HDL Cholesterol

Reagent for quantitative In-vitro-determination of HDL-cholesterol in blood

HDL 321

Order No. **HDL 321**
Contents: 20 tests
20 pipette tips, 500 µL

Additionally required:
CHO 142 for the determination of the Total-cholesterol

Method
Cholesterol determination in the supernatant after the precipitation of LDL and VLDL by means of poly tungstic acid and magnesium ions.[1)]

Sample material
Capillary or venous EDTA blood (fresh).
Set capillary blood immediately in reaction tube "R".
Stability of the sample in reaction tube "R":
at +15 to +25°C: 6 hours

Reagent
Content / concentrations:
1. Starter reagent (caps in PE-bottle)
   Cholesterol oxidase (CHOD) from brevi bacterium > 350 U/L, Peroxidase (POD) > 4kU/L, 4-Aminophenazone 0.20 mmol/L
2. Buffer solution (pre-portioned in round cuvettes)
   Lipoprotein lipase/cholesterol esterase from microorg. >1200 U/L, 4-Chlorophenol 13.5 mmol/L, Sodium azide <0.1 %, Triton X-100< 1%, PIPES-buffer pH 7.6, 113 mmol/L
3. Precipitation reagent (reaction tube "R")
   Sodium chloride 154 mmol/L, Magnesium chloride 18.9 mmol/L, Phosphotungstic acid 0.26 mmol/L

Safety information
The buffer solution (round cuvette) contains Sodium azide (<0.1 %) and Triton X-100. Do not swallow and avoid contact with skin and mucous membranes. If desired a safety data sheet will be provided.[2)]

Storage and shelf life
Reagents can be kept at a temperature between +2°C and +8°C until the expiry date indicated on the packaging.

Measurement conditions

Measurement device:	Vario Photometer Diaglobal
Meas. wavelength:	520nm
Temperature:	Room temperature
Additionally required:	Mini centrifuge

Measurement range
10 - 120 mg/dL (0.1 - 3.1 mmol/L)

Tips
The determination of cholesterol as a whole as well as of HDL cholesterol happens in the menu <HDL>. Here, the waiting periods which come about during the HDL 321-measurement may be used for the determination of cholesterol as a whole. Besides single measurements, the measurement of small series (up to n = 6) is feasible as well.

Working instructions
- Withdraw 60µL capillary blood from finger pulp or earlobe and insert in reaction tube "R" with pre-pipetted precipitation reagent. Transfer blood in reagent solution by mixing strongly.
- Allow reaction tube "R" to stand for 5 minutes.
- Insert reaction tube "R" in Mini centrifuge and centrifuge for 5 minutes.
- Pipette 500µL supernatant in round cuvette HDL 321.
- Screw cap with starter reagent onto this round cuvette and mix.
  Cuvette (= analysis cuvette) is ready to be measured after 5 minutes.
- Select the <CHO/HDL> test.
- First determine cholesterol as a whole, see package insert CHO 142.
- Afterwards measure HDL cholesterol.
- Set the photometer's zero point (blank value) using a non-processed single test cuvette HDL 321 from the kit.
- Remove cuvette.
- After 5 minutes waiting time insert the prepared analysis cuvette into photometer.
- Read the result.

Calculation
Concentration c of the HDL cholesterol in the plasma:
c (mg/dL) = F1 x ABS x 1/(1-HCT)

F1 = calculation factor, HCT = Haematocrit
The calculation formula is programmed in the Vario Photometer. There is no need for a separate determination of the HCT count. The determination of cholesterol as a whole causes a measurand that is proportional to the HCT count and used by the device's software for the HDL calculation.

Quality assurance
For quality assurance we recommend special control sera from company Roche, www.roche.de:
PreciControl ClinChem Multi 1 / Multi 2 (4 x 5 mL)
Order-No.: 05 947 626 190 / 05 947 774 190
Method: Precipitation with phosphotungstic acid and magnesium ions

Reference values[3)]

	No risk	Moderate risk	High risk	
Men	> 55	55 - 35	< 35	mg/dL
	> 1.45	1.45 - 0.90	< 0.90	mmol/L
Women	> 65	65 - 45	< 45	mg/dL
	> 1.68	1.68 - 1.15	< 1.15	mmol/L

Summary
The HDL (High density lipoproteins) are responsible for the transportation of cholesterol from peripheral cells back to the liver. The determination of HDL cholesterol is significant clinically.

Indications / diagnostic significance:[3)]
- Early diagnosis of arteriosclerotic risk (determination of the antiatherogen cholesterol ratio)
- Therapeutic control of treatment with lipid-lowering drugs
- Result checking of activities in the fitness sector and recreational sport

High HDL concentrations in serum have a protective effect on the coronary heart disease, whereas low HDL cholesterol - especially in association with high triglycerides - increases the cardiovascular risk. When medicating with lipid-lowering drugs, it is necessary to evoide a HDL decrease. For prevention it is important that endurance sport leads to an increase of HDL cholesterol.
Different determination methods are available (ultra centrifugation, HPLC, electro-phorese, precipitation methods). Especially the precipitation methods have become important in the routine.
The Diaglobal test HDL 321 was developed in particular for the examination of capillary blood and allows the determination of HDL cholesterol on location.

Measurement principle
VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated by poly tungstic acid and magnesium ions in the diluted sample. The HDL cholesterol, which remains in the supernatant, is determined after centrifugation, which separates precipitate and erythrocytes.[1)]
The determination is based on the CHOD-PAP method.
When calculating the result, the haematocrit value of the sample is taken into consideration.

Performance parameters
Specificity / interferences[4)]
Bilirubin (> 10 mg/dL) and strong haemolysis (> 2.0 g/dL) interfere. For additional information see package insert CHO 013 / 015 and CHO 142 respectively.
A complete precipitation is not guaranteed anymore with triglyceride values > 400 mg/dL (4.56 mmol/L).

Inaccuracy
The reproducibility was checked using venous human blood.

In series [n = 20]	Average [mg/dL]	Standard deviation [mg/dL]	VK [%]
Sample 1	34.5	1.3	3.8
Sample 2	60.9	1.7	2.8
Interrupted series [n = 20]	**Average [mg/dL]**	**Standard deviation [mg/dL]**	**VK [%]**
Sample 1	36.2	1.6	4.3
Sample 2	59.8	2.1	3.5

Analytic sensitiveness
Lower detection limit: 10 mg/dL (0.5 mmol/L)

Comparison of methods
A comparison of the Diaglobal test HDL 321 (y, sample material blood) and another commercially available test (x, sample material plasma) based on the PWS method resulted in the following correlation according to the Passing/Bablok[5)] process:

y = 0.947x + 3.29
r = 0.990

n = 40
Concentration range: 30.0 - 110 mg/dL

Information on disposal
Waste code number 180106:
Vials with reagent are considered hazardous waste. Do not allow reagent to reach surface water or sewage system.
Dispose of in accordance with official regulations.
Non-contaminated and completely empty packaging can be recycled.
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Bibliography
1. Assmann G, Schriewer H, Schmitz G et al. Qualification of high density lipoprotein cholesterol by precipitation with phosphotungstic acid/$MgCl_2$. Clin Chem 1983:29:2026-2030
2. http://www.diaglobal.de/de/service/downloads/index.html
3. Thomas L. Labor und Diagnose. 4.Aufl. Marburg: Die Medizinische Verlagsgesellschaft 1995: 207
4. Sonntag O. Arzneimittelinterferenzen. Stuttgart, New York: Thieme Verlag, 1985:37
5. Passing H, Bablok W. A new biometric procedure for testing the equality of measurements from two different analytical methods. J Clin Chem Clin Biochem. 1983; 21:709-720