Code No. 997-01301

HDL-Cholesterol E

(Phosphotungstate-magnesium salt precipitation Method)
For Research Use Only. Not for use in diagnostic procedures.

Intended use

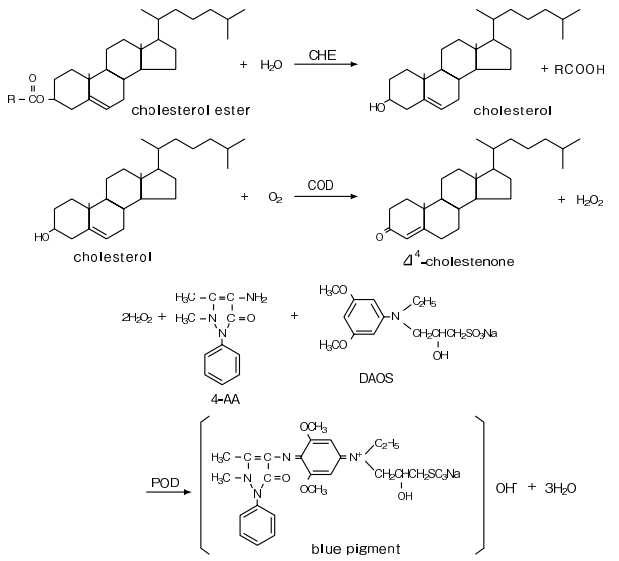
The HDL-Cholesterol E is an *in vitro* assay for the quantitative determination of HDL cholesterol in serum.

Summary and explanation of the test

Classically, the lipoproteins have been defined by physical separation via ultra-centrifugation, since they have different densities or gel filtration method, electropheresis method etc.
Given the relative difficulty of ultra centrifugation and the relative ease of the precipitation method – particularly when combined with a single-step enzymatic cholesterol determination – the precipitation method became available for both manual and automated measurement of HDL cholesterol. The HDL-Cholesterol E is composed of precipitant reagent (phosphotungstate / magnesium salt), color reagent (enzymes / couplers) and standard solution.

Principle of the method

When the Precipitating Reagent containing phosphotungstate and magunesium salt is added to the sample, lipoprotein except for HDL fraction is precipitated out and removed.
The remaining supernatant liquid is then tested for cholesterol by enzymatic colorimetric procedure.
The Working Solution containing cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine and DAOS, is added and then incubated at 37℃ for ten minutes. The ester-form cholesterol is transformed into free-form cholesterol which is then oxidized by cholesterol oxidase to form a corresponding quantity of hydrogen peroxide. The hydrogen peroxide thus produced yields a blue color complex upon oxidase condensation with DAOS and 4-aminoantipyrine in the presence of peroxidase.
By measuring the absorbance of the blue color complex, the original HDL cholesterol concentration of the specimen can be calculated when compared with the absorbance of the cholesterol standard.



Reagents

(1) Precipitating Reagent 1 × 20 mL
Phosphotungstate including magnesium chloride
Store at 2-10℃.
(2) Color Reagent (when reconstituted) 2 × for 150 mL
0.95 mmol/L 3,5-dimethoxy-N-ethyl-N-(2-hydroxy-3-sulfopropyl) · aniline sodium (DAOS)
0.19 mmol/L 4-Aminoantipyrine (4-AA)
1.7 U/mL Cholesterol esterase (CHE) (*Pseudomonas sp.*)
0.39 U/mL Cholesterol oxidase (COD) (*Streptomyces sp.*)
5.8 U/mL Peroxidase (POD) (Horse radish)
4.4 U/mL Ascorbate oxidase (AOD) (pumpkin)
Store at 2-10℃.
(3) Buffer Solution 2 × 150 mL
50 mmol/L Good's buffer (MES) (pH6.1)
Store at 2-10℃.
(4) Standard Solution 1 × 10 mL
50.0 mg/dL Cholesterol (Equivalent to 100 mg/dL HDL-Cholesterol)
Store at 2-10℃.

Warnings and precautions

(1) For Research Use Only. Not for use in diagnostic procedures.
(2) Do not use the reagents described above in any procedures other than those described herein. Performance cannot be guaranteed if the reagents are used in other procedures or for other purposes.
(3) Operate the instruments according to the operator's manuals under appropriate conditions. Consult the instrument manufacturer for details.
(4) Store the reagents under the specified conditions. Do not use reagents past the expiration date stated on each reagent container label.
(5) Do not use reagents which were frozen in error. Such reagents may give false results.
(6) After opening the reagents, it is recommended to use them immediately. To store opened reagents, cap the bottles and keep them under the specified conditions.
(7) Do not use the containers and other materials in the package for any purpose other than those described herein.
(8) The vial is stoppered at reduced pressure. Slowly remove the stopper to prevent the powder in the vial from releasing.
(9) Recommend to use Spitz test tubes to mix sample and Precipitating Reagent.
Decantation can be done after centrifugation to separate the supernatant.
(10) Color reaction will finish in about 2 minutes. A five minutes incubation at 37℃ is sufficient.
(11) Developed color and optical density are stable even after 2 hours.
(12) Standard solution is 50 mg/dL cholesterol solution and corresponds to 100 mg/dL HDL-Cholesterol when measured according to the standard procedure.
(13) When discarding the reagents, dispose of them according to local or national regulations.
(14) All the devices including reagents and reagent bottles that come in contact with specimens should be considered potentially infectious.
(15) If the reagents come in contact with the mouth, eyes, or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
(16) Do not mouth pipette directly. Use a safety device for pipetting.
(17) Be careful not to cut yourself with the aluminum cap when removing it from the vial.

