

For Veterinary use only
Customer and Technical Service 1-800-822-2947

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1. Intended Use

The VetScan[®] Avian/Reptilian Profile Plus reagent rotor used with the VetScan Whole Blood Analyzer utilizes dry and liquid reagents to provide *in vitro* quantitative determinations of aspartate Aminotransferase (AST), bile acids (BA), creatine kinase (CK), uric acid (UA), glucose (GLU), total calcium (CA⁺⁺), phosphorus (PHOS), total protein (TP), albumin (ALB), globulin* (GLOB), potassium (K⁺), sodium (Na⁺) in heparinized whole blood, heparinized plasma, or serum.¹

* Calculated Value

2. Summary and Explanation of Tests

NOTE: Samples should be run as “Other” species (animal type) when running the Avian/Reptilian Profile Plus Rotor. The albumin (ALB) method has specific calibration factors, which are stored in this key function. Please refer to the VetScan Operator’s Manual for additional information.

The VetScan Avian/Reptilian Profile Plus reagent rotor and the VetScan Whole Blood Analyzer comprise an *in vitro* diagnostic system that aids the veterinarian in diagnosing the following disorders:

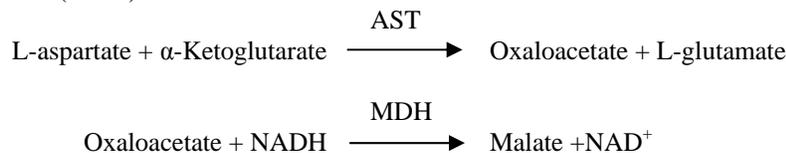
Aspartate Aminotransferase (AST)	Liver disease, muscle damage.
Bile Acids (BA)	Hepatobiliary disease; portosystemic vascular anomaly (PSVA); extrahepatic shunting.
Creatine Kinase (CK)	Muscle damage, used in conjunction with AST to differentiate between liver and muscle damage.
Uric Acid (UA)	Best indicator of renal health in almost all birds and reptiles.
Glucose (GLU)	Severe liver disease; sepsis; anorexia; pancreatic disease.
Phosphorous (PHOS)	Renal and nutritional disease; fluid balance.
Calcium (CA⁺⁺)	Egg production; bone and renal disease
Total Protein (TP)	Liver, gastrointestinal, and kidney disease; dehydration.
Albumin (ALB)	Liver and kidney disease.
Globulin (GLOB)	Globulin recovery is calculated from the TP and ALB. Dehydration; antigenic stimulation.
Potassium (K⁺)	Indicator of cell lysis, and fluid balance.
Sodium (Na⁺)	Indicator of fluid balance and dehydration.

As with any diagnostic test procedure, all other test procedures including the clinical status of the patient should be considered prior to final diagnosis.

3. Principles of Procedure

Aspartate Aminotransferase (AST)

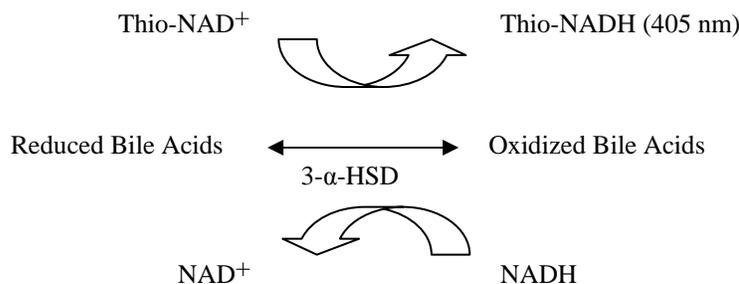
The Abaxis AST method is a modification of the IFCC reference method.^{3,4} This method catalyzes the reaction of L-aspartate and α -ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD^+ by the enzyme malate dehydrogenase (MDH).



The rate of absorbance change caused by the conversion of NADH to NAD^+ is determined bichromatically at 340 nm and 405 nm. This rate is directly proportional to the amount of AST present in the sample.

