

PHOSPHORUS UV

PH 0500 CH

4 x 125 ml

SUMMARY OF TEST

Phosphorus in the form of inorganic or organic phosphate is an important and widely distributed element in the human body. An adult human has approximately 600 g of phosphate expressed as phosphorus, of which about 85% is in the skeleton and the rest principally in soft tissues. In the soft tissues, most phosphate is cellular. Although both inorganic and organic phosphate are present in cells, most is organic and incorporated into nucleic acids, phospholipids, phosphoproteins, and high-energy compounds involved in cellular integrity and metabolism. Plasma contains approximately 2.5 to 4.5 mg/dl (0.81 - 1.45 mmol/l) of inorganic phosphate, the fraction measured in routine biochemical analysis. Serum phosphate concentrations are dependent on meals and variation in the secretion of hormones such as PTH and may vary widely (up to 1.5 mg/dl). Intracellular function: phosphate has several diverse actions in cells. It has a critical role as a high-energy phosphate bond in adenosine triphosphate (ATP) and other high-energy compounds. These energy sources maintain many physiological functions such as muscle contractility, neurological function, and electrolyte transport. Phosphate is a constituent of cyclic adenine and guanine nucleotides as well as NADP, which are important in many enzyme systems. Phosphate is also an essential element in phospholipid cell membranes, nucleic acids, and phosphoproteins. Intracellular phosphate is therefore involved in the regulation of intermediary metabolism of proteins, fats, and carbohydrates, as well as in gene transcription and cell growth. Phosphate is critical for important enzyme systems including 2,3-diphosphoglycerate (oxygen-carrying capacity of hemoglobin), adenylate cyclase, and 1- α -25-hydroxyvitamin-D-hydroxylase.

Extracellular function: extracellular phosphate maintains the critical intracellular concentration and provides substrate for bone mineralization. An acute decline in the serum phosphate concentration may result in rapid complications such as rhabdomyolysis and altered red blood cell function.

Skeletal function: inorganic phosphate is a major component of hydroxyapatite, thereby playing an important part in structural support of the body. The cellular demands for metabolic function in bone cells are similar to those in other cells. The skeleton also serves as a storehouse for phosphate.

Phosphate in serum exists as both the monovalent and divalent phosphate anions. Approximately 10% of the phosphate in serum is protein-bound; 35% is complexed with sodium, calcium, and magnesium; and the remainder, or 55%, is free. Although both inorganic and organic phosphate are present in blood, only inorganic phosphate is measured. The organic esters are primarily located within the cellular elements of blood.

Phosphate levels have been determined by a number of procedures, including the vanadate-molybdate and enzymatic methods. Vanadate and molybdate form a yellow complex with phosphate at acid pH. The present method is based on the molybdate unreduced complex, which is measured at 340 nm.

PRINCIPLE OF THE METHOD

The phosphate ions react with ammonium molybdate to form a phosphomolybdate complex. The colorless phosphomolybdate complex can be measured directly by ultraviolet (UV) absorption at 340 nm. An acid pH is necessary for the formation of complexes.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

Reagent A: 4 x 125 ml (liquid) blue cap

Composition: ammonium molybdate 0.4 mmol/l, sulphuric acid 0.21 mol/l, surfactant.

Standard: inorganic phosphorus 5 mg/dl - 5 ml

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Use reagent ready to use.

Stability: up to expiration date on labels at 2-8°C.

Stability since first opening of vials: \geq 60 days at 2-8°C.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum is the preferred specimen. Although heparinized plasma is acceptable, levels of inorganic phosphate are about 0.2 to 0.3 mg/dl lower than in serum. Anticoagulants such as citrate, oxalate, and EDTA interfere with formation of the phosphomolybdate complex and should not be used.

Inorganic phosphate in whole blood specimens may either decrease or increase with time, depending on the type of specimen, temperature, and duration of storage. Levels in plasma or serum are increased by prolonged storage with cells at room temperature or 37°C; it is important to promptly separate serum or plasma from erythrocytes. Hemolyzed specimens are unacceptable because erythrocytes contain high concentrations of organic phosphate esters, which can be hydrolyzed to inorganic phosphate during storage. Inorganic phosphate increases by 4 to 5 mg/dl per day in hemolyzed specimens stored at 4°C. Glucose phosphate, creatine phosphate, and other organic phosphates may also be hydrolyzed by assay conditions, resulting in overestimation of inorganic phosphate levels.

Phosphate is considered to be stable in serum that has been separated from the clot for days at 4°C and months when frozen.

Urine samples should be collected in 6 mol/l HCl, 20-30 ml for a 24 hours specimen, to avoid precipitation of phosphate complexes.

Dilute urine samples 1:20 with purified water before assay.

TEST PROCEDURE

Wavelength:	340 nm		
Lightpath:	1 cm		
Temperature:	25, 30 or 37°C		
dispense:	blank	standard	sample
reagent	2 ml	2 ml	2 ml
water	20 μ l	-	-
standard	-	20 μ l	-
sample	-	-	20 μ l
Mix, incubate at 25, 30 or 37°C for 5 minutes. Read absorbances of standard (As) and samples (Ax) against reagent blank.			

RESULTS CALCULATION

serum/plasma sample:

phosphorus mg/dl = $Ax/As \times 5$ (standard value)

random urine sample:

phosphorus mg/dl = $Ax/As \times 5 \times 20$ (standard value and dilution factor)

24 hours urine sample:

phosphorus g/24h = $Ax/As \times 5 \times 20 \times \text{urine volume}$
1000
(standard value, dilution factor and diuresis in decilitres)

EXPECTED VALUES

serum/plasma (adults): 2.5 - 4.5 mg/dl (0.81 - 1.45 mmol/l)
serum/plasma (children): 4.0 - 7.0 mg/dl (1.29 - 2.26 mmol/l)

urine (nonrestricted diet): 0.4 - 1.3 g/24h (12.9 - 42.2 mmol/24h)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml
with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml
with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 20 mg/dl.

If the limit value is exceeded, it is suggested to dilute sample 1+9 with distilled water and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 0.1 mg/dl.

Interferences

no interference was observed by the presence of:

bilirubin \leq 40 mg/dl

hemoglobin \leq 100 mg/dl

Hemolysis interferes. Both positive and negative interferences with lipemic samples has been observed.

Precision

intra-assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	3.50	0.05	1.50
sample 2	5.87	0.11	1.90

inter-assay (n=20)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	3.41	0.08	2.40
sample 2	5.84	0.11	1.90

Methods comparison

a comparison between Chema and a commercially available product gave the following results:

phosphorus Chema = x
phosphorus competitor = y
n = 105

y = 1.005x + 0.109 mg/dl r = 0.975

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

REFERENCES








Yee H.Y. - Clin. Chem. 14, 898 (1968).

Tietz Textbook of Clinical Chemistry, Second Edition, Bur-tis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions