

Clinical Evaluation of an Enzymatic Point-of-Care Phosphorus Assay Using the ABAXIS Piccolo®

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Abstract

Measurement of phosphorus concentrations in blood is typically performed using the reaction of phosphate ion with ammonium molybdate to form a phosphomolybdate complex. We evaluated a rapid and precise enzymatic, endpoint phosphorus assay based on the reaction of phosphorus and sucrose. A series of reactions, driven by sucrose phosphorylase, phosphoglucomutase and glucose-6-phosphate dehydrogenase results in the formation of NADH that is measured at 340 nm. The assay can be performed using 90 µL of serum, plasma, or whole blood in the ABAXIS Piccolo® point-of-care analyzer. We evaluated the performance of the Piccolo enzymatic phosphorus assay by assessing precision, linearity, effect of interfering substances, and comparison to two high-throughput phosphomolybdate-based methods performed in the clinical laboratory.

Precision was evaluated in accordance with NCCLS EP5-A guidelines with modifications based on NCCLS EP18-P. The linear range of the assay was assessed using NCCLS EP6-P guidelines. The effect of endogenous and exogenous interfering substances was evaluated in accordance with NCCLS EP7-P guidelines. The analytical performance was evaluated against an Ortho Vitros® 950 and Beckman LX20 analyzer using patient serum samples assayed by all three methods.

Within-run and total precision of the Piccolo method was very good. Within-run CVs were 1.1% and 1.0% at phosphorus concentrations of 4.3 and 7.6 mg/dL, respectively. Total precision was 1.8% and 1.1% at these same concentrations. The assay demonstrated linearity from 0.2 to 20.0 mg/dL. Commonly encountered endogenous interferents showed less than 10% bias at hemoglobin concentrations up to 400 mg/dL, bilirubin up to 50 mg/dL, and lipemia up to 1100 mg/dL. The assay was also evaluated for interference from 38 commonly encountered drugs and metabolites using supra-therapeutic concentrations. Only, nitrofurantoin caused a 19% positive bias at 20 mg/dL and oxaloacetate at 132 mg/dL caused a 14% decrease in expected results.

Method comparison studies were performed against phosphomolybdate-based methods using a Beckman LX20 and Vitros 950. The Piccolo showed excellent agreement with these two methods as shown below:

ABAXIS Piccolo = 1.024 Vitros - 0.2	r = 0.993 (n = 90)
ABAXIS Piccolo = 0.982 Beckman - 0.0	r = 0.991 (n = 60)

In summary, the enzymatic phosphorus assay performed using the Piccolo point-of-care analyzer was precise, demonstrated a robust dynamic range, and showed excellent agreement with high-throughput, phosphomolybdate-based methods. In addition, the Piccolo enzymatic method is relatively free of interference effects from icterus, lipemia, and elevated protein concentrations that often plague phosphomolybdate-based phosphorus methods.

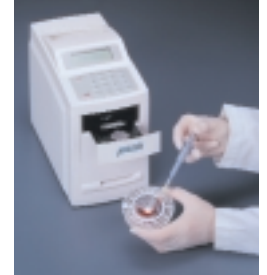
Introduction

We evaluated an, enzymatic, automated point-of-care method for phosphorus determinations using the ABAXIS Piccolo®.

A. The ABAXIS Piccolo system consists of (see picture):

1. The Piccolo Point-of-Care Blood Analyzer

- It is 24.2 cm (9.5 in) high, 15.3 cm (6 in) wide, and 29.2 cm (11.5 in) deep.
- It weighs 6.9 kg (15 pounds).



2. Single-use disposable reagent discs

- A clear plastic disc, 8 cm in diameter and 2 cm in depth
- The reagent disc contains an aqueous diluent in the center and dry reagent beads in cuvettes around the disc periphery.

- Plasma separation, mixing, and critical volumetric measurements are performed by the disc.
- Fluid flow within the disc is controlled by centrifugal and capillary forces.

