Alinity c Phosphorus2 (Phos2)-28

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Phosphorus2 assay is used for the quantitation of phosphorus in human serum, plasma, or urine on the Alinity c system.

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

SUMMARY AND EXPLANATION OF THE TEST

Phosphorus is a major component of bone mineral, phospholipids in cell membranes, and nucleic acids. Phosphorus acts as a major pH buffer in serum and urine.[1] Phosphorus is essential for normal muscle contractility, neurologic function, electrolyte transport, and

oxygen carrying by hemoglobin (2,3 diphosphoglycerate).[2]

Serum phosphate is filtered at the glomerulus and is reabsorbed primarily in the proximal tubule. Parathyroid hormone (PTH) increases renal phosphate excretion by inhibiting the sodium-phosphate cotransporter in the proximal tubule, whereas vitamin D enhances intestinal phosphate absorption.[1]

Hypophosphatemia may be caused by decreased intake, impaired intestinal absorption, redistribution into cells or bones, and renal losses (as in Fanconi syndrome).[1] Clinical manifestation of severe hypophosphatemia include encephalopathy, dilated cardiomyopathy, generalized muscle weakness leading to respiratory failure, destruction of muscles (rhabdomyolysis), and hemolysis.[1] Hemolysis of red blood cells may be seen with serum phosphorus levels < 0.5 mg/dL.[3]

Hyperphosphatemia is caused by excessive phosphate intake, increased intestinal absorption, redistribution from intracellular stores, or impaired renal excretion. Acute levels can increase the risk for precipitation of calcium phosphate in the kidney and soft tissues.[1]

In chronic hyperphosphatemia (renal insufficiency) with a phosphate level > 6.5 mg/dL, mortality is higher due to the increased risk for the development of coronary and other vascular calcification, leading to increased systolic blood pressure and left ventricular hypertrophy.[1] A rapid increase in serum phosphorus can result in hypocalcemia, which can cause tetany, hypotension, seizures, and cardiac arrhythmias.[1]

A urine phosphorus-creatinine ratio and urine fractional excretion of phosphorus may provide useful information about therapies altering intestinal absorption or urine phosphorus handling.[4]

PRINCIPLES OF THE PROCEDURE

The Phosphorus2 assay is an automated clinical chemistry assay.

Inorganic phosphate reacts with ammonium molybdate to form a heteropolyacid complex.[5] The absorbance at 340 nm is directly proportional to the inorganic phosphorus level in the sample.

Methodology: Phosphomolybdate

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Phosphorus2 Reagent Kit 04U03

Volumes (mL) listed in the following table indicate the volume per cartridge.

REF	04U0320	04U0330
Tests per cartridge	280	700
Number of cartridges per kit	4	4
Tests per kit	1120	2800
R1	20.7 mL	44.4 mL
R2	20.0 mL	44.0 mL
R2 Active ingredient: ammonium molybdate tetrahydrate 2.90 g/L. Preservative: ProClin 300.		

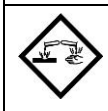
Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials and all consumables contaminated with potentially infectious materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents.[6], [7], [8], [9]

The following warnings and precautions apply to: R1


DANGER	Contains sulfuric acid.
H314	Causes severe skin burns and eye damage.
H290	May be corrosive to metals.
Prevention	
P234	Keep only in original container.
P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P301+P330+P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water / shower.
P310	Immediately call a POISON CENTER or doctor / physician.
P390	Absorb spillage to prevent material damage.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: R2

WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
H402*	Harmful to aquatic life.

H412	Harmful to aquatic life with long lasting effects.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P273	Avoid release to the environment.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) has been implemented.

Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

For the most current hazard information, see the product Safety Data Sheet.

Safety Data Sheets are available at www.corelaboratory.abbott or/and MSD folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

·Upon receipt, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.

·If a reagent cartridge is dropped, place in an upright position for 2 hours before use to allow bubbles that may have formed to dissipate.

·Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	28 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, refer to the Alinity ci-series Operations Manual, Section 5.

