Quantitative determination of Cholesterol in Serum or Plasma. Enzymatic colorimetric method CHOD-PAP, endpoint.

**METHOD AND PRINCIPLE**

By enzymatic hydrolysis, the enzyme Cholesterol Esterase (CHE) frees Cholesterol from fatty acids. This fraction, together with the free part in the plasma, is oxidized by enzyme Cholesterol Oxidase (CHO) producing Hydrogen Peroxide ($H_2O_2$). The latter compound, by reaction catalyzed by the enzyme Peroxidase (POD) reacts with 4-amino-antipyrine to form the red quinone which can be measured photometrically (Trinder's reaction). The measurement of this Absorbance (optical density) is directly proportional to the cholesterol initially contained in the sample under examination. The use of a known calibrator makes quantitative analysis possible.

**CHE**

\[ \text{Cholesterol ester} + H_2O \rightarrow \text{Cholesterol} + \text{Fatty acid} \]

**CHOD**

\[ \text{Cholesterol} + O_2 \rightarrow \text{Cholesten-3-one} + H_2O_2 \]

**POD**

\[ 2H_2O_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \rightarrow \text{Quinone-imine} + 4H_2O \]

**CLINICAL SIGNIFICANCE**

Cholesterol exists in the human blood as a free sterol and in an esterified form. The knowledge of the plasma level of lipids (cholesterol and triglycerides) together with lipoproteins of high and low density (HDL and LDL) aids in the detection of many conditions bound to metabolic disorders of high risk. The imbalance in the level of lipoproteins in plasma leads to hyperlipoproteinemias, a group of disorders that affects lipid levels in serum, causing coronary heart disease (CHD) and atherosclerosis, conditions in which the cholesterol levels are important tools in their diagnosis and classification.

Jaundice of the obstructive type usually is accompanied by an elevated total serum cholesterol with a normal ester fraction. Diabetes, hypothyroidism, and certain types of kidney disease are other disorders that may exhibit the same cholesterol disturbance. Low total cholesterol values with normal ester fractions are noted mainly in hyperthyroidism and malnutrition.

**REAGENT COMPOSITION**

**Reagent (R1)**

- Good's buffer, pH 6.7: 50 mmol/L
- Phenol: 5 mmol/L
- 4-Aminoantipyrine: 0.3 mmol/L
- Cholesterol Esterase (CHE): ≥ 300 U/L
- Cholesterol Oxidase (CHOD): ≥ 200 U/L
- Peroxidase (POD): ≥ 12000U/L

**Reagent (R2)**

- Standard (Cholesterol): see value on label

**REAGENT PREPARATION AND STABILITY**

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Blank Absorbance > 0.150 at 546 nm in 1 cm cuvette.

After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

**SPECIMEN**

Serum, heparin plasma or EDTA plasma

Avoid hemolysate or lipemic samples. Separate the serum from the clot quickly.

Stability: 3 days at 20° - 25°C; 7 days at 2° - 8°C; 3 months at -20°C.

Discard contaminated specimens. Freeze only once.

**PROCEDURE**

- **Wavelength:** 546 nm (510-550)
- **Temperature:** 37° C
- **Measurement:** against distilled water

 Pipette as follows:

| Working Reagent R1 | 1000 µL |
| Sample, Std / Cal / H_2O | 10 µL |

Mix, incubate for 5 minutes. Read absorbance within 60 minutes.

**CALCULATION**

\[ \text{Cholesterol} = \frac{\text{Abs Sample} - \text{Abs Blank Reagent}}{\text{Abs Std/Cal} - \text{Abs Blank Reagent}} \times \text{Conc.Std/Cal} \]

Conversion Factor: Cholesterol [mg/dL] x 0.02586 = Cholesterol [mmol/L]

**CALIBRATION**

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

As an alternative to the standard included in the package, it is possible to use:

- Chemistry Multicalibrator - REF CAL1010 (10x3 mL)

**QUALITY CONTROL**

Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples utilizing MTD Diagnostics Quality Control Material:

- Chemistry Control N - REF CNN1010 10x5 mL (Level 1)
- Chemistry Control P - REF CDPN1020 10x5 mL (Level 2)
**EXPECTED VALUES**

Desirable: ≤ 200 mg/dL (5.2 mmol/L)
Borderline high risk: 200 – 240 mg/dL (5.2 – 6.2 mmol/L)
High risk: > 240 mg/dL (>6.2 mmol/L)

The European Task Force on Coronary Prevention recommends to lower TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-Cholesterol to less than 115 mg/dL (3.0 mmol/L).

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient’s medical history, clinical examination and other results.

**PERFORMANCE**

**PRECISION:**
- Low Level: Samples = 20; Average = 135; S.D. = 1.36; CV = 1.01%
- High Level: Samples = 20; Average = 218; S.D. = 3.10; CV = 1.41%

**ACCURACY (CORRELATION):** A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

\[ y = 1.005 x - 0.281 \]
\[ r = 0.999 \]

**SENSITIVITY:** 7 mg/dL

**LINEARITY:** 7 – 600 mg/dL

**SPECIFICITY / INTERFERENCES**
No interference was observed by bilirubin up to 20 mg/dL, haemoglobin up to 500 mg/dL and lipemia up to 1000 mg/dL triglycerides. For further information on interfering substances refer to Young DS.

**NOTES**

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.

2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

**PRECAUTIONS / DANGER SYMBOLS**

4-AMINOANTIPYRINE 0.3 mmol/L – CAS 83-07-8 Xn R22 (H302)

H301 – Toxic if swallowed
H302 – Harmful if swallowed
H311 – Toxic in contact with skin.
H314 – Causes severe skin burns and eye damage
H331 – Toxic if inhaled

The product does not contain any other hazardous substances or mixtures according to EC Regulation No. 1272/2008 (CLP) or their concentrations are such that they are not considered to be persistent, bioaccumulative or toxic (PBT). Therefore, it is not subject to the special labeling required by the aforementioned regulation. The product is labeled according to the directive for CE marking (98/79 / EC). Sodium Azide less than 0.1%.

However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:

S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S66 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

**FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.**

**BIBLIOGRAPHY**


