

For Veterinary use only

Customer and Technical Service 1-800-822-2947

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1. Intended Use

The VetScan[®] Large Animal Profile reagent rotor, used with the VetScan Whole Blood Analyzer, utilizes dry and liquid reagents to provide *in vitro* quantitative determinations of albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium (CA⁺⁺), creatine kinase (CK), gamma glutamyl transferase (GGT), globulin*(GLOB), magnesium (MG), inorganic phosphorus (PHOS), total protein (TP) and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.¹

* Calculated Value

2. Summary and Explanation of Tests

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

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

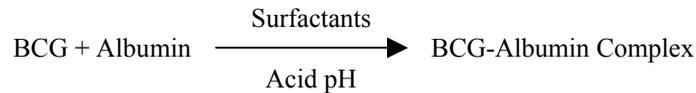
Albumin	Liver and kidney disease
Alkaline phosphatase	Liver, bone, parathyroid and intestinal diseases
Aspartate aminotransferase	Liver disease including hepatitis and viral jaundice; shock
Calcium	Parathyroid, bone and chronic renal diseases; tetany
Creatine Kinase	Myocardial infarction, progressive muscular dystrophy, dermatomyositis, convulsions, heart disease, hypothyroidism, severe exercise, intramuscular injection, physical inactivity, and decreased muscle mass
Gamma glutamyl transferase	Liver disease, primary and secondary liver tumors
Magnesium	Kidney disease and malnutrition
Phosphorus	Kidney disease, hypoparathyroidism and nutritional disorders
Total protein	Liver, kidney, bone marrow diseases; metabolic and nutritional disorders
Urea Nitrogen	Renal and metabolic diseases

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

Albumin (ALB)

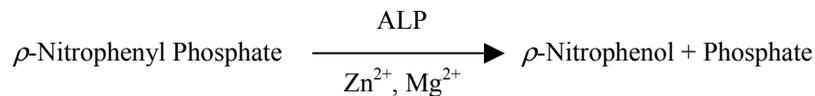
Dye binding techniques are the most frequently used methods for measuring albumin. Bromocresol green (BCG) is the most commonly used of the dye binding methods but may over-estimate albumin concentration, especially at the low end of the normal range.²



Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as the difference in absorbance between 600 nm and 550 nm.

Alkaline Phosphatase (ALP)

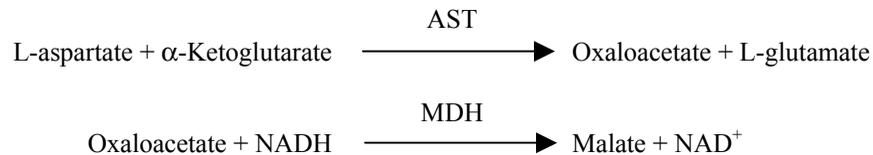
The Abaxis procedure is modified from the American Association of Clinical Chemistry (AACC)³ and the International Federation of Clinical Chemistry (IFCC)⁴ methods, which uses ρ -NPP as a substrate and a metal-ion buffer. In this reaction, ALP hydrolyzes ρ -NPP in a metal ion buffer and forms ρ -nitrophenol and phosphate.



The amount of ALP in the sample is proportional to the rate of increase in absorbance at 405 nm.

Aspartate Aminotransferase (AST)

The Abaxis AST method is a modification of the IFCC reference method.^{5,6} 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 catalyst MDH.



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

Calcium (Ca^{++})

Calcium in the patient sample binds with Arsenazo III to form a calcium-dye complex.^{7,8}



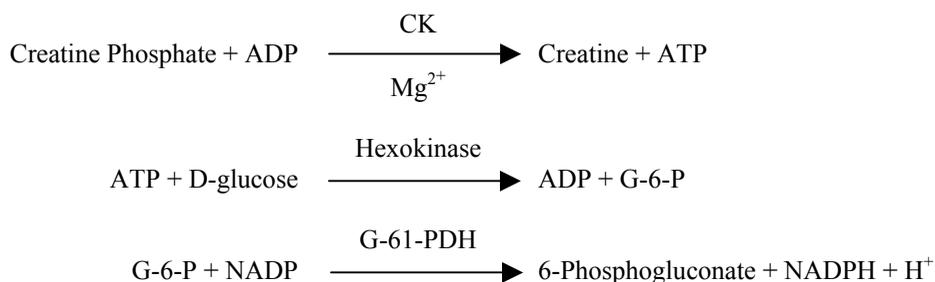
The endpoint reaction is monitored at 405 nm, 467 nm, and 600 nm. The amount of calcium in the sample is proportional to the absorbance.

Creatine Kinase (CK)

Creatine Kinase catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP).⁹

The CK measurement procedure used by Abaxis is a modified version of the IFCC.¹⁰ Key modifications are sample volume fraction, buffer and temperature. N-acetyl cysteine (NAC) has been added to reactivate the CK.¹¹ 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 inhibit CK. P¹, P⁵-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.

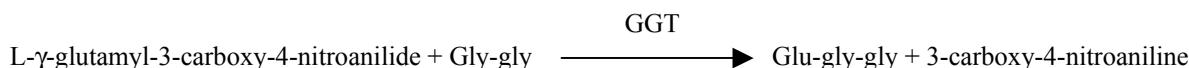
Creatine Kinase catalyzes the formation of creatine and adenosine triphosphate (ATP) from creatine phosphate P¹, P⁵-di (adenosine 5')penta phosphate (ADP) at pH 6.7. With hexokinase as a catalyst, ATP reacts with D-glucose to form ADP and D-glucose-6-phosphate (G-6-P), which is reacted with nicotinamide 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.

Gamma Glutamyl Transferase (GGT)

Abaxis has modified the IFCC method which uses the L- γ -glutamyl-3-carboxy-4-nitroanilide and glycylglycine¹² as the other substrate¹³ to react at 37° C. The addition of sample containing gamma glutamyl transferase to the substrates L- γ -glutamyl-3-carboxy-4-nitroanilide and glycylglycine (gly-gly) causes the formation of L- γ -glutamyl-glycylglycine (glu-gly-gly) and 3-carboxy-4-nitroaniline.

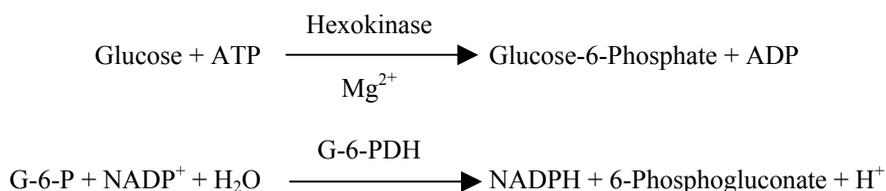


The absorbance of this rate reaction is measured at 405 nm. The production of 3-carboxy-4-nitroaniline is directly proportional to the GGT activity in the sample.

Magnesium (MG)

The hexokinase activation method for magnesium is the best fit system in terms of sensitivity, precision and accuracy.¹⁴

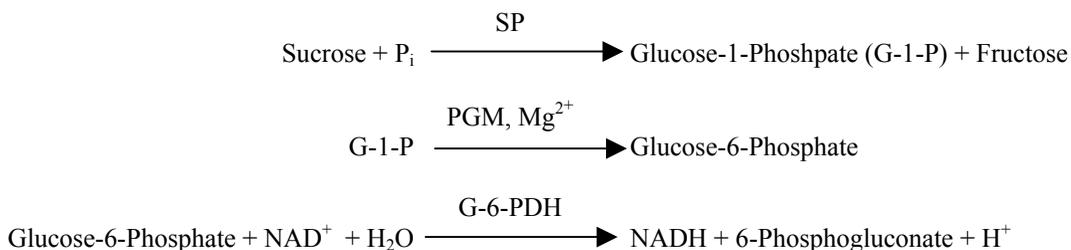
The enzymatic magnesium method can be written as follow:



The rate limiting reaction is the hexokinase reaction. Magnesium from serum activates hexokinase, which in turn catalyzes the breakdown of glucose to form glucose-6-phosphate (G-6-P) and ADP. Glucose-6-phosphate reacts with NADP⁺ to form NADPH and 6-phosphogluconate in the presence of glucose-6-phosphate dehydrogenase (G-6-PDH). This is a first-order rate reaction. Magnesium concentration is determined by measuring the increase in absorbance of NADPH at 340 nm.

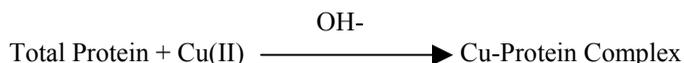
Phosphorus (PHOS)

The most applicable enzymatic method for the Abaxis system uses sucrose phosphorylase coupled through phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PDH).^{15,16} Using the enzymatic system for each mole of phosphorus present in the sample, one mole of NADH is formed. The amount of NADH formed can be measured as an endpoint at 340 nm.



Total Protein (TP)

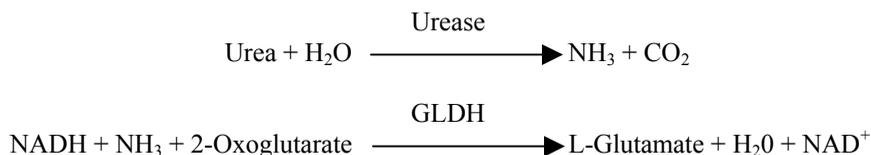
In the biuret reaction, the protein solution is treated with cupric [Cu(II)] ions in a strong alkaline medium. Sodium potassium tartate and potassium iodide are added to prevent the precipitation of copper hydroxide and the auto-reduction of copper, respectively.¹⁷ The Cu(II) ions react with peptide 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 and the absorbance is measured as the difference in absorbance between 550 nm and 850 nm.

Urea Nitrogen (BUN)

A coupled-enzymatic reaction is used by the Abaxis system. In this reaction, urease hydrolyzes urea into ammonia and carbon dioxide.¹⁸ Upon combining ammonia with 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD⁺.



The rate of change of the absorbance difference between 340 nm and 405 nm is caused by the conversion of NADH to NAD⁺ and is directly proportional to the amount of urea present in the sample.

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 Large Animal Profile 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 ALP, AST, CK, GGT, and urea nitrogen (BUN). 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.

Table 1: Reagents

Components	Contents
Albumin Reagent	
Bromcresol purple	2 µg
Buffer, surfactants, excipients and preservatives	
Alkaline Phosphatase Reagent	
Magnesium chloride	3 µg
Zinc sulfate	3 µg
ρ -NPP	56 µg
Buffers, surfactants and excipients	
Aspartate Aminotransferase Reagent (AST)	
L-aspartic acid	426 µg
Lactate dehydrogenase (LDH) (microbial)	0.03 U
β -nicotinamide adenine dinucleotide, reduced (NADH)	5 µg
Malate dehydrogenase (MDH) (porcine heart)	0.01 µg
α -ketoglutarate	28 µg
Buffers, surfactants, excipients and preservatives	
Calcium Reagent	
Arsenazo III, sodium salt	3 µg
Buffers, surfactants and excipients	
Creatine Kinase Reagent	
Adenosine diphosphate	31 µg
Adenosine monophosphate	33 µg
P^1 , P^5 -di(adenosine-5')pentaphosphate	0.2 µg
Magnesium Acetate, Tetrahydrate	69 µg
Hexokinase	95904 U
Glucose-6-phosphate dehydrogenase	79920 U
NADP Sodium Salt	104 µg
EDTA, disodium	12 µg
N-acetyl cysteine	52 µg
Phosphocreatine	122 µg
Buffer, surfactants, excipients and preservatives	
Gamma Glutamyl Transferase	
Glycylglycine	317 µg
L-glutamic acid γ -(3-carboxy-4-nitroanilide)	30 µg
Buffer, surfactants, excipients and preservatives	
Magnesium	
EDTA, disodium	0.00032 mg
NADP, sodium	0.0296 mg
Hexokinase	0.0120 U
Glucose-6-phosphate dehydrogenase	0.0220 U
Phosphorus	
NAD (free acid)	0.043 mg
Magnesium Acetate, Tetrahydrate	0.007 mg
Glucose-1,6-diphosphate	0.001 mg
Glucose-6-phosphate dehydrogenase	0.023 U
Phosphoglucomutase (rabbit)	0.035 U
Sucrose phosphorylase	0.070 U

Table 1: Reagents (continued)

Components	Contents
Total Protein Reagent	
Sodium potassium tartrate	343 µg
Cupric sulfate	134 µg
Potassium iodide	28 µg
Surfactants, excipients and preservatives	
Total Protein Blank	
Sodium potassium tartate	343 µg
Potassium iodide	28 µg
Surfactants, excipients 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 can not 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. Do not allow the rotors to remain at room temperature longer than 48 hours. 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 can not 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). To use reagent rotors, remove the rotors from their sealed foil pouches from the refrigerator. Ensure that the cumulative time that the rotors are unrefrigerated (in their sealed pouches) does not exceed 48 hours. Open the pouch and remove the rotor just prior to use.

