



INORGANIC PHOSPHORUS

OSR6122

4 x 15 mL

R1

4 x 15 mL

R2

OSR6222

4 x 40 mL

R1

4 x 40 mL

R2

Intended Use

System reagent for the quantitative determination of Inorganic Phosphorus in human serum and urine on Beckman Coulter AU analyzers.

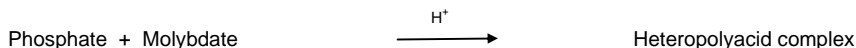
Summary

Measurements of inorganic phosphorus are used in the diagnosis and treatment of various disorders including parathyroid gland and kidney diseases, and vitamin D imbalance.

In 1925, Fiske and Subbarow¹ described a method for the determination of phosphorus in protein-free blood filtrates using ammonium molybdate. Aminoaphtholsulfonic acid was employed as a reducing agent. Many reducing agents have since been introduced, among them ferrous sulfate (first introduced by Summer²). This methodology offered the advantage of working in a slightly acid medium, thereby permitting greater specificity in dealing with mixtures of phosphates and phosphate esters. Taussky and Shorr³ in 1953, improved the methodology by using ferrous ammonium sulfate, which is more stable than ferrous sulfate. In 1957, Dryer, Tammes, and Routh⁴ increased the sensitivity of the reaction by measuring the absorbance at 340 nm rather than the previously recommended 650 to 750 nm.

Methodology

This Inorganic Phosphorus method is based on a modification of the method developed by Daly and Ertingshausen⁵ in which inorganic phosphate 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 Phosphorus level in the sample.



System Information

For AU400/400[®]/480, AU600/640/640[®]/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents

Final Concentration of Reactive Ingredients:

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

Also contains preservatives.

Precautions

1. For *in vitro* diagnostic use.
2. **WARNING!** Corrosive, strong acid. Avoid ingestion or contact with skin or eyes. In case of contact, immediately flush affected areas with water and seek medical attention. Obtain medical attention immediately for eye contact or ingestion.

Preparation of Reagents

The Inorganic Phosphorus reagent is ready to use. No preparation is required.

Storage and Stability

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagent is stable for 30 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration

Visible signs of microbial growth, turbidity, precipitate, or change of color in the Phosphorus reagent may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation

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

Timed, 24 hour, collection is recommended for urine specimens. Urine specimens should be manually prediluted 1:10 with water before analysis on the AU600 in Standard Measure Mode.

NOTE: There is a diurnal variation in urine phosphate excretion, with the highest output occurring in the afternoon.⁶

EDTA, fluoride oxalate or citrate should not be used when measuring inorganic phosphorus.

Sample Storage and Stability

Serum samples are stable 8 hours when stored at room temperature (15 - 25°C) or more than one week when stored at 2 - 8°C.⁷

Urine may contain larger quantities of organic phosphates, which can decompose on exposure to elevated temperatures. When acidified with HCl, urine phosphate is stable for more than 6 months.⁸

Inorganic Phosphorus

Interfering Substances

Results of studies⁹ show that the following substances interfere with phosphorus determinations. The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant interference up to 40 mg/dL Bilirubin
*Hemolysis: No significant interference less than 10% up to 350 mg/dL Hemolysate
Lipemia: No significant interference less than 10% up to 900 mg/dL Intralipid**

* Hemolysis must be avoided as Phosphate may be split off from labile esters in the erythrocytes.¹⁰

** Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young¹¹ for a compilation of reported interferences with this test.

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

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided

Inorganic Phosphorus Reagent

Materials Required But Not Provided

Chemistry Calibrator (Cat # DR0070)
Urine Calibrator (Cat # DR0090)

Stability Of Final Reaction Mixture

The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration

The frequency of calibration is every 30 days. Calibration of this Inorganic Phosphorus procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070), which is traceable to the New England Reference Laboratory (NERL) standard for serum specimens. For urine specimens use Urine Calibrator (Cat # DR0090).

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control

During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Appropriate qualified urine controls, should be established and utilized during urine analysis.

Results

Automatically printed out for each sample in mg/dL at 37°C. For SI units (mmol/L) the result must be multiplied by 0.323.

Dynamic Range

The Inorganic Phosphorus procedure is linear from 1.0 to 20.0 mg/dL for serum determinations and from 10 to 200 mg/dL for urine (prediluted) determinations. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Expected Values

	<u>Serum</u> ¹²	<u>Urine</u> ⁸
Adults	2.5 - 5.0 mg/dL	0.3 - 1.3 g/24 hours
Children	4.0 - 7.0 mg/dL	0.5 - 0.8 g/24 hours

Beckman Coulter Determined Reference Range¹³ 3.7 - 7.2 mg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Inorganic Phosphorus Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision¹⁴

Estimates of precision, based on CLSI recommendations,⁶ are consistent with typical performance. The within run precision for serum samples is less than 3% CV and total precision is less than 5% CV. Assays of control sera were performed and this data reduced following CLSI guidelines above.

Serum

N = 100 Mean, mg/dL	Within run		Total	
	SD	CV%	SD	CV%
2.01	0.04	1.9	0.04	2.1
5.7	0.0	0.6	0.05	0.9

Inorganic Phosphorus

Urine

N = 100	Within run		Total		
	Mean, mg/dL	SD	CV%	SD	CV%
	30.8	0.2	0.8	0.5	1.6
	78.1	0.5	0.6	2.08	2.7

Method Comparison¹⁴

Serum

Patient samples were used to compare this Inorganic Phosphorus Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	AU600
Slope	0.962
Intercept	0.11
Correlation Coeff. (r)	1.000
No. of Samples (n)	177
Range (mg/dL)	0.5-17.9

Urine

Patient samples were used to compare this Inorganic Phosphorus Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	AU600
Slope	0.992
Intercept	0.09
Correlation Coeff. (r)	0.9996
No. of Samples (n)	171
Range (mg/dL)	2.6-199.8

Sensitivity

Typical change in absorbance for 1 mg/dL Inorganic Phosphorus is 30 mAbsorbance.

References

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6. CLSI/NCCLS, Evaluation Protocol EP5-A, 1999.
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10. Tietz, N.W. (ed), Fundamentals of Clinical Chemistry, 3rd Edition, W.B., Saunders, 1987.
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12. Tietz, N.W.(ed), Textbook of Clinical Chemistry, W.B., Saunders, 1986.
13. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
14. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA



