Code No. 997-01801

Phospholipids C

(Choline oxidase – DAOS method)

For Research Use Only. Not for use in diagnostic procedures.

Intended use

The Phospholipids C is an in vitro assay for the quantitative determination of phospholipids in serum.

Summary and explanation of the test

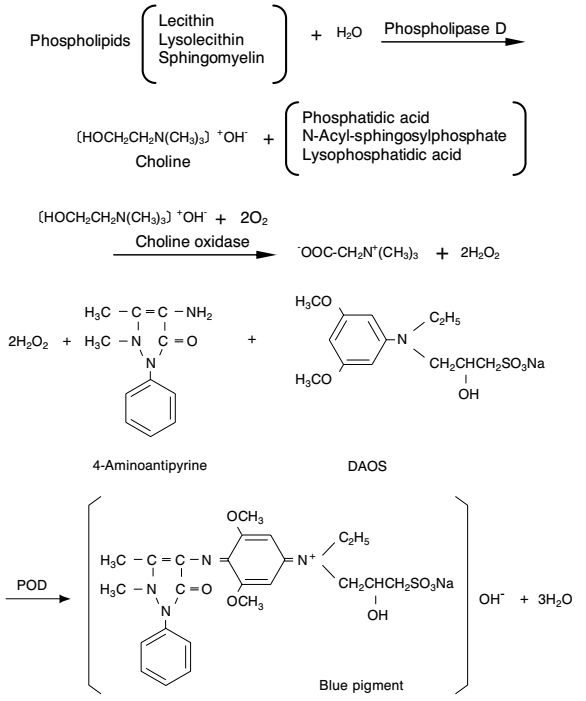
Phospholipids play an important function in the composition of cell membranes and in the emulsification and absorption of fat in the body.
Serum phospholipids are formed in the liver, where they become bound to apolipoproteins, which makes them soluble in plasma.

In the past, methods used to measure phospholipids required extractions with organic solvents followed by acid-digestion to release the phosphorus, which then would be measured by a colorimetric method. Such methods were complicated and not easily adaptable to automation.

The Wako Phospholipids C assay is an enzymatic method utilizing N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-domethoxyaniline (DAOS) in a reaction that produces a blue pigment, which can be measured spectrophotometrically. This assay has been shown not to be significantly influenced by coexisting substances such as ascorbic acid and bilirubin.

Principle of the method

When a sample is added to the Color Reagent, phospholipids (lecithin, lysolecithin, sphingomyelin) in the sample are hydrolyzed by Phospholipase D to produce choline, which in turn is oxidized by choline oxidase to betaine and hydrogen peroxide. The hydrogen peroxide produced causes DAOS and 4-aminoantipyrine to undergo a quantitative oxidative condensation catalyzed by peroxidase (POD), producing a blue pigment.
The amount of phospholipids in the sample is determined by measuring the absorbance of the blue color.



Reagents

(1) Buffer Solution 8 × 50 mL
50 mmol/L Good's buffer (pH7.5)
Store at 2-10℃.

(2) Color Reagent 8 × for 50 mL
When reconstituted
0.47 U/mL Phospholipase D (*microorganism*)
2.16 U/mL Choline oxidase (*microorganism*)
4.2 units/mL Peroxidase (POD, *horseradish*)
0.77 mmol/L N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (DAOS)
0.24 mmol/L 4-Aminoantipyrine
3.9 units/mL Ascorbate oxidase (*cucurbita sp.*)
Store at 2-10℃.

(3) Standard Solution 2 × 10 mL
54 mg/dL Choline chloride
(Equivalent to as 300 mg/dL phospholipids)
Store at 2-10℃.

Warnings and precautions

(1) For Research Use Only. Not for use in diagnostic procedures.
(2) Not to be used internally in humans or animals.
(3) Do not use the reagents past the expiration date stated on each reagent container label.
(4) Do not use reagents described above for any purpose other than described herein.
(5) Do not use the reagents which were frozen by mistake. Such reagents may give false results.
(6) 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.
(7) 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.
(8) The vial is stoppered at reduced pressure. Slowly remove the stopper to prevent the powder in the vial from releasing.

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 deterioration.

Instruments

A spectrophotometer capable of measuring absorbance at 600 nm is required. Refer to the manual provided by the instrument's manufacturer for information on proper use and maintenance procedures.

Specimen collection and preparation

(1) Use serum as a specimen.
(2) Hemolysis, ascorbic acid and bilirubin do not have significant influence on the assay.
(3) Lecithin, lysolecithin and sphingomyelin of phospholipids are measured, but kephalin in the sample is not measured.

Warning/Biohazard

Since all specimens are potentially infectious, they should be handled at the Biosafety Level 2 as recommended for any potentially infectious body fluid in the USA Centers for Disease Control/USA National Institutes of Health manual ''Biosafety in Microbiological and Biomedical Laboratories'', and in accordance with any other local or national regulations relating to the safe handling of such materials.

Procedure

Materials supplied

Refer to the section entitled ''Reagents''.

Materials required but not supplied

Test tubes
Pipettes, capable of accurately dispensing 0.02 mL
Transfer pipette, 3.0 mL
Water bath, capable of maintaining 37 ℃
Spectrophotometer or Colorimeter, capable of measuring absorbance at 600 nm.

For further assistance call Wako Diagnostics Technical Service Department at 1-877-714-1924.

Reagent preparation

Color Solution : Dissolve one bottle of Color Reagent with one bottle of Buffer Solution. After preparation, the reagent is stable for 1 week at 2-10℃.

Test procedure

Temperature: 37℃

	Sample (Bl)	Standard (Std)	Reagent Blank (Bl)
Specimen	Serum 0.02 mL	Standard 0.02 mL	– *1
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 the Reagent Blank at 600 nm.*2			

*1 0.02 mL deionized water may be omitted as it does not significantly affect the absorbance of the reaction solution.
*2 If double wavelength photometry is used, the main wavelength is 600 nm and the sub wavelength is 700 nm.

Calibration

Prepare a diluted standard solution by adding distilled or deionized water to the Standard according to Table 1.
Assay each standard as outlined in the 'Test procedure'.
Plot the absorbance for each standard versus the phospholipids concentration to construct a calibration curve.

Table 1

No.	Standard	Distilled or Deionized water	Assay volume	Phospholipid Concentration
1	1.0 mL	1.0 mL	0.02 mL	150 mg/dL
2	Undiluted	–––	0.02	300
3	Undiluted	–––	0.04	596.1 *1

*1 The test sample volume is usually 0.02 mL, but 0.04 mL is taken in this case. The total volume is therefore increased slightly.
The concentration of phospholipids must be corrected accordingly as indicated in the table above.

Calculation

(1) Calculation method from calibration curve
Calculate phospholipids concentration from the prepared calibration curve.

(2) Calculation method from expression

$$\text{Phospholipids (mg/dL)} = \frac{Es}{Estd} \times 300$$

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the low and high ranges 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 an unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and an acceptable range.

Limitations of the procedure

The linearity of Phospholipids C is up to 1000 mg/dL.

Ordering information

Code No.	Product	Package
997-01801	Phospholipids C Buffer Solution 8 × 50 mL Color Reagent 8 × for 50 mL Standard Solution 2 × 10 mL	120 tests

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997-01801F

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

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