | µL | 10 | - | - |
| **Sample** | µL | - | 10 | - |
| **Blanck** | µL | - | - | 10 |

Mix gently then incubate at 37°C for 10 minutes. Read the absorbances of the sample and the calibrator, subtracting the absorbance of the reagent white.

**The reaction volumes can be varied proportionally while the calculation remains unchanged.**

**Results**

The concentration of cholesterol is obtained through the following formula:

$$\frac{\text{D. O. Sample}}{\text{D.O. Calib.Serum}} \times \text{Concentration Calib.} = \text{Cholesterol mg/dL}$$

- **REFERENCE RANGE**

Serum or plasma:

- Normal values: < 200 mg/dL (< 5.2 mmol/L)
- Borderline values: 200 - 240 mg/dL (5,2 - 6,2 mmol/L)
- High values: > 240 mg/dL (> 6,2 mmol/L)

Each laboratory should establish its own normal values according to the population in which it operates.

**Reagents included in the kit**

The reagent is described above.

**Materials required but not supplied in the kit**

Calibrators and controls.

**Sclavo Diagnostics International**
**Via Po 26-28 – Loc. Pian dei Mori – 53018 Sovicille (SI) (Italy)**
**Phone +39 0577 39041 - Fax +39 0577 390 444**

**Cholesterol PAP –** Instructions for use (IFU) Distributed by: ISE S.r.l. CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

IVD CE

6 x 50 mL • REF A-R0100000501
3 x 18,5 mL • REF R3330000014

**NORMAL VALUES**
Serum or plasma:
- Normal values: < 200 mg/dL (< 5.2 mmol/L)
- Borderline: 200 - 240 mg/dL (5.2 - 6.2 mmol/L)
- High values: > 240 mg/dL (> 6.2 mmol/L)

Each laboratory should calculate its own normal values on the basis of its local population.

**ANALYTICAL CHARACTERISTICS / PERFORMANCE**
**Linearity**
The method is linear up to 600 mg/dL of Cholesterol, in the test conditions reported.

**Accuracy-Recovery**
The recovery of Cholesterol added to normal samples at known concentrations showed an accuracy of 90%.

**Interference**
The high dilution of the sample with the reagent reduces to a minimum the interference by lipids. Bilirubin below 5.8 mg/dL does not interfere in the reaction, haemoglobin interferes at concentrations above 10 g/L. For other interfering substances, make reference to the bibliography reported below (4).

**Precision of the method**

| **Within-run precision** | | | | | |
|---|---|---|---|---|---|
| Range | U.M. | Mean | S.D. | C.V. (%) | No. |
| Low | mg/dL | 107 | 3.41 | 3.18% | 18 |
| High | mg/dL | 246 | 7.16 | 2.91% | 18 |
| **Between-run precision** | | | | | |
| Range | U.M. | Mean | S.D. | C.V. (%) | No. |
| Low | mg/dL | 107 | 2.19 | 2.05% | 18 |
| High | mg/dL | 246 | 7.16. | 2.91% | 18 |

**Sensitivity**
At λ 505 nm a concentration of about 7.0 mg/dL of Cholesterol in the conditions established for this test.

**Comparative method**
The Cholesterol method was compared with a similar commercial method. Samples tested = No. 46; Y = 0.938x + 11.7; correlation coefficient = 0.989.

**Disposal of reagent**
Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.


**Manufacturer:**
**Sclavo Diagnostics International**
Via Po 26-28 – Loc. Pian dei Mori – 53018 Sovicille (SI) (Italy)
Phone +39 0577 39041 - Fax +39 0577 390 444

**Distributor:**
**I.S.E S.r.l.**
Via Delle Driadi, 45 – 00133 Roma
Tel.+39 077 4579365; FAX +39 077 4579305
E-mail: info@logotech-ise.com
www.logotech-ise.com


| **Symbols used in IFU and Packaging** | |
|---|---|
| IVD In vitro diagnostic medical device vitro | Manufacturer |
| REF Catalogue Number | Instruction for use |
| LOT Lot Number | Temperature limitation |
| Expiration date | |

**References**


1. Butris CA and Ashwood ER (Ed.), Tietz Fundamentals of Clinical Chemistry, 5st Edition, W.B. Saunders Company, Philadelphia, 2001, p.463-467.
2. Thomas L. (Ed.) Clinical Laboratory Diagnostics; use and assessment of Clinical Laboratory Results, 1st. Edition, TH-Books Verlagsgesellschaft mbH, Frankfurt/Main, Germany 1998, pp. 366-370.
3. Guder WG, Narayanan S., Wisser H., Zavata B. List of analytes; preanalytical variables. Brochure in: Samples: from patient t o the laboratory. Git Verlag GmbH, Darmstadt, 1996.
4. Young D, Effects of drugs on clinical laboratory tests. 5st Edition, AACC Press, Washington, DC, 3-817 - 3-830.
5. Third Report of the National Cholesterol education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of high blood Cholesterol in adults (ATP III), NIH Publication no. 02-5215, 2002.


| REVISION | DATE | CHANGE |
|---|---|---|
| Rev.A | 12/2022 | New Issue for IVDR Regulation (UE) 2017/746 compliance |