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Phosphorus2 assay file must be installed on the Alinity c system prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
mg/dL	0.323	mmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Specimen Type	Collection Vessel	Special Conditions
Serum	Serum tubes Serum separator tubes	
Plasma	Dipotassium EDTA tubes Lithium heparin tubes Lithium heparin separator tubes Sodium heparin tubes	
Urine (random specimens or timed specimens collected over intervals shorter than 24 hours)*10*	Clean glass or plastic container	
Urine (24 hour)*10*	Clean glass or plastic container	Urine specimens should be collected in 6 mol/L HCl, 20 to 30 mL, to avoid precipitation of phosphate complexes.*10*

·Liquid anticoagulants may have a dilution effect resulting in lower concentration values for individual specimens.

The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

Do not use:

·heat-inactivated specimens

·pooled specimens

·grossly hemolyzed specimens

·specimens with obvious microbial contamination

·specimens with fungal growth

·For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.

For accurate results, plasma specimens should be free of platelets and other particulate matter. Ensure centrifugation is adequate to remove platelets.

·To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

·Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.

·Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

·they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low-speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

·Frozen specimens must be completely thawed before mixing.

·Mix thawed specimens thoroughly by low-speed vortex or by inverting 10 times.

·Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.

·If specimens are not mixed thoroughly, inconsistent results may be obtained.

·Recentrifuge specimens.

Recentrifugation of Specimens

·Transfer specimens to a centrifuge tube and centrifuge.

·Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Collection Vessel	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature (20 to 25°C)	Serum tubes Serum separator tubes Lithium heparin plasma tubes Lithium heparin separator tubes Sodium heparin tubes Dipotassium EDTA tubes	24 hours	
	2 to 8°C	Serum tubes Serum separator tubes Lithium heparin plasma tubes Lithium heparin separator tubes Sodium heparin tubes Dipotassium EDTA tubes	3 days	
	-20°C	Serum tubes Serum separator	30 days[11]	Avoid multiple freeze/thaw cycles.[11]

Specimen Type	Temperature	Collection Vessel	Maximum Storage Time	Special Instructions
		tubes Lithium heparin plasma tubes Lithium heparin separator tubes Sodium heparin tubes Dipotassium EDTA tubes		
Urine	Room temperature (20 to 25°C)	Clean glass or plastic container	4 days	Acidify to pH < 5
	2 to 8°C	Clean glass or plastic container	7 days	Acidify to pH < 5
	-20°C	Clean glass or plastic container	1 month	Acidify to pH < 5 Avoid multiple freeze/thaw cycles.

For additional information on sample handling and processing, refer to CLSI GP44-A4.[12] The storage information provided here is based on references or data maintained by the manufacturer.

Stored specimens must be inspected for particulates. If present, mix with a low-speed vortex or by inversion and centrifuge the specimen to remove particulates prior to testing.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

04U03 Phosphorus2 Reagent Kit

Materials Required but not Provided

·Phosphorus2 assay file

·04V6201 Consolidated Chemistry Calibrator

·Controls containing phosphorus

·Saline (0.85% to 0.90% NaCl) for specimen dilution

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

·If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.

·Minimum sample cup volume is calculated by the system and printed on the Order List report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Minimum sample volume requirements:

·Sample volume for single test: 3.6 µL (serum/plasma); 16.0 µL (urine).

NOTE: This amount does not include the dead volume plus the additional over-aspiration volume. **For total sample volume requirements, refer to the Alinity ci-series Operations Manual, Section 4.**

·Refer to the Consolidated Chemistry Calibrator package insert REF 04V6201 and/or commercially available control material package insert for preparation and usage.

·For general operating procedures, **refer to the Alinity ci-series Operations Manual, Section 5.**

·For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, **Section 9. Perform maintenance more frequently when required by laboratory procedures.**

Sample Dilution Procedures

Serum/Plasma

The standard dilution factor, applied automatically by the system software to all results for the Phosphorus2 assay, is 1:1.73. Samples with a phosphorus value exceeding 25.3 mg/dL (8.17 mmol/L) are flagged with the code "> 25.3 mg/dL" ("> 8.17 mmol/L"). Automated or manual sample dilutions have not been evaluated for the Phosphorus2 assay.

Urine

The standard dilution factor, applied automatically by the system software to all results for the Phosphorus2 assay, is 1:17.33. Samples with a phosphorus value exceeding 186.2 mg/dL (60.14 mmol/L) are flagged with the code "> 186.2 mg/dL" ("> 60.14 mmol/L"). Automated or manual sample dilutions have not been evaluated for the Phosphorus2 assay.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Calibration is stable for approximately **14 days (336 hours)** but is required with each change in reagent cartridge. Verify calibration with at least 2 levels of controls according to the laboratory quality procedure. If control results fall outside acceptable ranges, recalibration may be necessary.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

- At least 2 levels of controls (low and high) are to be run every day testing performed.
- Run all levels of control with each cartridge change.
- If quality control results do not meet the acceptance criteria defined by laboratory quality controls procedure, sample results may be suspect. Follow the laboratory quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to “Basic QC Practices” by James O. Westgard, Ph.D. for guidance on laboratory quality control practices.[13]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Phosphorus2 (Phos2) assay utilizes the Linear data reduction method to generate a calibration and results for both the serum/plasma and urine applications.

Urine sample quantification (Phos2-U) is achieved using the calibration generated with the Phosphorus2 (Phos2) assay parameter file.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

Reportable Interval

Based on representative data for the limit of quantitation (LoQ) and the limit of detection (LoD), the ranges over which results can be reported are provided below according to the definitions from CLSI EP34, 1st ed.*14*

Serum

	mg/dL	**mmol/L**
Analytical Measuring Interval (AMI)[a]	**0.5 - 25.3**	0.16 - 8.17
Reportable Interval[b]	0.2 - 25.3	0.06 - 8.17

[a] AMI: The AMI extends from the LoQ to the upper limit of quantitation (ULoQ). This is determined by the range of values in mg/dL (mmol/L) that demonstrated acceptable performance for linearity, imprecision, and bias.

[b]The reportable interval extends from the LoD to the upper limit of the AMI.

NOTE: The default Low Linearity value of the assay file corresponds to the lower limit of the AMI. Samples with a phosphorus value below 0.5 mg/dL (0.16 mmol/L) are reported as "< 0.5 mg/dL" ("< 0.16 mmol/L").

Urine

	mg/dL	mmol/L
Analytical Measuring Interval (AMI)[a]	2.4 - 186.2	0.78 - 60.14
Reportable Interval[b]	1.0 - 186.2	0.32 - 60.14

[a] AMI: The AMI extends from the LoQ to the upper limit of quantitation (ULoQ). This is determined by the range of values in mg/dL (mmol/L) that demonstrated acceptable performance for linearity, imprecision, and bias.

[b]The reportable interval extends from the LoD to the upper limit of the AMI.

NOTE: The default Low Linearity value of the assay file corresponds to the lower limit of the AMI. Samples with a phosphorus value below 2.4 mg/dL (0.78 mmol/L) are reported as "< 2.4 mg/dL" ("< 0.78 mmol/L").

LIMITATIONS OF THE PROCEDURE

·Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.

·Substances that demonstrated interference with the Phosphorus2 assay are listed in the SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity, Interference section of this package insert.

·Potential interference has not been evaluated for substances other than those described in the SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity, Interference section of this package insert.

·In very rare cases, gammopathy may cause unreliable results.*15*

EXPECTED VALUES

Manufacturer provided reference ranges will be adopted. Efforts will be made to verify in house

Reference Range (Serum)

Category	Range (mg/dL)	Range (mmol/L)
Children*3*	4.0-7.0	1.29-2.26
Adults**3*	2.5-4.5	0.81-1.45

For Pediatrics specific reference ranges, Canadian Caliber Studies

(https://caliperdatabase.org) will use both, Kit insert and CALIPER

* Adult **plasma** reference intervals are approximately 0.2 mg/dL (0.06 mmol/L) lower than for serum.*16*

NOTE: The system will flag results based only on the serum reference range in the assay file.

Reference Range (Urine)*17*

Category	Range	Range
24 Hour (adults)	0.4-1.3 g/day	12.9-42.0 mmol/day
Random (adult males)	5-189 mg/dL	1.6-61 mmol/L
Random (adult females)	7-148 mg/dL	2.3-48 mmol/L

24-Hour Urinary Excretion

To convert results from mg/dL to g/day (24-hour urinary excretion)

24-hour excretion = [(V × c) ÷ 100 000] g/day

Where:

V = 24-hour urine volume (mL)

c = analyte concentration (mg/dL)

To convert results from mmol/L to mmol/day (24-hour urinary excretion)

24-hour excretion = [(V × c) ÷ 1000] mmol/day

Where:

V = 24-hour urine volume (mL)

c = analyte concentration (mmol/L)

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity c system and the ARCHITECT c System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity c system.

Precision

Within-Laboratory Precision

Serum

A study was performed based on guidance from CLSI EP05-A3.[18] Testing was conducted using 3 lots of the Phosphorus2 reagents, 3 lots of the Consolidated Chemistry Calibrator, 1 lot of commercially available controls, and 1 instrument. Two controls and 3 human serum panels were tested in a minimum of 2 replicates, twice per day on 20 days on 3 reagent lot/calibrator lot combinations, where a unique reagent lot and a unique calibrator lot are paired. The performance from a representative combination is shown in the following table.

			Within-Run (Repeatability)		Within-Laboratory[a]	
Sample	**n**	**Mean (mg/dL)**	**SD**	**%CV**	**SD (Range[b])**	**%CV (Range[b])**
Control Level 1	80	3.4	0.03	0.8	0.03 (0.03-0.05)	1.0 (1.0-1.4)
Control Level 2	80	7.4	0.03	0.3	0.06 (0.06-0.06)	0.8 (0.8-0.8)
Panel A	80	1.1	0.02	2.0	0.03 (0.02-0.05)	2.5 (1.8-4.2)
Panel B	80	8.9	0.04	0.5	0.06 (0.06-0.06)	0.7 (0.7-0.7)
Panel C	80	22.8	0.13	0.6	0.16 (0.14-0.19)	0.7 (0.6-0.8)

[a]Includes within-run, between-run, and between-day variability.

[b] Minimum and maximum SD or %CV across the 3 reagent lot/calibrator lot combinations.

			Within-Run (Repeatability)		Within-Laboratory[a]	
Sample	**n**	**Mean (mmol/L)**	**SD**	**%CV**	**SD (Range[b])**	**%CV (Range[b])**
Control Level 1	80	1.10	0.006	0.5	0.009 (0.009-0.009)	0.8 (0.8-0.8)

Sample	n	Mean (mmol/L)	Within-Run (Repeatability) SD	Within-Run (Repeatability) %CV	Within-Laboratory[a] SD (Range[b])	Within-Laboratory[a] %CV (Range[b])
Control Level 2	80	2.38	0.008	0.3	0.019 (0.015-0.019)	0.8 (0.6-0.8)
Panel A	80	0.36	0.005	1.5	0.008 (0.007-0.008)	2.4 (2.1-2.4)
Panel B	80	2.88	0.009	0.3	0.021 (0.017-0.021)	0.7 (0.6-0.7)
Panel C	80	7.37	0.044	0.6	0.053 (0.046-0.061)	0.7 (0.6-0.8)

[a]Includes within-run, between-run, and between-day variability.

[b] Minimum and maximum SD or %CV across the 3 reagent lot/calibrator lot combinations.

Urine

A study was performed based on guidance from CLSI EP05-A3.*18* Testing was conducted using 3 lots of the Phosphorus2 reagents, 3 lots of the Consolidated Chemistry Calibrator, 1 lot of commercially available controls, and 1 instrument. Two controls and 3 urine panels were tested in a minimum of 2 replicates, twice per day on 20 days on 3 reagent lot/calibrator lot combinations, where a unique reagent lot and a unique calibrator lot are paired. The performance from a representative combination is shown in the following table.

Sample	n	Mean (mg/dL)	Within-Run (Repeatability) SD	Within-Run (Repeatability) %CV	Within-Laboratory[a] SD (Range[b])	Within-Laboratory[a] %CV (Range[b])
Control Level 1	80	24.8	0.12	0.5	0.24 (0.24-0.26)	0.9 (0.9-1.0)
Control Level 2	80	48.1	0.26	0.5	0.31 (0.26-0.41)	0.7 (0.5-0.8)
Panel A	80	5.2	0.08	1.5	0.21	4.1

Sample	n	Mean (mg/dL)	Within-Run (Repeatability) SD	Within-Run (Repeatability) %CV	Within-Laboratory[a] SD (Range[b])	Within-Laboratory[a] %CV (Range[b])
					(0.18-0.21)	(3.4-4.1)
Panel B	80	92.2	0.63	0.7	1.09 (1.09-2.15)	1.2 (1.2-2.3)
Panel C	80	161.3	0.93	0.6	1.11 (1.11-1.78)	0.7 (0.7-1.1)

[a]Includes within-run, between-run, and between-day variability.

[b] Minimum and maximum SD or %CV across the 3 reagent lot/calibrator lot combinations.

Sample	n	Mean (mmol/L)	Within-Run (Repeatability) SD	Within-Run (Repeatability) %CV	Within-Laboratory[a] SD (Range[b])	Within-Laboratory[a] %CV (Range[b])
Control Level 1	80	8.02	0.037	0.5	0.076 (0.076-0.082)	0.9 (0.9-1.0)
Control Level 2	80	15.52	0.083	0.5	0.099 (0.085-0.132)	0.6 (0.5-0.8)
Panel A	80	1.66	0.026	1.6	0.066 (0.057-0.066)	4.0 (3.4-4.0)
Panel B	80	29.78	0.202	0.7	0.351 (0.351-0.695)	1.2 (1.2-2.3)
Panel C	80	52.10	0.301	0.6	0.360 (0.360-0.574)	0.7 (0.7-1.1)

[a]Includes within-run, between-run, and between-day variability.

[b] Minimum and maximum SD or %CV across the 3 reagent lot/calibrator lot combinations.

Reproducibility

Serum

A study was performed based on guidance from CLSI EP05-A3.[18] Testing was conducted using 1 lot of the Phosphorus2 reagents, 1 lot of the Consolidated Chemistry Calibrator, 1 lot of commercially available controls, and 3 instruments. Each instrument was operated by a different technician, and each technician prepared an individual sample set. Two controls and 3 human serum panels were tested in a minimum of 3 replicates at 2 separate times per day on 5 different days.

			Repeatability		Within-Laboratory[a]		Reproducibility[b]	
Sample	**n**	**Mean (mg/dL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	90	3.4	0.02	0.5	0.02	0.7	0.02	0.7
Control Level 2	90	7.4	0.03	0.4	0.04	0.5	0.04	0.5
Panel A	90	1.1	0.02	1.4	0.02	1.6	0.02	1.7
Panel B	90	8.9	0.03	0.4	0.04	0.4	0.05	0.5
Panel C	90	22.8	0.06	0.3	0.15	0.6	0.15	0.7

[a]Includes repeatability (within-run), between-run, and between-day variability.

[b]Includes repeatability (within-run), between-run, between-day, and between-instrument variability.

			Repeatability		Within-Laboratory[a]		Reproducibility[b]	
Sample	**n**	**Mean (mmol/L)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	90	1.11	0.006	0.5	0.008	0.7	0.008	0.7
Control Level 2	90	2.39	0.010	0.4	0.012	0.5	0.013	0.5
Panel A	90	0.35	0.006	1.6	0.006	1.7	0.006	1.8
Panel B	90	2.88	0.010	0.4	0.011	0.4	0.016	0.5
Panel C	90	7.36	0.020	0.3	0.047	0.6	0.048	0.7

[a]Includes repeatability (within-run), between-run, and between-day variability.

[b]Includes repeatability (within-run), between-run, between-day, and between-instrument variability.

Urine

A study was performed based on guidance from CLSI EP05-A3.[18] Testing was conducted using 1 lot of the Phosphorus2 reagents, 1 lot of the Consolidated Chemistry Calibrator, 1 lot of commercially available controls, and 3 instruments. Each instrument was operated by a different technician, and each technician prepared an individual sample set. Two controls and 3 urine panels were tested in a minimum of 3 replicates at 2 separate times per day on 5 different days.

			Repeatability		Within-Laboratory[a]		Reproducibility[b]	
Sample	**n**	**Mean (mg/dL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	90	25.6	0.25	1.0	0.26	1.0	0.39	1.5
Control Level 2	90	48.7	0.35	0.7	0.37	0.8	0.59	1.2
Panel A	90	4.8	0.16	3.2	0.18	3.8	0.25	5.2
Panel B	90	91.4	0.49	0.5	0.53	0.6	1.38	1.5
Panel C	90	160.0	0.74	0.5	0.91	0.6	2.51	1.6

[a]Includes repeatability (within-run), between-run, and between-day variability.

[b]Includes repeatability (within-run), between-run, between-day, and between-instrument variability.

			Repeatability		Within-Laboratory[a]		Reproducibility[b]	
Sample	**n**	**Mean (mmol/L)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	90	8.26	0.080	1.0	0.085	1.0	0.125	1.5
Control Level 2	90	15.72	0.113	0.7	0.121	0.8	0.189	1.2

Sample	n	Mean (mmol/L)	Repeatability		Within-Laboratory[a]		Reproducibility[b]	
			SD	%CV	SD	%CV	SD	%CV
Panel A	90	1.56	0.050	3.2	0.059	3.8	0.080	5.1
Panel B	90	29.52	0.158	0.5	0.174	0.6	0.446	1.5
Panel C	90	51.67	0.237	0.5	0.294	0.6	0.809	1.6

[a]Includes repeatability (within-run), between-run, and between-day variability.

[b]Includes repeatability (within-run), between-run, between-day, and between-instrument variability.

Accuracy

A study was performed to estimate the bias of the Phosphorus2 assay relative to standard reference material **(NIST SRM 2186I Standard)**. Testing was conducted using 3 concentrations of the standard across 1 lot of the Phosphorus2 reagents, 1 lot of the Consolidated Chemistry Calibrator, and 1 instrument. The bias ranged from -0.2% to 0.0% for serum and -5.1% to -0.1% for urine.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.*19* Testing was conducted using 3 lots of the Phosphorus2 reagents on each of 2 instruments over a minimum of 3 days. The maximum observed limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) values are summarized below.

Serum

	mg/dL	mmol/L
LoB[a]	0.1	0.03
LoD[b]	0.2	0.06
LoQ[c]	**0.5**	0.16

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met and was determined from n ≥ 60 replicates of low-analyte level samples.

Urine

	mg/dL	mmol/L
LoB[a]	0.5	0.16
LoD[b]	1.0	0.32
LoQ[c]	2.4	0.78

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ presented in the table is in alignment with the LoQ for the Phosphorus2 assay on the ARCHITECT c System. The observed LoQ on the Alinity c system was 2.2 mg/dL (0.71 mmol/L). This LoQ is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met and was determined from n ≥ 60 replicates of low-analyte level samples.

Linearity

A study was performed based on guidance from CLSI EP06-A.*20*

This assay is linear across the analytical measuring interval of **0.5 to 25.3** mg/dL (0.16 to 8.17 mmol/L) for serum, and 2.4 to 186.2 mg/dL (0.78 to 60.14 mmol/L) for urine.

Analytical Specificity

Interference

These studies were performed on the ARCHITECT c System.

Serum

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07, 3rd ed.*21* Each substance was tested at 2 levels of the analyte (approximately 3.5 mg/dL and 5.0 mg/dL).