Physical or chemical indications of instability

The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

Specimen collection and preparation

(1) Samples
(a) Sample analysis should be done immediately after collection.
(b) Sample is stable at 4℃ for two days and at −20℃ for six months.
(2) Interfering substances
(a) Citrate gives slightly negative influence on the assay.
(b) Sodium fluoride may give negative influence on the assay.
(c) Anticoagulants such as heparin, oxalate, EDTA have no influence on the assay when they are used in their usual amounts.
Hemolysis, ascorbic acid and bilirubin do not have significant influence on the assay.

Warning/Biohazard

Since all specimens are potentially infectious, they should be handled at the Biosafety Level 2 asrecommended for any potentially infectious body fluid in the USA Centers for DiseaseControl/USA National Institutes of Health manual ''Biosafety in Microbiological and BiomedicalLaboratories'', and in accordance with any other local or national regulations relating to the safehandling of such materials.

Materials required but not supplied

Pipettes (0.05 mL and 3.0 mL), Water bath or heating block capable of maintaining 37℃, Spectrophotometer or colorimeter, capable of measuring absorbance at 600 nm.

Reagent preparation

(1) Precipitating Reagent : Use as supplied.
(2) Color Solution : Dissolve one bottle (for 150 mL) of Color Reagent in one bottle of Buffer solution and use as Color Solution. After opening the bottle, store at 2-10℃ and use within 4 weeks.

Test procedure

Temperature : 37℃

(1) Separation of HDL Cholesterol
1. Accurately pipette 0.2 mL of serum into the test tubes. Then pipette 0.2 mL Precipitating Reagent into each tube and mix well.
2. Allow to stand for 10 minutes at room temperature and then subject the sample to centrifugal separation for 10 to 15 minutes at 3,000 r.p.m.
3. Take the supernatant immediately after the centrifugation and use it as sample for HDL-Cholesterol assay.

(2) HDL-cholesterol assay

	Sample (serum)	Standard	Reagent Blank
Specimen	Supernatant 0.05 mL	Standard 0.05 mL	Nothing*
Color Solution	3.0 mL	3.0 mL	3.0 mL
Mix well and incubate about 5 minutes at 37℃. Measure the absorbance of sample (Es) and Standard (Estd) against Reagent Blank at 600 nm. If double wavelength photometry is used, main wavelength 600 nm and sub wavelength 700 nm are applied.			

*0.05 mL deionized water does not influence practically, thus omitted.

(3) Calibration
Prepare working standard solutions according to the table below and assay each sample by the text procedure to get absorbance of each. Plot the net absorbance for each, versus the HDL-Cholesterol concentration in mg/dL on graph paper.

No.	Standard	Distilled water	Assay Volume	Concentration
1	1.0 mL	1.0 mL	0.05 mL	50 mg/dL
2	1 mL	–	0.05	100
3	No. 1	–	0.15	145.2*
4	1 mL	–	0.10	196.8*

*Sample portion is normally 0.05 mL, but in this case 0.1 mL or 0.15 mL of Std. Solution is taken. These figures are corrected ones.

Results

From the above calibration curve
Calculate as follows :
HDL-Cholesterol (mg/dL) = Es / Estd × 100 (mg/dL)

Quality control

A quality control program is recommended for all laboratories. The analysis of control material in both the low and high ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

Performance characteristics

Measurable range

Up to 200 mg/dL HDL-Cholesterol (In the case of using the standard procedure).

References

(1) Burstein, M., Scholnick, H. R. and Morfin, R. : J. Lipid es., **11**, 583-595 (1970).
(2) Kawai T., et al. HDL-Cholesterol Kisoto Rinshou : 131-149 (1980) (Japanese).

Ordering information

Code No.	Product	Package
997-01301	HDL-Cholesterol E	90 Tests

Issue date : Sep. 15, 2016

997-01301F

Manufactured by
Wako Pure Chemical Industries, Ltd.
1-2 Doshomachi 3-Chome, Chuo-Ku Osaka 540-8605 Japan
Tel : +81-6-6203-3749
Fax : +81-6-6203-1917
www.wako-chem.co.jp

Distributed by
Wako Diagnostics
Wako Life Sciences, Inc.
1025 Terra Bella Ave., Suite A
Mountain View, CA 94043 USA
Tel : 877-714-1924
Fax : 804-271-0449
www.wakodiagnostics.com

16.09.15K00