Liquick Cor-PHOSPHORUS

DIAGNOSTIC KIT FOR DETERMINATION OF INORGANIC PHOSPHORUS CONCENTRATION

Kit name	Cat. No
Liquick Cor-PHOSPHORUS 500	3-321
Liquick Cor-PHOSPHORUS "bulk"	3-290

INTRODUCTION

Phosphorus is present in all body cells as a component of nucleic acids, phospholipids and phosphoproteins. Phosphorus is essential for intracellular storage and conversion of energy (ATP, creatine phosphate) and participates in carbohydrates metabolism. In the blood phosphorus is present as a mixture of inorganic phosphates HPO_4^{-2} and $H_2PO_4^-$. Besides phosphorus and calcium constitute mineral portion of bone. Continuous flux of phosphorus in organism is controlled by parathyroid hormone (PTH), vitamin D and calcitonin. Phosphorus serum level abnormalities are caused usually by disorders of vitamin D metabolism or parathyroid and kidney diseases.

METHOD PRINCIPLE

Direct phosphomolybdate reaction without deproteinization. Phosphate ions form with molybdate ions in acid solution proportional amounts of unreduced phosphomolybdate complex. The concentration of the complex formed is determined by measuring its absorbance.

REAGENTS

Package

	Liquick Cor-PHOSPHORUS 500	Liquick Cor-PHOSPHORUS "bulk"
1-PHOSPHORUS	4 x 500 ml	--*

*reagent volume is printed on the label.

Reagent preparation and stability

The reagent is ready to use.

The reagent is stable up to the kit expiry date printed on the package when stored at 2-8°C. On board stability of the reagents depends on type of analyser used for analysis. Protect from light and avoid contamination!

Concentrations in the test

ammonium molybdate	0.4 mmol/l
sulphuric acid	150 mmol/l
hydrochloric acid	100 mmol/l
detergents	

Warnings and notes

- Product for in vitro diagnostic use only.
- Contaminated glassware is the greatest source of error. Disposable plastic ware is recommended for the test.
- The reagent is usable when its absorbance is less than 0.200 (read against distilled water, wavelength λ=340 nm, cuvette l = 1 cm, at temp. 37°C).
- 1-PHOSPHORUS meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Ingredients:

1-PHOSPHORUS contains sulfuric acid (VI) and hydrochloric acid.

Danger

H314 Causes severe skin burns and eye damage.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing.

P305 +P351 +P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 - Immediately call a POISON CENTER or doctor.

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 340 nm;
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN

Serum, heparinized plasma (recommended: heparine lithium, sodium or ammonium salt) free from hemolysis, 24-hours urine.

Serum is the preferred specimen. Level of inorganic phosphate in heparinized plasma is about 0.2 to 0.3 mg/dl (0.06 – 0.10 mmol/l) lower than in serum.

Serum should be separated from red blood cells as soon as possible after blood collection, because erythrocytes contain several times higher phosphate concentration than normal serum.

Urine preparation: to prevent phosphate precipitation in urine, specimens should be collected in HCl, 20-30 ml of 6 mol/l for 24-h specimen. Then dilute 1 part of acidified urine with 10 parts of distilled water. Multiply the result by the dilution factor.

Serum and plasma can be stored up to 7 days at 2-8ºC. For longer storage samples should be frozen at -20ºC.

24-hours urine samples can be stored up to 7 days at 2-8ºC. Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength	340 nm
temperature	37°C
cuvette	1 cm

Pipette into the cuvette:

	reagent blank (RB)	test (T)	standard (S)
1-PHOSPHORUS	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

standard	-	-	10 µl
sample	-	10 µl	-

Mix well, incubate for 5 min. at the determination temperature. Read the absorbance of test A(T) and standard A(S) against reagent blank (RB). The absorbance is stable within 30 minutes.

Calculation

$$\text{phosphorus concentration} = \frac{A(T)}{A(S)} \times \text{standard concentration}$$

REFERENCE VALUES [7]

serum / plasma	mg/dl	mmol/l
age: 0 – 10 d	4.5 – 9.0	1.45 – 2.91
10 d – 24 mo	4.5 – 6.7	1.45 – 2.16
24 mo – 12 y	4.5 – 5.5	1.45 – 1.78
12 – 60 y	2.7 – 4.5	0.87 – 1.45
> 60 y males	2.3 – 3.7	0.74 – 1.20
> 60 y females	2.8 – 4.1	0.90 – 1.32
24-hours urine	g/24h	mmol/24h
	0.4 – 1.3	12.9 – 42.0

It is recommended for each laboratory to establish its own reference ranges for local population.

Phosphorus concentration in 24-hours urine – calculation

phosphorus concentration in	=	phosphorus concentration in	x	urine volume of 24-hours	÷	1000

24-hours urine [g/24h]	sample of 24-hours urine [mg/dl]	urine [dl/24h]

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) for determination in serum or CORMAY URINE CONTROL LEVEL 1 (Cat. No 5-161) and LEVEL 2 (Cat. No 5-162) for determination in urine with each batch of samples.

For the calibration of manual assay the PHOSPHORUS STANDARD (Cat. No 5-120) is recommended.

For calibration of the automatic analysers systems CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174 and 5-176) and LEVEL 2 (Cat. No 5-175 and 5-177) is recommended.

Calibration stability depends on type of analyser used for analysis. The calibration curve should be prepared with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using a Multi + analyser for manual assay and an automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity (Multi +):** 0.95 mg/dl (0.307 mmol/l).
  Sensitivity (Biolis 24i Premium): 0.21 mg/dl (0.068 mmol/l).

- **Linearity (Multi +):** up to 18.0 mg/dl (5.81 mmol/l).
  Linearity (Biolis 24i Premium): up to 18.5 mg/dl (5.98 mmol/l).
  For higher concentration dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

- **Specificity / Interferences**
  Haemoglobin up to 0.16 g/dl, ascorbate up to 62 mg/l, bilirubin up to 15 mg/dl and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision (Multi +)**

Repeatability (run to run) n = 5	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	3.62	0.03	0.73
level 2	6.46	0.14	2.18

- **Precision (Biolis 24i Premium)**

Repeatability (run to run) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	3.65	0.03	0.91
level 2	6.70	0.08	1.15

Reproducibility (day to day) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	4.45	0.09	2.10
level 2	7.38	0.09	1.25

- **Method comparison**

A comparison between phosphorus values determined at Multi + (y) and at ADVIA 1650 (x) using 24 samples gave following results:
y = 0.9641 x + 0.0513 mg/dl;
R = 0.986 (R – correlation coefficient)

A comparison between phosphorus values determined at Biolis 24i Premium (y) and at ADVIA 1650 (x) using 24 samples gave following results:
y = 0.9382 x + 0.236 mg/dl;
R = 0.984 (R – correlation coefficient)

TRACEABILITY

PHOSPHORUS STANDARD is traceable to the spectrofotometric reference method.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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