B. Multiple analyses can be performed simultaneously as a panel with results for all analytes available in approximately 12.5 minutes.

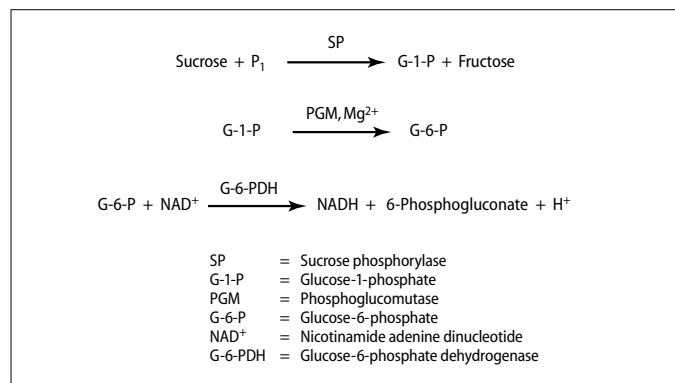
C. The test system requires approximately 90 µL of heparinized whole blood, heparinized plasma, or serum. One 90-µL sample can provide up to 14 results, depending on the test panel selected.

D. Phosphorus Assay Principle: This assay is based on the enzymatic determination of phosphorus: (see Figure 1).

1. Phosphorus in the specimen is metabolized with sucrose by sucrose phosphorylase to form glucose-1-phosphate (G-1-P) and fructose.
2. The formed G-1-P reacts in an intermediate step with phosphoglucomutase (PGM) in the presence of Mg²⁺ to produce glucose-6-phosphate (G-6-P).
3. In the final indicator reaction, G-6-P and NAD⁺ reacts with glucose-6-phosphate dehydrogenase (G-6-PDH) to produce NADH and 6-phosphogluconate.
4. The amount of phosphorus in the patient sample is proportional to the amount of NADH produced. The amount of NADH produced is measured as an endpoint bichromatically at 340 nm and 405 nm.

Introduction (cont.)

Figure 1. Assay Principle



Materials and Methods

We evaluated the ABAXIS Piccolo phosphorus assay by assessing:

A. Precision

1. Within-run and total precision was evaluated in accordance with NCCLS EP5-A guidelines with modifications based on NCCLS EP18-P.
2. Two levels of control sera were assayed in duplicate in the morning and afternoon across two Piccolo analyzers over at least five days at each of two sites (OHSU and Abaxis). The results were combined for the calculation of precision (n=80 for each level).

B. Linearity

1. The linear range of the assay (0.2-20.0 mg/dL) was assessed using NCCLS EP6-P guidelines.
2. A human serum pool was supplemented to achieve a concentration of approximately 20.0 mg/dL. A phosphate-free serum pool was also prepared.
3. Pools containing 6 levels of phosphorus were prepared by preparing admixtures of the low and high phosphorus pools.
4. Each pool was measured 8 times using 8 different Piccolo instruments.

C. Effects of Potential Interferences

1. The potential effects of endogenous and exogenous interferents were evaluated in accordance with NCCLS EP7-P guidelines.
2. Possible endogenous interferents including bilirubin, triglycerides (Intralipid® was used to simulate lipemia), and hemolysis were tested.
3. In addition, 38 exogenous substances and drugs were assessed for their potential interference.

Materials and Methods (cont.)

D. Method Comparison

1. We assessed method agreement between the Piccolo phosphorus assay with two high-throughput laboratory-based methods according to NCCLS EP9-A guidelines.
2. A distribution of patient samples was measured using the ABAXIS Piccolo and Ortho Vitros 950 (n=90) and the Beckman LX20 (n=60) analyzer.
3. All measurements were completed using samples drawn within the previous 4 hours.
4. Data were evaluated using Deming regression analysis.

E. Effect of Sample Type

1. The effect of sample type (whole blood vs serum vs plasma) was investigated by analysis of heparinized whole blood and serum collected simultaneously from healthy individuals.
2. Heparinized whole blood, plasma obtained from the whole blood sample, and serum were randomized and analyzed on the same analyzer.
3. Mean recovery of phosphorus from plasma and serum, relative to that obtained in whole blood was calculated and compared.

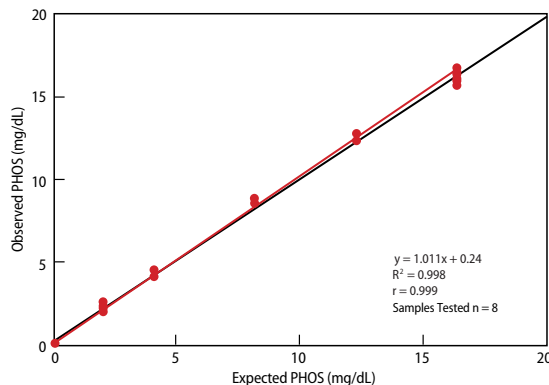
Results

Table 1. Precision of Piccolo Phosphorus Method

n=80

	Within-run	Total
Level 1		
Mean (mg/dL)	4.3	4.3
SD	0.047	0.077
%CV	1.1	1.8
Level 2		
Mean (mg/dL)	7.6	7.6
SD	0.076	0.084
%CV	1.0	1.1

Figure 2. Linearity of Piccolo PHOS Assay



Results (cont.)

Effects of Endogenous Substances

Certain endogenous substances such as hemoglobin, bilirubin, and triglycerides may affect the measured concentration of some analytes. The Piccolo assesses the level of these potential interferents in all samples. An index value that corresponds to the relative concentration of hemoglobin, bilirubin, and turbidity due to lipemia is printed on the bottom of each result card to inform the operator about the levels of these potential interferents. The Piccolo Point-of-Care Chemistry System suppresses any results that are affected by >10% interference from hemoglobin, bilirubin, and triglycerides. "HEM," "LIP," or "ICT" respectively, is printed on the result card in place of the result. These levels of endogenous substances are given in Table 2.

Table 2. Maximum Concentration of Endogenous Interferents that will Cause Greater than 10% Bias in Expected Phosphorus Result.

Analyte	Hemolysis (Hemoglobin) mg/dL	Lipemia (Triglycerides) mg/dL	Icterus (Bilirubin) mg/dL
Phosphorus	400	1100	50

Table 3. Exogenous Substances with No Significant Effect on Piccolo PHOS Method

Analyte	Therapeutic Range (mg/dL)	Maximum Concentration Tested Showing <10% Interference (mg/dL)
Acetaminophen	2–10	100
Acetoacetate	0.05–3.60	102
Acetylsalicylic acid	1–2	50
Ampicillin	0.5	30
Ascorbic acid	0.8–1.2	20
Caffeine	---	10
Cephalothin (Keflin)	10	400
Chloramphenicol	1.0–2.5	100
Cimetidine	0.1–1.0	16
Dopamine	0.3–1.5	19
Epinephrine	---	1
Erythromycin	0.2–2.0	10
Glutathione	---	30
Heparin	---	28,000
Hydrochlorothiazide	---	7.5
Ibuprofen	0.5–4.2	50
Isoniazide	0.1–0.7	4
Ketoprofen	---	50
L-dopa	---	5
Lidocaine	0.15–0.6	1
Lithium lactate	6–12	84
Methicillin	---	100
Methotrexate	0.1	0.5
Metronidazole	0.1	5
Nafcillin	---	1
Nitrofurantoin	0.2	20
Oxacillin	---	1
Penicillin G	---	100
Phenytoin	1–2	3
Proline	---	4
Pyruvate	0.3–0.9	44
Rifampin	0.4–3.0	0.5
Salicylic acid	15–30	50
Sulfadiazine	2–4	150
Sulfanilamide	10–15	50
Theophylline	1–2	20

Results (cont.)

Table 4. The Following Exogenous Substances Were Found to Have an Affect on the Piccolo PHOS Method.

Analyte	Therapeutic Range (mg/dL)	Maximum Concentration Tested Showing <10% Interference (mg/dL)	% Change
Nitrofurantoin	0.2	20	19% inc.
Oxaloacetate	---	132	14% dec.

Figure 3. Linear Regression: ABAXIS Piccolo vs Ortho Vitros 950 Phosphorus

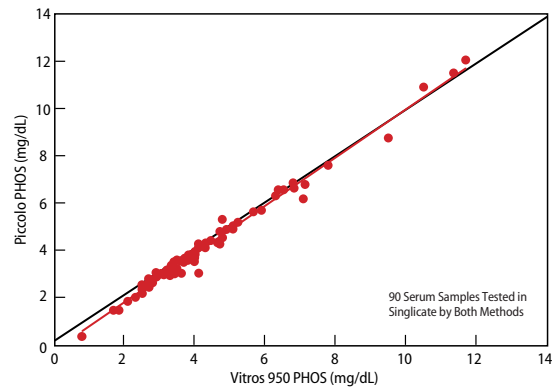
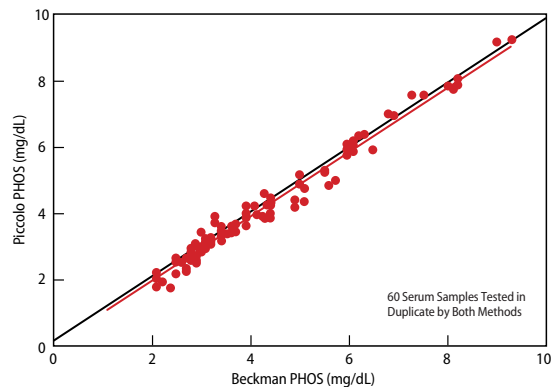


Figure 4. Linear Regression: ABAXIS Piccolo vs Beckman LX-20 Phosphorus



Results (cont.)

Table 5. Summary of Phosphorus Correlation Data

Method Y Axis	Method Method	Slope* X Axis	Intercept*	Correlation Coefficient (r)
ABAXIS Piccolo	Ortho Vitros 950	1.024	-0.2	0.993
ABAXIS Piccolo	Beckman LX20	0.982	0.0	0.991

*Deming Regression Analysis

Table 6. Effect of Sample Type on Phosphorus Recovery

Sample Type: Whole Blood	Average (SD) Percent Recovery
Versus Plasma	99.54% (0.92%)
Versus Serum	108.87% (3.32%)

- Essentially identical recovery of phosphorus from whole blood and plasma was observed.
- Serum, as expected, showed higher recovery of phosphorus due to release of phosphorus from erythrocytes during the clotting process.
- The average difference between phosphorus in whole blood or plasma, and that observed in serum was 0.30 mg/dL.

Conclusions

- We found the ABAXIS enzymatic phosphorus assay to be accurate and precise.
- The enzymatic method showed excellent agreement with phosphomolybdate-based phosphorus assays found on high-throughput analyzers.
- Use of a neutral pH in the enzymatic assay minimizes hydrolysis of phosphate esters that occurs with molybdate-based phosphorus methods.
- The method demonstrated excellent linear response up to 20 mg/dL.
- The method was not affected by a wide variety of exogenous interferences that might be encountered in some settings.
- Endogenous interferences such as bilirubin that interfere with molybdate-based phosphorus methods do not interfere with the enzymatic method unless grossly increased.
- This enzyme-based phosphorus method is very effective, especially when combined with electrolytes and other markers of renal function in a self-contained disposable disc.
- The small sample volume required (90 μ L) to perform up to 14 tests and the short analysis time (12.5 min) make this point-of-care analyzer very useful in a wide variety of clinical settings.