BECKMAN COULTER CE

AU

Instructions For Use

IN PHOS
Inorganic Phosphorous

REF OSR6122 4 x 15 mL R1, 4 x 15 mL R2
OSR6222 4 x 40 mL R1, 4 x 40 mL R2

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Photometric UV test for the quantitative determination of inorganic phosphorous in human serum, plasma and urine on Beckman Coulter analysers.

SUMMARY AND EXPLANATION

Reference[1,2,3,4]

In plasma and serum the majority of phosphate exists in the inorganic form (Pi), approximately 15% bound to protein and the remainder in complexed and free forms. Serum phosphate concentrations are dependent on diet and variation in the secretion of hormones such as PTH. Intracellularly phosphate occurs primarily as organic phosphate however a small but extremely important fraction exists as inorganic phosphate which, because it is a substrate for oxidative phosphorylation, participates in reactions concerned with generation of metabolic energy. About 85% of extracellular phosphate occurs in the Pi form as hydroxyapatite thereby playing an important role in bone structure.

Hypophosphataemia (phosphate depletion) is relatively common in hospitalised patients and is found in up to 30% of surgical patients. Hypophosphataemia is caused by a decreased intake or absorption of phosphate such as occurs in Vit D deficiency, malabsorption, use of oral phosphate binders and primary PTH excess; increased excretion such as occurs in secondary PTH excess, post renal transplant and re-feeding starved patients; and from redistribution of phosphate e.g. hyperalimentation, recovery from diabetic ketoacidosis and respitatory alkalosis.

Hyperphosphataemia is caused by increased intake such as occurs in intravenous therapy and phosphate enemas; reduced excretion such as occurs in acute and chronic renal failure, low PTH or resistance to PTH and vitamin D toxicity; and redistribution of phosphate that occurs in tumour lysis, rhabdomyolysis and heat stroke.

METHODOLOGY

Reference[5,6]

Inorganic phosphorous reacts with molybdate to form a heteropolyacid complex. The use of a surfactant eliminates the need to prepare a protein free filtrate. The absorbance at 340/380 nm is directly proportional to the inorganic phosphorous concentration in the sample.

CHEMICAL REACTION SCHEME

$$7\ H_3PO_4 + 12\ (Mo_7O_{24})^{6-} + 72\ H^+ \longrightarrow 7\ H_3PO_4(MoO_3)_{12} + 36\ H_2O$$

SPECIMEN

TYPE OF SPECIMEN

Serum and heparinised plasma: Stable in serum for 4 days when stored at 2…8°C and 1 day when stored at 15…25°C.[7]

Serum samples, free from hemolysis, are the recommended specimens. Remove serum from clot as soon as possible to minimize hemolysis.

Urine:[8] Acidified with 6M HCl. Collect timed 24-hour specimen using standard laboratory procedures. Store at 2…8°C.

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Sulphuric acid	200 mmol/L
Ammoniumheptamolybdate	0.35 mmol/L
Glycine	50 mmol/L

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

GHS HAZARD CLASSIFICATION

Inorganic Phosphorous R1	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	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/physician.
		Sulfuric Acid 5 - 10%
		Polyoxyethylated Octyl Phenol 0.5 - 1%
Inorganic Phosphorous R2	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	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/physician.
		Sulfuric Acid 5 - 10%
		Polyoxyethylated Octyl Phenol 0.5 - 1%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagents are ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 30 days.

CALIBRATION

CALIBRATOR REQUIRED

Use System Calibrator Cat. No. 66300 for serum and plasma application and Urine Calibrator Cat. No. B64606 for urine application.

The inorganic phosphorous values of both calibrators are traceable to a Beckman Coulter Master Calibrator.

Recalibrate the assay every 30 days, or when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used for the serum application.

Biorad Liquichek Urine Chemistry Controls Cat. No. 397 and 398 or other control materials with values determined by this Beckman Coulter system may be used for the urine application.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration/blanking is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement.

CALCULATIONS

The Beckman Coulter analysers automatically compute the inorganic phosphorous concentration of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Reference[2]

Serum	Adults	0.81 – 1.45 mmol/L (2.5 – 4.5 mg/dL)
	Children	1.29 – 2.26 mmol/L (4.0 – 7.0 mg/dL)
Urine	On non-restricted diet	12.9 – 42.0 mmol/d (0.4 – 1.3 g/day)

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval

according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

INTERFERENCES

Results of serum studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 3% or 0.1 mmol/L up to 40 mg/dL or 684 µmol/L bilirubin
Lipemia:	Interference less than 10% or 0.1 mmol/L up to 800 mg/dL Intralipid

Hemolysis must be avoided as Phosphate may be split off from labile esters in the erythrocytes.[9]

Results of urine studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 5% or 1 mmol/L up to 40 mg/dL or 684 µmol/L bilirubin
Haemolysis:	Interference less than 5% or 1 mmol/L up to 5 g/L haemoglobin

In very rare cases gammopathy, especially monoclonal IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Eltrombopag and its metabolites may interfere with this assay causing erroneously high patient results.

Patients receiving a high-dose liposomal Amphotericin B therapy (L-AMB) may generate false low results with this reagent[10].

Refer to Young[11] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within a concentration range of 0.32 – 6.40 mmol/L (1 – 20 mg/dL) for serum.

The test is linear within a concentration range of 0 – 113 mmol/L (0 – 350 mg/dL) for urine.

SENSITIVITY

The lowest detectable level using serum settings on a DxC 700 AU analyser was estimated at 0.11 mmol/L.

The lowest detectable level using urine settings on an AU2700 analyser was estimated at 0.48 mmol/L.

The lowest detectable level represents the lowest measurable level of Inorganic phosphorous that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Patient serum samples were used to compare this Inorganic phosphorous assay on the AU600 against another commercially available inorganic phosphorous assay. Results of linear regression analysis were as follows:

y = 0.968x – 0.055	r = 1.000	n = 118	Sample range = 0.44 – 6.41 mmol/L

Patient urine samples were used to compare this Inorganic phosphorous assay on the AU2700 against another commercially available inorganic phosphorous assay. Results of linear regression analysis were as follows:

y = 0.936x + 0.170	r = 0.999	n = 100	Sample range = 3.42 – 53.07 mmol/L

PRECISION

The following data was obtained on a DxC 700 AU using 3 serum pools analysed over 20 days.

n = 80	**Within-run**		**Total**	
Mean mmol/L	**SD**	**CV%**	**SD**	**CV%**
0.80	0.01	1.11	0.01	1.36
1.60	0.01	0.65	0.02	0.95
3.58	0.02	0.47	0.02	0.63

The following data was obtained on an AU640 using 3 urine pools analysed over 20 days.

n = 80	**Within-run**		**Total**	
Mean mmol/L	**SD**	**CV%**	**SD**	**CV%**
9.54	0.13	1.41	0.28	2.99
32.96	0.23	0.71	0.51	1.55
87.35	0.62	0.71	1.14	1.30

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	**Description**
PHO1N	Inorganic Phosphorous (Serum)
PHO1N	Inorganic Phosphorous (Urine)

Setting Sheet Footnotes
User defined
Serum: † System Calibrator Cat. No.: 66300.

Urine: † Urine Calibrator Cat. No: B64606. Ensure relevant value sheet is used.

* Values set for working in SI units (mmol/L). To work in mg/dL multiply by 3.1.

REVISION HISTORY

Updated Specimen Section

Updated Interference section

Updated References section

Preceding version revision history

Added new languages

REFERENCES

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9. Tietz, N.W., Fundamentals of Clinical Chemistry, 3rd Edition, W.B. Saunders, 1987.

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