PHOS2

cobas®

Phosphate (Inorganic) ver.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058610190	08058610500	Phosphate (Inorganic) ver.2 (750 tests)	System-ID 2099 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

PHOS2: ACN 20990 (Serum/plasma)

PHOS2U: ACN 20991 (Urine)

Intended use

In vitro test for the quantitative determination of phosphorus in human serum, plasma and urine on **cobas c** systems.

Summary

Phosphate measurements, performed with this assay, in human serum, plasma and urine are used as an aid in diagnosis and monitoring of phosphate imbalances such as hyper- or hypophosphatemia.

The large majority (85 %) of phosphate is contained in the skeleton combined with calcium as hydroxyapatite, about 15 % is contained in soft tissue and only < 0.1 % in the extracellular fluid. Phosphate homeostasis is a complex process involving the kidneys, intestine, and skeleton. Phosphate occurs in blood in the form of inorganic phosphate and in organically bound phosphoric acid. The small amount of extracellular organic phosphate is found almost exclusively in the form of phospholipids.[1]

The ratio of phosphate to calcium in the blood is approximately 6 : 10.[1] An increase in the level of phosphate causes a decrease in the calcium level. The mechanism is influenced by interactions between parathormone and vitamin D. Hyperphosphatemia originates from excessive phosphate intake or renal reabsorption, reduced phosphate excretion or transcellular shifting.[2] Clinical conditions such as hypoparathyroidism, vitamin D intoxication and most commonly, renal failure with decreased glomerular phosphate filtration (like in chronic kidney disease, CKD), give rise to hyperphosphatemia.[3,4,5] Hypophosphatemia is the result of inadequate phosphorus intake, reduced intestinal absorption, excessive urinary excretion, or redistribution of phosphate to the intracellular compartments.[2,6] Clinical conditions such as rickets, hyperparathyroidism and Fanconi's syndrome are associated with hypophosphatemia.[7,8,9]

The method presented here for the determination of inorganic phosphate is based on the reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate without reduction. The addition of an accelerator gives rise to a more rapid rate of reaction and the application of sample blanking yields more precise results.

Test principle[10]

Molybdate UV.

Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula $(NH_4)_3[PO_4(MoO_3)_{12}]$ with ammonium molybdate in the presence of sulfuric acid.

Phosphate + ammonium molybdate $\xrightarrow{H_2SO_4}$ ammonium phosphomolybdate

The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration and is measured photometrically.

Reagents - working solutions

R1 Sulfuric acid: 0.36 mol/L; detergent

R3 Ammonium molybdate: 3.5 mmol/L; sulfuric acid: 0.36 mol/L; sodium chloride: 150 mmol/L

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H290 May be corrosive to metals.

Prevention:

P234 Keep only in original packaging.

Response:

P390 Absorb spillage to prevent material damage.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and K_2-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could

affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Urine

Collect in detergent-free containers. Acidify with hydrochloric acid after collection (pH < 3).[11,12]

Stability in serum/plasma:[13]	24 hours at 15-25 °C
	4 days at 2-8 °C
	1 year at (-15)-(-25) °C

Freeze only once.

Stability in urine:[11,12]	6 months at 2-8 °C (when acidified)
24-hour urine:	Store cooled during collection.

Centrifuge samples containing precipitates before performing the assay.

If stabilizers are added to the sample, the sample index feature must not be used.

See the limitations and interferences section for details about possible sample interferences.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition

Reporting time	10 min	
Wavelength (sub/main)	700/340 nm	
Reagent pipetting		Diluent (H_2O)
R1	58 µL	18 µL
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.6 µL	–	–
Decreased	3.2 µL	20 µL	60 µL
Increased	1.6 µL	–	–

Application for urine

Test definition

Reporting time	10 min	
Wavelength (sub/main)	700/340 nm	
Reagent pipetting		Diluent (H_2O)
R1	58 µL	18 µL
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.6 µL	10 µL	100 µL
Decreased	1.6 µL	5 µL	105 µL
Increased	1.6 µL	10 µL	100 µL

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Application for serum/plasma (ACN 20990)

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change Full calibration - as required following quality control procedures

Application for urine (ACN 20991)

Transfer of calibration from serum/plasma application (ACN 20990)

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against NERL primary reference material.

Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte concentration of each sample in the unit mmol/L (mg/dL, mg/L).

Conversion factors:	mmol/L x 3.10 = mg/dL
	mmol/L x 31 = mg/L

Limitations - interference[11]

Criterion: Recovery within ± 10 % of initial value at a phosphate concentration of 0.87 mmol/L in serum and 13 mmol/L in urine.

Serum/plasma

Icterus:[14] No significant interference up to an I index of 40 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 684 µmol/L or 40 mg/dL and approximate unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[14] No significant interference up to an H index of 300 (approximate hemoglobin concentration: 186 µmol/L or 300 mg/dL).

Note: This interference results from inorganic phosphates produced by the action of phosphatases on organic phosphates, both of which are released from the red cells upon hemolysis.

Lipemia (Intralipid):[14] No significant interference up to an L index of 1250. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[15,16]

Exception: Phospholipids contained in liposomal drug formulations (eg AmBisome) may be hydrolyzed in the test due to the acidic reaction pH and thus lead to elevated phosphate results.[17]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[18]

Urine

Hemolysis: No significant interference up to an H index of 750 (approximate hemoglobin concentration: 466 µmol/L or 750 mg/dL).

Urea: No significant interference from urea up to a concentration of 1500 mmol/L (9009 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

Serum/plasma

0.10-6.46 mmol/L (0.31-20.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Urine

1.1-92 mmol/L (3.4-285 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Serum/plasma

Limit of Blank	= 0.1 mmol/L (0.31 mg/dL)
Limit of Detection	= 0.1 mmol/L (0.31 mg/dL)
Limit of Quantitation	= 0.1 mmol/L (0.31 mg/dL)

Urine

Limit of Blank	= 1.1 mmol/L (3.4 mg/dL)
Limit of Detection	= 1.1 mmol/L (3.4 mg/dL)
Limit of Quantitation	= 1.1 mmol/L (3.4 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 30 %. It has been determined using low concentration phosphate samples.

Expected values

Serum/plasma

mmol/L

Adults:[19]

0.81-1.45 mmol/L

Children:[20]

Age	*Male*	*Female*
1-30 d	1.25-2.25	1.40-2.50
1-12 m	1.15-2.15	1.20-2.10
1-3 y	1.00-1.95	1.10-1.95
4-6 y	1.05-1.80	1.05-1.80
7-9 y	0.95-1.75	1.00-1.80
10-12 y	1.05-1.85	1.05-1.70
13-15 y	0.95-1.65	0.90-1.55
16-18 y	0.85-1.60	0.80-1.55

mg/dL

Adults:[19]

2.5-4.5 mg/dL

Children:[20]

Age	*Male*	*Female*
1-30 d	3.9-6.9	4.3-7.7
1-12 m	3.5-6.6	3.7-6.5
1-3 y	3.1-6.0	3.4-6.0
4-6 y	3.3-5.6	3.2-5.5
7-9 y	3.0-5.4	3.1-5.5
10-12 y	3.2-5.7	3.3-5.3
13-15 y	2.9-5.1	2.8-4.8
16-18 y	2.7-4.9	2.5-4.8

Urine

mmol/L, mmol/d

1st morning urine[21]	13-44 mmol/L
24-hour urine[11]	13-42 mmol/d

mg/dL, g/d

1st morning urine[21]	40-136 mg/dL*
24-hour urine[11]	0.4-1.3 g/d

* calculated by unit conversion factor

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Serum/plasma

Repeatability	*Mean* *mmol/L*	*SD* *mmol/L*	*CV* *%*
PCCC1[a)]	1.34	0.00636	0.5

PCCC2[b)]	2.35	0.00939	0.4
Human serum 1	0.256	0.00301	1.2
Human serum 2	1.11	0.00546	0.5
Human serum 3	1.68	0.00732	0.4
Human serum 4	3.75	0.0150	0.4
Human serum 5	5.81	0.0176	0.3

Intermediate precision	*Mean mmol/L*	*SD mmol/L*	*CV %*
PCCC1[a)]	1.34	0.0109	0.8
PCCC2[b)]	2.39	0.0203	0.9
Human serum 1	0.256	0.00571	2.2
Human serum 2	1.11	0.00788	0.7
Human serum 3	1.71	0.00872	0.5
Human serum 4	3.75	0.0168	0.4
Human serum 5	5.81	0.0236	0.4

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

Urine

Repeatability	*Mean mmol/L*	*SD mmol/L*	*CV %*
Control 1[c)]	8.38	0.0533	0.6
Control 2[c)]	16.7	0.0970	0.6
Human urine 1	2.99	0.0380	1.3
Human urine 2	12.1	0.0764	0.6
Human urine 3	27.7	0.164	0.6
Human urine 4	45.2	0.238	0.5
Human urine 5	79.6	0.430	0.5

Intermediate precision	*Mean mmol/L*	*SD mmol/L*	*CV %*
Control 1[c)]	8.38	0.0670	0.8
Control 2[c)]	16.5	0.113	0.7
Human urine 1	3.05	0.0481	1.6
Human urine 2	11.9	0.0957	0.8
Human urine 3	27.7	0.203	0.7
Human urine 4	45.2	0.361	0.8
Human urine 5	79.6	0.587	0.7

c) commercially available control material

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Inorganic phosphate values for human serum, plasma and urine samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Serum/plasma

Sample size (n) = 75

Passing/Bablok[22]	Linear regression
y = 1.013x + 0.00734 mmol/L	y = 1.011x + 0.00582 mmol/L
τ = 0.989	r = 1.000

The sample concentrations were between 0.390 and 5.86 mmol/L.

Urine

Sample size (n) = 70

Passing/Bablok[22]	Linear regression
y = 0.986x + 0.0391 mmol/L	y = 0.993x - 0.0281 mmol/L
τ = 0.996	r = 1.000

The sample concentrations were between 1.21 and 91.8 mmol/L.

Inorganic phosphate values for human serum, plasma and urine samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Serum/plasma

Sample size (n) = 77

Passing/Bablok[22]	Linear regression
y = 1.015x - 0.009 mmol/L	y = 1.016x - 0.008 mmol/L
τ = 0.989	r = 1.000

The sample concentrations were between 0.43 and 6.19 mmol/L.

Urine

Sample size (n) = 73

Passing/Bablok[22]	Linear regression
y = 1.022x - 0.103 mmol/L	y = 1.018x - 0.092 mmol/L
τ = 0.981	r = 1.000

The sample concentrations were between 1.59 and 90.0 mmol/L.

Inorganic phosphate values for human serum, plasma and urine samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Serum/plasma

Sample size (n) = 74

Passing/Bablok[22]	Linear regression
y = 1.000x - 0.0100 mmol/L	y = 0.996x + 0.000118 mmol/L
τ = 0.976	r = 1.000

The sample concentrations were between 0.167 and 6.31 mmol/L.

Urine

Sample size (n) = 75

Passing/Bablok[22]	Linear regression
y = 0.963x - 0.0167 mmol/L	y = 0.959x + 0.0679 mmol/L
τ = 0.995	r = 1.000

The sample concentrations were between 1.52 and 91.0 mmol/L.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

References

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Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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