

Phospholipids

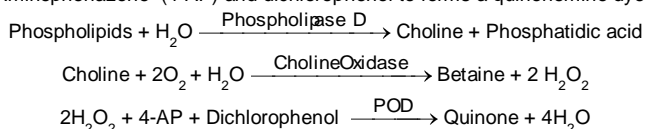
CHO-POD. Enzymatic colorimetric

Quantitative determination of phospholipids IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

Phospholipids are hydrolysed by phospholipase D and the liberated choline is subsequently oxidized by choline oxidase (CHO) to betaine with the simultaneous production of hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide couples oxidatively the 4 – Aminophenazone (4-AP) and dichlorophenol to forms a quinonemine dye:



The intensity of the colour formed is proportional to the phospholipids concentration^{1,2}.

CLINICAL SIGNIFICANCE

Phospholipids are a complex lipid containing phosphorus. Their function as the principal components of cell membranes makes phospholipids essential for all vital cell processes. The determination of serum phospholipids is an important clinical test in diagnosis of liver diseases, especially obstructive jaundice^{1,2}. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REAGENTS

R 1 Buffer	TRIS pH 7.55	50 mM
	Dichlorophenol	2.1 mM
R 2 Enzymes	Phospholipase D	400 U/L
	Choline oxidase (CHO)	2200 U/L
	Peroxidase (POD)	3600 U/L
	4 – Aminophenazone (4-AP)	1 mmol/L
PHOSPHOLIPIDS CAL	Phospholipids aqueous primary standard 300 mg/dL	

PREPARATION

Working reagent (WR):
Dissolve (→) the contents of 1 vial R 2 Enzymes in 10 mL of R 1 Buffer. Cap and mix gently to dissolve contents.
The reagent is stable after reconstitution 3 weeks in the refrigerator (2-8°C) or 7 days at 15-25°C.
Protect from the sunlight.

STORAGE AND STABILITY

All the 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 contaminations prevented during their use.
Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm ≥ 0.16.

ADDITIONAL EQUIPMENT

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

SAMPLES

Serum or plasma.
Stability of the sample: 3 days at 2-8°C.

PROCEDURE

- Assay conditions:
Wavelength: 505 nm. (490-550)
Cuvette: 1 cm. light path
Temperature..... 37°C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard ^(Note 1,2) (µL)	--	10	--
Sample (µL)	--	--	10

- Mix and incubate for 5 min. at 37°C.
- Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

$$\frac{(A) \text{ Sample}}{(A) \text{ Standard}} \times 300 \text{ (Standard conc.)} = \text{mg/dL phospholipids in the sample}$$

Conversion factor: mg/dL x 0.0129 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINCONTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

The serum phospholipids concentration in normal healthy individuals is in about the same concentration range as total cholesterol. The ratio of phospholipids to cholesterol remains 1/1. Any change in cholesterol concentration results in a corresponding change in phospholipids in similar direction. Adult: 125-275 mg/dL^{1,6}.
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 2.54 mg/dL to linearity limit of 600 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
	Mean (mg/dL)	SD	CV (%)	
Mean (mg/dL)	121	2.12	1.74	126
SD	2.12	2.03	1.74	225
CV (%)	1.74	0.91	1.74	2.92
				4.61
				2.31
				2.05

Sensitivity: 1 mg/dL = 0.0014 A.

Accuracy: Results obtained using SPINREACT reagents did not show systematic differences when compared with other commercial reagents.

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.998

Regression equation: y=0.9979x + 1.2518

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

INTERFERENCES

No influence of ascorbic acid, glucose, bilirubin, uric acid or hemoglobin was found within the range of physiological concentration².
A list of drugs and other interfering substances with phospholipids determination has been reported by Young et. al^{3,4}.

NOTES

- PHOSPHOLIPIDS CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
- 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.
- SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

- Naito K N. Lipids. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 918-919 and 570-572.
- Takeyama M., et al. A new enzymatic method for determination of serum choline-containing phospholipids. Clin Chem 1977; Acta 79; 93-98.
- Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
- Young DS. Effects of disease on Clinical Lab. Tests, 4th ed. AACC 2001.
- Burtis A. et al. Tietz Textbook of Clinical Chemistry, 3rd ed. AACC 1999.
- Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed. AACC 1995.

PACKAGING

Ref:1001140 R1: 1 x 50 mL, R2: 5 → 10 mL, CAL: 1 x 5 mL