Phospholipids. CHO-POD. Enzymatic colorimetric Ref.:PID-029
Quantitative Determination of phospholipids
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC 4 x 10 mL

PHOSPHOLIPIDS

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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:

$$\text{Phospholipids} + H_2O \xrightarrow{\text{Phospholipase D}} \text{Choline} + \text{Phosphatidic acid}$$

$$\text{Choline} + 2O_2 + H_2O \xrightarrow{\text{Choline Oxidase}} \text{Betaine} + 2\,H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{Dichlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + 4H_2O$$

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 (2-8ºC) or 7 days at 15-25ºC.
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 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.

PHOSPHOLIPIDS CAL Once open is stable up to 1 month when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
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 2-8ºC.

PROCEDURE

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

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

4. Mix and incubate for 5 min. at 37ºC.
5. 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.
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/L 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)	121	221	126	225
SD	2.12	2.03	2.92	4.61
CV (%)	1.74	0.91	2.31	2.05

Sensitivity: 1 mg/dL = 0.0014 A.
Accuracy: Results obtained using BSM reagents did not show systematic differences when compared with other commercial reagents.

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 concentration[2].
A list of drugs and other interfering substances with phospholipids determination has been reported by Young et. al[3,4].

NOTES

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.
3. **BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

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