Bile Acids (BA)

In the presence of the thio-derivative of nicotinamide adenine dinucleotide (Thio- NAD^+) the enzyme 3- α -Hydroxysteroid Dehydrogenase (3- α -HSD) reversibly oxidizes bile acids to oxidized bile acids (3- α -keto forms) with the concomitant conversion of Thio- NAD^+ to its reduced form (Thio- NADH). In a cycling reaction, the oxidized bile acids are returned to their reduced state when excess NADH is present. The NADH is converted to NAD^+ . In the Abaxis system, Thio- NAD^+ , NADH, and 3- α -HSD are supplied as dry reagent beads. The cycling reaction amplifies the levels of bile acids from the sample. The rate of increase in absorbance at 405 nm (Thio- NADH) is measured and is proportional to the concentration of bile acids in the sample. The rate is measured bichromatically at 405 and 500 nm.

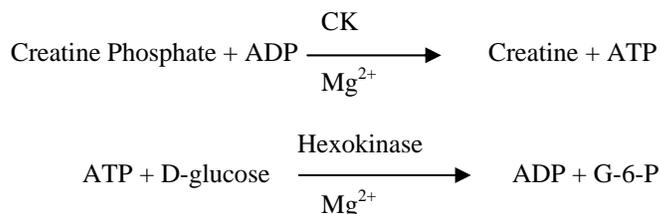


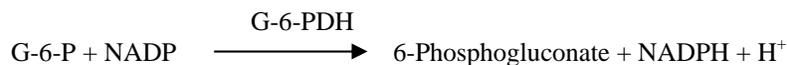
Creatine Kinase (CK)

Creatine kinase catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP).⁷

The CK measurement procedure used by Abaxis is a modified version of the IFCC.⁸ Key modifications are sample volume fraction, buffer and temperature. N-acetyl cysteine (NAC) has been added to reactivate the CK.⁹ Magnesium is used as a cofactor for both CK and hexokinase. EDTA has been added as a stabilizer for NAC and for the removal of various cations, such as calcium and iron, that inhibits CK. P^1 , P^5 -di(adenosine-5') pentaphosphate and adenosine monophosphate (AMP) have also been added to inhibit adenylate kinase, another skeletal muscle and erythrocyte enzyme that reacts with the substrates used to measure CK.

Creatine Kinase catalyzes the formation of creatine and adenosine triphosphate (ATP) from creatine phosphate P^1 , P^5 – di (adenosine 5') pentaphosphate (ADP) at pH 6.7. With hexokinase as a catalyst, ATP reacts with D-glucose to form ADP and D-glucose-6-phosphate (G-6-P), which is reacted with nicotinamide adenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase (G-6-PDH) to produce G-6-P and NADPH.

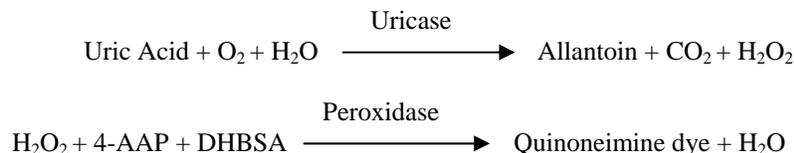




The formation of NADPH is measured as a change in absorbance at 340 nm relative to 405 nm. This absorbance change is directly proportional to creatine kinase activity in the sample.

Uric Acid (UA)

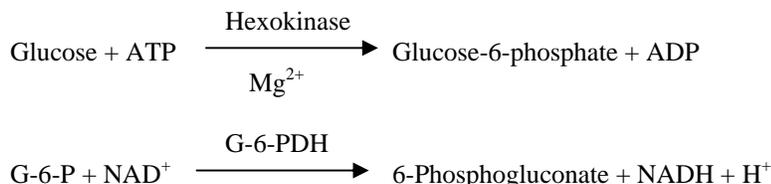
The standard clinical chemistry technique for this assay is a uric acid-specific enzyme uricase.²⁴ The uricase method is coupled through a Trinder finish.²⁵ In this method, uricase catalyzes the oxidation of uric acid to allantoin and hydrogen peroxide. Peroxidase catalyzes the reaction among hydrogen peroxide (H₂O₂), 4-aminotantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBSA) into a red quinoneimine dye. Sodium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimize the potential interference of bilirubin and ascorbic acid.



The amount of uric acid in the sample is directly proportional to the absorbance of the quinoneimine dye. The final absorbance of this endpoint reaction is measured bichromatically at 515 nm and 600 nm.

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu¹⁰ and Somogyi-Nelson^{11,12}). The lack of specificity in copper-reduction techniques led to the development of quantitative procedures using the enzymes hexokinase and glucose oxidase. The glucose test incorporated into the Avian/Reptilian Reagent Rotor is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.¹³ The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine dinucleotide (NAD⁺) to NADH.



Total Calcium (CA⁺⁺)

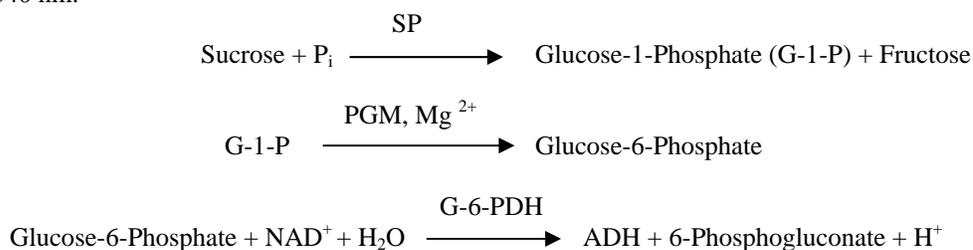
Calcium in the patient sample binds with arsenazo III to form a calcium-dye complex.^{5,6}



The endpoint reaction is monitored at 405 nm, 467 nm, and 600 nm. The amount of calcium in the sample is proportional to the absorbance.

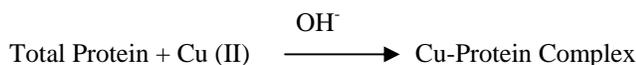
Phosphorus (PHOS)

The most applicable enzymatic method for the Abaxis system uses sucrose phosphorylase (SP) coupled through phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G-6-PDH).^{14,15} Using the enzymatic system for each mole of inorganic phosphorus present in the sample, one mole of NADH is formed. The amount of NADH formed is measured as an endpoint at 340 nm.



Total Protein (TP)

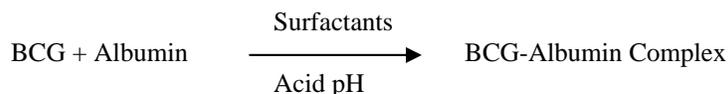
In the biuret reaction, the protein solution is treated with cupric [Cu(II)] ions in a strong alkaline medium. Sodium potassium tartate and potassium iodide are added to prevent the precipitation of copper hydroxide and the auto-reduction of copper, respectively.²² The Cu (II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-Protein complex.



The amount of total protein present in the sample is directly proportional to the absorbance of the Cu-protein complex. The total protein test is an endpoint reaction

Albumin (ALB)

Dye binding techniques are the most frequently used methods for measuring albumin. Bromocresol green (BCG) is the most commonly used of the dye binding methods but may over-estimate albumin concentration, especially at the low end of the normal range.²

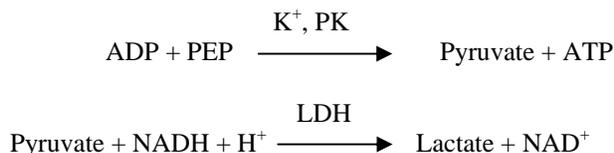


Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured bichromatically at 630 nm and 405 nm.

Potassium (K⁺)

Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. The Abaxis enzymatic method is based on the activation of pyruvate kinase (PK) with potassium and shows excellent linearity and negligible susceptibility to endogenous substances.^{16, 17, 18} Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamate dehydrogenase respectively.¹⁸

In the coupled-enzyme reaction, PK dephosphorylates phosphoenolpyruvate (PEP) to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD⁺. The rate of change in absorbance due to the conversion of NADH to NAD⁺ is directly proportional to the amount of potassium in the sample.



Sodium (Na⁺)

Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.^{19, 20, 21} In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol and galactose



and the absorbance is measured as the difference in absorbance between 550 nm and 850 nm.

4. Principle of Operation

See the VetScan Chemistry Analyzer Operator's Manual, for the Principles and Limitations of the Procedure.

5. Description of Reagents

Reagents

Each VetScan Avian/Reptilian Profile Plus reagent rotor contains dry test specific reagent beads. A dry sample blank reagent (comprised of buffer, surfactants, excipients and preservatives) is included in each reagent rotor for use in calculating concentrations of albumin, alanine aminotransferase, calcium, creatine kinase, glucose, potassium, sodium, and urea nitrogen. A dedicated sample blank is included in the rotor to calculate the concentration of total protein levels. Each reagent rotor also contains a diluent consisting of surfactants and preservatives.

Warnings and Precautions

- For *In vitro* Diagnostic Use
- The diluent container in the reagent rotor is automatically opened when the analyzer drawer closes. A rotor with an opened diluent container cannot be re-used. Ensure that the sample or control has been placed into the rotor before closing the drawer.
- Reagent beads may contain acids or caustic substances. The operator does not come into contact with the reagent beads when following the recommended procedures. In the event that the beads are handled (e.g., cleaning up after dropping and cracking a reagent rotor), avoid ingestion, skin contact, or inhalation of the reagent beads.
- Reagent beads and diluent contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Reagents will not come into contact with lead and copper plumbing when following recommended procedures. However, if the reagents do come into contact with such plumbing, flush with a large volume of water to prevent azide buildup.

Instructions for Reagent Handling

Reagent rotors may be used directly from the refrigerator without warming. Open the sealed foil pouch and remove the rotor being careful not to touch the bar code ring located on the top of the reagent rotor. Use according to the instructions provided in the VetScan System Operator's Manual. A rotor not used within 20 minutes of opening the pouch should be discarded. Rotors in opened pouches cannot be placed back in the refrigerator for use at a later time.

Storage

Store reagent rotors in their sealed pouches at 2-8°C (36-46°F). Do not expose opened or unopened rotors to direct sunlight or temperatures above 32°C (90°F). Do not allow the rotors sealed in their foil pouches to remain at room temperature longer than 48 hours prior to use. Open the pouch and remove the rotor just prior to use.

Indications of Reagent Rotor Instability or Deterioration

- All reagents contained in the reagent rotor, when stored as described above, are stable until the expiration date printed on the rotor pouch. Do **not** use a rotor after the expiration date. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on the VetScan Whole Blood Analyzer display if the reagents have expired.

Indications of Reagent Rotor Instability or Deterioration Continued

- A torn or otherwise damaged pouch may allow moisture to reach the unused rotor and adversely affect reagent performance. Do not use a rotor from a damaged pouch.

6. Instrument

See the VetScan System Operator's Manual for complete information on using the analyzer.

7. Sample Collection and Preparation

The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma, serum or serum control. The reagent rotor sample chamber can contain up to 120 µL of sample.

- Specimen collected in a heparinized micropipette should be dispensed into the reagent rotor **immediately** following sample collection.
- Use only lithium heparin (green stopper) evacuated specimen collection tubes for whole blood or plasma samples. Use no-additive (red stopper) evacuated specimen collection tubes or serum separator tubes (red or red/black stopper) for serum samples.
- Whole blood samples obtained by venipuncture must be homogenous before transferring a sample to the reagent rotor. Gently invert the collection tubes several times just prior to sample transfer. Do **not** shake the collection tube. Shaking can cause hemolysis.
- The test must be started within 10 minutes of transferring the sample into the reagent rotor.

- Whole blood venipuncture samples should be run within 60 minutes of collection; if this is not possible, separate the sample and transfer it into a clean test tube.²⁶ Run the separated plasma or serum sample within 5 hours of centrifugation. If this is not possible, refrigerate the sample in a stoppered test tube at 2-8°C (36-46°F) for no longer than 48 hours. A plasma or serum sample can be stored at -10°C (14°F) for up to 5 weeks in a freezer that does not have a self-defrost cycle.

Known Interfering Substances

- The only anticoagulant recommended for use with the VetScan Whole Blood Analyzer is lithium heparin.
- Physical interferents (hemolysis, icterus, and lipemia) may cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each result card to inform the operator about the levels of interferents present in each sample. The VetScan Whole Blood Analyzer suppresses any results that are affected by >10% interference from hemolysis, lipemia, or icterus. “HEM”, “LIP”, “ICT” is printed on the result card in place of the result.
- Creatine kinase is inactivated both by bright daylight and by increasing specimen pH owing to loss of carbon dioxide. Specimens should be stored in the dark in tightly closed tubes accordingly.²⁷
- Glucose concentrations are affected by the length of time since the patient has eaten and by the type of sample collected from the patient. To accurately interpret glucose results, samples should be obtained from a patient that has been fasted for at least 12 hours.²⁸
- Bile Acids concentrations can be affected by the length of time since the patient has eaten, however pre- and post-prandial measurements in birds can be challenging due to variability in storage and ingestion of crop contents.
- The potassium assay in the VetScan system is a coupled pyruvate kinase (PK) / lactate dehydrogenase (LDH) assay. Therefore, in cases of extreme muscle trauma or highly elevated levels of creatine kinase (CK), the VetScan may recover a falsely elevated potassium (K+) value. In such cases, unexpected high potassium recoveries need to be confirmed utilizing a different methodology.

8. Procedure

Materials Provided

- One VetScan Avian/Reptilian Profile Plus Reagent Rotor PN: 500-1041 (a box of 12 rotors PN: 500-0041-12)

Materials Required but not Provided

- VetScan Whole Blood Chemistry Analyzer

Test Parameters

The VetScan System operates at ambient temperatures between 15°C and 32°C (59-92°F). The analysis time for each VetScan Avian/Reptilian Profile Plus Reagent Rotor is less than 14 minutes. The analyzer maintains the reagent rotor at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure

The complete sample collection and step-by-step operating procedures are detailed in the VetScan System Operator’s Manual.

Calibration

The VetScan Whole Blood Analyzer is calibrated by the manufacturer before shipment. The barcode printed on the barcode ring provides the analyzer with rotor-specific calibration data. Please see the VetScan System Operator’s Manual.

Quality Control

Controls may be run periodically on the VetScan Whole Blood Analyzer to verify the accuracy of the analyzer. Abaxis recommends that a serum-based commercially available control be run. Run controls on the reagent rotor in the same manner as for patient samples. See the VetScan System Operator’s Manual to run controls.

9. Results

The VetScan Whole Blood Analyzer automatically calculates and prints the analyte concentrations in the sample. Details of the endpoint and rate reaction calculations are found in the VetScan System Operator’s Manual.

10. Limitations of Procedure

General procedural limitations are discussed in the VetScan Systems Operator’s Manual.

- **If a result for a particular test exceeds the assay range, the sample should be analyzed by another approved test method or sent to a referral laboratory. Do not dilute the sample and run it again on the VetScan Whole Blood Analyzer.**

- Samples with hematocrits in excess of 60% packed red cell volume may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down and the plasma then re-run in a new reagent rotor.
- Not all Avian/Reptilian species have been studied. As a result, unknown matrix effects are possible.
- The Avian/Reptilian Profile Plus reagent rotor has been designed for bird and reptile samples only.

Warning: Extensive testing of the VetScan has shown that in very rare instances, sample dispensed into the reagent rotor may not flow smoothly into the sample chamber. Due to the uneven flow, an inadequate quantity of sample may be analyzed and several results may fall outside your established reference ranges. The sample may be re-run using a new reagent rotor.

11. Expected Values

The most definitive normal ranges are those established for your patient population. Test results should be interpreted in conjunction with the patient's clinical signs. To customize specific normal ranges in your VetScan for the "Other" bank, refer to your VetScan System Operator's Manual under the Menu Key functions.

For literature references on normal ranges for birds and reptiles please refer to the following references:

- 1) Fudge A. Laboratory Medicine / Avian and Exotic Pets; Philadelphia, PA, W.B. Saunders Company: 2000.
- 2) Johnson-Delaney C. Exotic Companion Medicine Handbook, Volume I & II; Lake Worth, FL: 2000.
- 3) Altman B, et al. Avian Medicine and Surgery; Philadelphia, PA, W.B. Saunders Company: 1997.

12. Performance Characteristics

Linearity

The chemistry for each analyte is linear over the dynamic range listed below when the VetScan System is operated according to the recommended procedure (see the VetScan System Operator's Manual). The Dynamic Range table referenced below represents the spectrum that the VetScan System can detect. **The intervals below do not represent normal ranges.**

Table 1: VetScan Dynamic Ranges

Analyte	Dynamic Ranges	
	Common Units	SI Units
AST	5-2000 U/L	5-2000 U/L
BA	35 – 200 µmol/L	35 – 200 µmol/L
CK	5-14000 U/L	5-14000 U/L
UA	0.3-25.0 mg/dL	18-1488 µmol/L
GLU	10-700 mg/dL	0.6-38.9 mmol/L
CA ⁺⁺	4 -16 mg/dL	1.0-4.0 mmol/L
PHOS	0.2-20.0 mg/dL	0.06-6.46 mmol/L
TP	2-14 g/dL	20-140 g/L
ALB_BCG	1-6.5 g/dL	10-65 g/L
GLOB*	0-13.0 g/dL	0-130 g/L
K+	1.5-8.5 mmol/L	1.5-8.5 mmol/L
NA+	110-170 mmol/L	110-170 mmol/L

*Calculated Value

Precision

Precision studies were conducted using the NCCLS EP5-A²⁹ Guidelines with modifications based on NCCLS EP18-P³⁰ for unit-use devices. Results for within-run and total precision were determined by testing bi-level controls. Albumin, aspartate aminotransferase, calcium, creatine kinase, globulin, glucose, phosphorus, sodium, total protein, and uric acid determinations were performed at one site. Controls were run in duplicate twice each day over a four-week period; potassium was performed at two sites over 20 days. Bile Acids were determined at one site over 5 days.

Table 2: Precision

Analyte	Sample Size	Within-Run	Total
Albumin-BCG (g/dL)	n=80		
Control 1			
Mean		3.9	3.9
SD		0.13	0.14
%CV		3.3	3.6
Control 2			
Mean		2.3	2.3
SD		0.09	0.10
%CV		3.9	4.3
Aspartate Aminotransferase (U/L)	n=80		
Control 1			
Mean		47	47
SD		0.98	0.92
%CV		2.1	2.0
Control 2			
Mean		145	145
SD		1.83	1.70
%CV		1.3	1.2
Bile Acids (µmol/L)	n=40		
Control 1			
Mean		101.0	101.0
SD		4.40	4.60
%CV		4.4	4.5
Control 2			
Mean		172.4	172.4
SD		8.47	8.47
%CV		4.9	4.9
Calcium (mg/dL)	n=80		
Control 1			
Mean		8.6	8.6
SD		0.21	0.25
%CV		2.4	2.9
Control 2			
Mean		11.8	11.8
SD		0.39	0.40
%CV		3.3	3.4
Creatine Kinase (U/L)	n=120		
Control 1			
Mean		105	105
SD		2.89	3.74
%CV		2.8	3.6
Control 2			
Mean		469	469
SD		12.23	28.32
%CV		2.6	6.0

Table 2: Precision (Continued)

Analyte	Sample Size	Within-Run	Total
Globulin (g/dL)	n=80		
Control 1			
Mean		3.2	3.2
SD		0.13	0.14
%CV		4.1	4.4
Control 2			
Mean		2.0	2.0
SD		0.07	0.07
%CV		3.5	3.5
Glucose (mg/dL)	n=80		
Control 1			
Mean		66	66
SD		0.76	1.03
%CV		1.1	1.6
Control 2			
Mean		278	278
SD		2.47	3.84
%CV		0.9	1.4
Phosphorus (mg/dL)	n=80		
Control 1			
Mean		6.9	6.9
SD		0.15	0.18
%CV		2.2	2.6
Control 2			
Mean		3.4	3.4
SD		0.14	0.17
%CV		4.1	4.9
Potassium (mmol/L)			
Control 1	n=120		
Mean		6.12	6.12
SD		0.32	0.35
%CV		5.2	5.7
Control 2			
Mean		4.1	4.1
SD		0.24	0.26
%CV		5.9	6.3
Sodium (mmol/L)	n=80		
Control 1			
Mean		143.5	143.5
SD		2.28	2.28
%CV		1.6	1.6
Control 2			
Mean		120.0	120.0
SD		2.13	2.13
%CV		1.8	1.8
Total Protein (g/dL)	n=80		
Control 1			
Mean		6.8	6.8
SD		0.05	0.08
%CV		0.8	1.2

Table 2: Precision (Continued)

Analyte	Sample Size	Within-Run	Total
Control 2			
Mean		4.7	4.7
SD		0.09	0.09
%CV		1.9	1.9
Uric Acid (mg/dL)	n=80		
Control 1			
Mean		3.8	3.8
SD		0.15	0.18
%CV		4.0	4.8
Control 2			
Mean		7.5	7.5
SD		0.24	0.29
%CV		3.2	3.9

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