No significant interference (interference within ± 10%) was observed at the following concentrations.

No Significant Interference (Interference within ± 10%)		
	Interferent Level	
Potentially Interfering Substance	**Default Units**	**Alternate Units**

No Significant Interference (Interference within ± 10%)		
	Interferent Level	
Potentially Interfering Substance	**Default Units**	**Alternate Units**
Bilirubin – conjugated	30 mg/dL	356 µmol/L
Bilirubin – unconjugated	60 mg/dL	1026 µmol/L
Hemoglobin	125 mg/dL	1.25 g/L
Total protein	15 g/dL	150 g/L
Triglycerides	750 mg/dL	8.47 mmol/L

Interference beyond ± 10% (based on 95% Confidence Interval [CI]) was observed at the concentrations shown below for the following substances.

Interference beyond ± 10% (based on 95% Confidence Interval [CI])					
Potentially Interfering Substance	**Interferent Level**		**Analyte Level**		**% Interference (95% CI)**
	Default Units	**Alternate Units**	**Default Units**	**Alternate Units**	
Bilirubin - conjugated	40 mg/dL	474 µmol/L	3.5 mg/dL	1.13 mmol/L	-12% (-13%, -11%)
Hemoglobin	250 mg/dL	2.50 g/L	3.5 mg/dL	1.13 mmol/L	14% (14%, 14%)
Triglycerides	1500 mg/dL	16.9 mmol/L	3.5 mg/dL	1.13 mmol/L	15% (14%, 16%)
Triglycerides	1500 mg/dL	16.9 mmol/L	5.0 mg/dL	1.62 mmol/L	10% (9%, 11%)

Potentially Interfering Exogenous Substances

A study was performed based on guidance from CLSI EP07, 3rd ed.[21] Each substance was tested at 2 levels of the analyte (approximately 3.5 mg/dL and 5.0 mg/dL).

No significant interference (interference within ± 10%) was observed at the following concentrations.

No Significant Interference (Interference within ± 10%)	
Potentially Interfering Substance	**Interferent Level**

	Default Units	Alternate Units
Acetaminophen	160 mg/L	1059 µmol/L
Acetylcysteine	150 mg/L	920 µmol/L
Acetylsalicylic acid	30 mg/L	167 µmol/L
Aminosalicylic acid (p-aminosalicylic acid)	47 mg/dL	3074 µmol/L
Amiodarone	5 mg/dL	77.5 µmol/L
Ampicillin-Na	80 mg/L	215 µmol/L
Ascorbic acid	60 mg/L	341 µmol/L
Biotin	4250 ng/mL	17.4 µmol/L
Ca-dobesilate	60 mg/L	143 µmol/L
Cefotaxime	53 mg/dL	1166 µmol/L
Cefoxitin	6600 mg/L	15.4 mmol/L
Cyclosporine	2 mg/L	1.66 µmol/L
Desacetylcefotaxime	6 mg/dL	145 µmol/L
Doxycycline	20 mg/L	45.0 µmol/L
Ibuprofen	220 mg/L	1067 µmol/L
Levodopa	8 mg/L	40.6 µmol/L
Mannitol	4 g/L	22.0 mmol/L
Methicillin (sodium)	8 mg/dL	198 µmol/L
Methotrexate	140 mg/dL	3080 µmol/L
Methyldopa	25 mg/L	118 µmol/L
Metronidazole	130 mg/L	759 µmol/L
Naproxen	36 mg/dL	1562 µmol/L
Nitroglycerin	0.015 mg/L	0.0660 µmol/L
Omeprazole	0.9 mg/dL	26.1 µmol/L
Phenylbutazone	330 mg/L	1069 µmol/L
Primidone	6 mg/dL	275 µmol/L
Promethazine	0.03 mg/dL	1.06 µmol/L

No Significant Interference (Interference within ± 10%)		
	Interferent Level	
Potentially Interfering Substance	**Default Units**	**Alternate Units**
Ranitidine	2 mg/dL	63.6 µmol/L
Rifampicin	50 mg/L	61.0 µmol/L
Sodium heparin	4 U/mL	NA
Theophylline (1,3-dimethylxanthine)	60 mg/L	333 µmol/L

NA = Not applicable

Interference beyond ± 10% (based on 95% Confidence Interval [CI]) was observed at the concentrations shown below for the following substances.

Interference beyond ± 10% (based on 95% Confidence Interval [CI])					
Potentially Interfering Substance	**Interferent Level**		**Analyte Level**		**% Interference (95% CI)**
	Default Units	**Alternate Units**	**Default Units**	**Alternate Units**	
Mannitol	5 g/L	27.5 mmol/L	3.5 mg/dL	1.13 mmol/L	-11% (-11%, -10%)
Mannitol	10 g/L	54.9 mmol/L	5.0 mg/dL	1.62 mmol/L	-10% (-11%, -10%)

Urine

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07, 3rd ed.[21] Each substance was tested at 2 levels of the analyte (approximately 30 mg/dL and 90 mg/dL).

No significant interference (interference within ± 10%) was observed at the following concentrations.

No Significant Interference (Interference within ± 10%)		
	Interferent Level	
Potentially Interfering Substance	**Default Units**	**Alternate Units**

No Significant Interference (Interference within ± 10%)		
	Interferent Level	
Potentially Interfering Substance	**Default Units**	**Alternate Units**
Acetic acid (8.5N)	6.25 mL/dL	531 mmol/L
Ascorbate	220 mg/dL	12.6 mmol/L
Boric acid	250 mg/dL	40.5 mmol/L
Glucose	1000 mg/dL	55.5 mmol/L
Hydrochloric acid (6N)	2.5 mL/dL	150 mmol/L
Nitric acid (6N)	5.0 mL/dL	300 mmol/L
Protein	50 mg/dL	0.500 g/L
Sodium carbonate	1.25 g/dL	118 mmol/L
Sodium fluoride	400 mg/dL	95.2 mmol/L
Sodium oxalate	60 mg/dL	4476 µmol/L

Potentially Interfering Exogenous Substances

A study was performed based on guidance from CLSI EP07, 3rd ed.[21] Each substance was tested at 2 levels of the analyte (approximately 30 mg/dL and 90 mg/dL).

No significant interference (interference within ± 10%) was observed at the following concentrations.

No Significant Interference (Interference within ± 10%)		
	Interferent Level	
Potentially Interfering Substance	**Default Units**	**Alternate Units**
Acetaminophen	16 mg/dL	1059 µmol/L
Acetylcysteine	15 mg/dL	920 µmol/L
Biotin	4250 ng/mL	17.4 µmol/L
Ibuprofen	22 mg/dL	1067 µmol/L
Mannitol	18 g/L	98.8 mmol/L

Interferences from medication or endogenous substances may affect results.[22]

Method Comparison

A study was performed based on guidance from CLSI EP09c, 3rd ed[23] using the Passing-Bablok regression method.

Phosphorus2 on the Alinity c system vs. Phosphorus on the ARCHITECT c System						
	n	**Units**	**Correlation Coefficient**	**Intercept**	**Slope**	**Concentration Range**
Serum	108	mg/dL	1.00	0.0	1.05	1.0-22.8
		(mmol/L)				(0.34-7.36)
Urine	110	mg/dL	1.00	-0.6	1.05	6.2-167.9
		(mmol/L)		(-0.21)		(2.00-54.22)

Phosphorus2 on the Alinity c system vs. Phosphorus2 on the ARCHITECT c System						
	n	**Units**	**Correlation Coefficient**	**Intercept**	**Slope**	**Concentration Range**
Serum	108	mg/dL	1.00	0.0	1.00	1.1-23.7
		(mmol/L)		(-0.02)	(1.01)	(0.36-7.67)
Urine	111	mg/dL	1.00	-0.3	1.01	4.4-174.1
		(mmol/L)		(-0.08)		(1.42-56.23)

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