Indications of Reagent Rotor Instability or Deterioration

- All reagents contained in a 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.
- 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.
- After opening the pouch, examine the desiccant packet that is included with the reagent rotor. A blue strip on the back of the desiccant packet indicates that the correct relative humidity has been maintained in the pouch. A pink strip means the rotor has been exposed to excess moisture in the pouch (e.g. through a puncture hole and the rotor should **not** be used).

6. Instrument

See the VetScan System Operator's Manual for complete information on using the analyzer, including installation, performance specifications, operational precautions and limits, service and maintenance.

7. Sample Collection and Preparation

The minimum required sample size is ~90 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 specimens 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.¹⁹ The sample may be separated into plasma or serum and stored in capped sample tubes at 2-8° C (36-46° F) if the sample can not be run within 60 minutes.

Known Interfering Substances

- The only anticoagulant recommended for use with the VetScan Whole Blood Analyzer is lithium heparin.
- Physical interferents (hemolysis, icterus, and lipemia) 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”, respectively 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. Accordingly, specimens should be stored in the dark in tightly closed tubes.²⁰

8. Procedure

Materials Provided

- One VetScan[®] Large Animal Reagent Rotor

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 (59-90° F). The analysis time for each VetScan[®] Large Animal 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. Reagent rotors used for controls should be prepared the same 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.

Interpretation of results is detailed in the VetScan Operator's Manual. Results are printed onto result cards supplied by Abaxis. The result cards have an adhesive backing for easy placement in the patient's files.

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 62-64% packed red cell volume may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down to get plasma then re-run in a new reagent rotor.

11. Expected Values

These normal ranges are provided as a guideline. 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.

Table 2: Bovine Reference Intervals

Analyte	Concentration
ALB_BCG	2.5–3.8 g/dL (25–38 g/L)
ALP	23–135 U/L
AST	66–211 U/L
CA ⁺⁺	7.9–9.6 mg/dL (1.97–2.39 mmol/L)
CK	83–688 U/L
GGT	12–48 U/L
GLOB*	4.0–5.5 g/dL (40–55 g/L)
MG	1.7–2.9 mg/dL (0.70–1.19 mmol/L)
PHOS	(4.1–9.2 mg/dL (1.3–3.0 mmol/L)
TP	6.6–9.3 g/dL (66–93 g/L)
BUN	6–20 mg/dL (2.14–7.14 mmol urea /L)

*Calculated Value

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).

Table 3: VetScan Dynamic Ranges

Analyte	Dynamic Ranges	
	Common Units	SI Units
ALB_BCG	1–6.5 g/dL	10–65 g/L
ALP	5–2400 U/L	5–2400 U/L
AST	5–2000 U/L	5–2000 U/L
CA ⁺⁺	4–16 mg/dL	1.0–4.0 mmol/L
CK	5–14000 U/L	5–14000 U/L
GGT	5–3000 U/L	5–3000 U/L
GLOB*	1–11 g/dL	10–110 g/L
MG	0–8 mg/dL	0–3.29 mmol/L
PHOS	0–20 mg/dL	0–6.46 mmol/L
TP	2–14 g/dL	20–140 g/L
BUN	2–180 mg/dL	0.7–64.3 mmol urea/L

*Calculated Value

Precision

Precision studies were conducted using the NCCLS EP5-A Guidelines. Results for within-run and total precision were determined by testing bi-level controls. Controls were run in duplicate twice each day for 20 days over a four week period. Precision was determined using Moni-trol[®] Level 1 and Level 2 Chemistry Controls (Dade International, Inc.). Results of the precision studies are shown in Table 4.

Table 4: Precision

Analyte		Within-Run (n=80)	Total (n=80)
Albumin-BCG (ALB, g/dL)			
Control 1			
	Mean	4.2	4.2
	SD	0.06	0.08
	%CV	1.4	1.9
Control 2			
	Mean	2.5	2.5
	SD	0.04	0.07
	%CV	1.5	3.0
Alkaline Phosphatase (ALP, U/L)			
Control 1			
	Mean	65	65
	SD	4.4	4.7
	%CV	6.7	7.3
Control 2			
	Mean	277	277
	SD	9.7	10.3
	%CV	3.5	3.7
Aspartate Aminotransferase (AST, U/L)			
Control 1			
	Mean	40	40
	SD	1.6	3.0
	%CV	3.9	7.5
Control 2			
	Mean	124	124
	SD	2.1	3.2
	%CV	1.7	2.6

Analyte		Within-Run (n=80)	Total (n=80)
Calcium (Ca⁺⁺, mg/dL)			
Control 1			
	Mean	10.4	10.4
	SD	0.5	0.5
	%CV	4.4	4.5
Control 2			
	Mean	8.5	8.5
	SD	0.3	0.3
	%CV	4.1	4.1
Gamma Glutamyl Transferase (GGT, U/L)			
Control 1			
	Mean	16	16
	SD	1.2	1.3
	%CV	7.6	8.0
Control 2			
	Mean	63	63
	SD	1.3	1.3
	%CV	2.0	2.0
Globulin (GLOB, g/dL)			
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
Magnesium (MG, mg/dL)			
Control 1			
	Mean	4.9	4.9
	SD	0.07	0.07
	%CV	1.4	1.4
Control 2			
	Mean	2.0	2.0
	SD	0.04	0.04
	%CV	2.0	2.1
Phosphorus (PHOS, mg/dL)			
Control 1			
	Mean	6.9	6.9
	SD	0.2	0.2
	%CV	2.2	2.6
Control 2			
	Mean	3.4	3.4
	SD	0.1	0.2
	%CV	4.1	4.9

Analyte		Within-Run (n=80)	Total (n=80)
Total Protein (TP, g/dL)			
Control 1			
	Mean	7.3	7.3
	SD	0.07	0.07
	%CV	0.9	1.0
Control 2			
	Mean	4.5	4.5
	SD	0.04	0.06
	%CV	1.0	1.4
Urea Nitrogen (BUN, mg/dL)			
Control 1			
	Mean	12	12
	SD	0.4	0.6
	%CV	3.4	5.4
Control 2			
	Mean	45	45
	SD	2.5	2.8
	%CV	5.5	6.2

Correlation

Field studies were conducted at a veterinary medicine teaching hospital. Heparinized bovine whole blood and serum samples were analyzed by the VetScan Whole Blood Analyzer and a comparative method. Whole blood and serum samples were grouped together for data analysis. Representative correlation statistics are shown in Table 5.

Table 5: Correlation of VetScan Analyzer Methods in the Large Animal Profile Rotor with Comparative Methods

Albumin (g/dL)

Correlation	0.74
Slope	0.80
Intercept	0.28
Sample Range	2.4-4.0
N	126
Comparative Method	Bayer Diagnostics BCG Reagent

ALP (U/L)

Correlation	0.97
Slope	0.83
Intercept	7
Sample Range	13-136
N	126
Comparative Method	Synermed IFCC – ρ -nitrophenol phosphate

AST (U/L)

Correlation	0.94
Slope	0.89
Intercept	-0.58
Sample Range	68-262
N	126
Comparative Method	Synermed IFCC modified

Calcium (mg/dL)	Correlation	0.89
	Slope	0.78
	Intercept	0.66
	Sample Range	5.2-9.8
	N	126
	Comparative Method	Randox Laboratories Arsenazo III
GGT (U/L)	Correlation	0.97
	Slope	1.13
	Intercept	0.7
	Sample Range	7-54
	N	126
	Comparative Method	Synermed Modified Szasz
GLOB (g/dL)	Correlation	0.94
	Slope	0.97
	Intercept	1.1
	Sample Range	3.1-6.8
	N	126
	Comparative Method	N/A (Calculated)
MG (mg/dL)	Correlation	0.98
	Slope	1.09
	Intercept	-0.1
	Sample Range	1.2-4.2
	N	126
	Comparative Method	Bayer Diagnostics Xylidyl
Phosphorus (mg/dL)	Correlation	0.99
	Slope	1.06
	Intercept	-0.5
	Sample Range	1.9-9.7
	N	126
	Comparative Method	Sigma modified unreduced
TP (g/dL)	Correlation	0.98
	Slope	1
	Intercept	0.5
	Sample Range	6-10
	N	126
	Comparative Method	Bayer Diagnostics Biuret Reagent
BUN (mg/dL)	Correlation	0.98
	Slope	0.99
	Intercept	1.4
	Sample Range	6-25
	N	126
	Comparative Method	Sigma Modified Talke & Shubert

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