# LIQUID CHOLESTEROL (CHOD/POD method)

IVD **For in -vitro diagnostic use only**

2℃ 8℃ **Store at 2-8℃** CE

**INTENDED USE**

For the measurement of cholesterol in human serum or plasma.

**INTRODUCTION**

Cholesterol is one of the lipids found in the blood stream. High level of cholesterol in blood - hypercholesterolemia - is a major risk factor of coronary heart disease, which may lead to heart attacks.

**METHODOLOGY**: CHOD/POD method.

**PRINCIPLE OF THE METHOD**

The enzyme cholesterol esterase is used to hydrolyze the cholesterol esters present in serum into free cholesterol and free fatty acids. The enzyme cholesterol oxidase, in the presence of oxygen, oxidizes cholesterol to cholest-4-en-3one and hydrogen peroxide. Hydrogen peroxide oxidizes phenol and 4-aminoantipyrine to produce red color that can be measured spectrophotometrically.

| | | |
|---|---|---|
| Cholesterol esters + $H_2O$ | CHE → | cholesterol +free fatty acids |
| Cholesterol + $O_2$ | CHOD → | cholest-4-en-3-one + $H_2O_2$ |
| $2H_2O_2$ + Phenol + 4-AA | POD → | Quinoneimine dye + $4H_2O$ |

The intensity of the color formed is proportional to the cholesterol concentration in serum.

**REAGENTS COMPOSITION**

| | | |
|---|---|---|
| **R** | PIPES PH 6.8 | 90mmol/L |
| | Phenol | 26mmol/L |
| | 4-Aminophenazone(4-AA) | 0.4mmol/L |
| | Cholesterol esterase(CHE) | 1000U/L |
| | Cholesterol oxidase(CHOD) | 300U/L |
| | Peroxidase(POD) | 650U/L |
| **Cholesterol STD** | Cholesterol aqueous primary standard 200mg/dl | |

**EQUIPMENTS NEEDED BUT NOT PROVIDED**

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

**PREPARATION**

- Reagents and standards provided are ready to use.

**STORAGE AND STABILITY**

- All components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminants during their use.
- Do not use reagents after the expiration date.
- SIGNS OF REAGENT DETERIORATION
  - Presence of particles and turbidity.
  - Blank absorbance against water at 505 nm ≥ 0.26

**PRECAUTION**

1. This reagent is for in vitro diagnostic and professional use.
2. Protective clothing should be worn when handling the reagents. Also, wash the area of contact with water immediately if contact occurs.
3. Do not pipette by mouth. Flash with water if contact occurs.
4. Specimens should be considered infectious and handled appropriately.
5. Do not use the reagents if damaged and discard the contents immediately.
6. Test materials and samples should be discarded in a biohazards container.
7. Wash hands and the test table top with water and soap once the testing is done.

**COLLECTING AND HANDLING OF SPECIMENS**

Use serum, or plasma preserved in EDTA.
Determination of lipid constituent in plasma or serum are normally done on blood drawn from patients fasting for 12 to 16 hours. Stability of the sample is 7 days at 2-8°C. Freezing at -20ºC will keep samples stable for 3 months. Freezing at -60°C provides the longest stable storage and may allow for Reproducible results even after a year or more.

**ASSAY PARAMETERS**

| **Reaction** | **End point** | **Sample Vol.** | **0.01ml** |
|---|---|---|---|
| **Wavelength** | 505 nm | **Reagent Vol.** | 1.0ml |
| **Zero Settings** | Reagent blank | **Standard** | 200mg/dl |
| **Incub. Temp.** | 37°C/R.T | **linearity** | Up to 600 mg/dl |
| **Incub. Time** | 5 min/10 min | | |
| **Reac. Slope** | increasing | | |
| **Units** | mg/dl | | |

**ASSAY PROCEDURE**

1. Wavelength..... 505 nm (500-550)
2. Cuvette.....1cm.light path
3. Tempe.....37ºC/25ºC
4. Adjust the instrument to zero with distilled water.
5. Pipette into clean dry test tubes labeled as Blank (B), Standard(S), and Test (T):

| | B | S | T |
|---|---|---|---|
| Reagent(ml) | 1.0 | 1.0 | 1.0 |
| Standard(µL) | - | 10 | - |
| Sample (µL) | - | - | 10 |

6. Mix well and incubate at 37C for 5min or at R.T. (25C) for 10min.

7. Measure the absorbance of the standard and test sample against blank.
8. After incubation the color is stable for at least 60 min.

**CALCULATIONS**

**Cholesterol (mg/dl) = (A) Sample X 200 mg/dl (STD Conc.)**

**(A) STD**

**Conversion factor**: mg/dL x 0.02586= mmol/L.

**QUALITY CONTROL**

To ensure adequate quality control, it is recommended that each run includes assayed normal and abnormal controls. If control values are found outside the defined range, check the instrument calibration, and reagent for problems.

**REFERENCE VALUES**

**Serum or plasma:**

| Classification | Total cholesterol (mg/dl) |
|---|---|
| Desirable | <200 |
| Borderline to high risk | 200-239 |
| High risk | ≥ 240 |

These values are for guidance purposes, each laboratory should establish its own reference range, according to its own geographic area.

**PERFORMANCE CHARACTERISTICS**

***Measuring range (Linearity):***

The assay is linear between 10 mg/dl and 600 mg/dl. If the results obtained were greater than linearity limit, dilute the sample to half with Nacl 9g/L and multiply the result by 2.

***Sensitivity*:**

1 mg/dl = 0.0016 (A)

***Accuracy:***

Results obtained using this reagent compared well with other commercial reagents.

***Precision:***

The results of the performance characteristics depend on the analyzer used.

| | Intra-assay (n=20 ) | | Inter-assay (n=20 ) | |
|---|---|---|---|---|
| **Mean (mg/dl)** | 84.256 | 199.395 | 99.58 | 217.02 |
| **STD** | 1.46 | 6.46 | 1.12 | 6.21 |
| **C.V%** | 1.73% | 3.2% | 1.12% | 2.86% |

**INTERFERENCES**

No interferences were observed to hemoglobin up to 5 g/L and up to 10mg/dl. A list of drugs and other substances interfering with cholesterol determination has been reported.

**NOTES**

- LCF (Lipid Clearing Factor) is integrated in the reagent that clears the turbidity caused by lipemic sample and thus avoids overestimation of Cholesterol.
- Calibration with the aqueous standard may cause a systematic error in Automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.

**REFERENCES**


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**Atlas Medical**
**Ludwig-Erhard Ring 3**
**15827 Blankenfelde-Mahlow**
**Germany**
**Tel: +49 - 33708 – 3550 30**
**Email: Info@atlas-medical.com**



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|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | *In Vitro* diagnostic medical device | | Caution |
| Σ | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |