VetScan® Comprehensive Diagnostic Profile

CE

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

January 2015
PN: 500-7123 Rev: E

1. Intended Use

The VetScan® Comprehensive Diagnostic Profile reagent rotor used with the VetScan Chemistry Analyzer utilizes dry and liquid reagents to provide *in vitro* quantitative determinations of alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), amylase (AMY) total calcium (CA^{++}), creatinine (CRE), globulin* (GLOB), glucose (GLU), phosphorus (PHOS), potassium (K^{+}), sodium (NA^{+}), total bilirubin (TBIL), total protein (TP), and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

* Calculated Value

2. Summary and Explanation of Tests

The VetScan Comprehensive Diagnostic Profile reagent rotor and the VetScan Chemistry Analyzer comprise an *in vitro* diagnostic system that aids the veterinarian in diagnosing the following disorders:

Alanine aminotransferase:	Liver diseases, including viral hepatitis and cirrhosis; heart diseases.
Albumin:	Liver and kidney diseases.
Alkaline phosphatase:	Liver, bone, parathyroid, and intestinal diseases.
Amylase:	Kidney and pancreatic disease.
Calcium:	Parathyroid, bone and chronic renal disease; tetany.
Creatinine:	Renal disease.
Globulin:	Globulin concentration will increase with dehydration and should also increase with antigenic stimulation.
Glucose:	Diabetes**,** hyperglycemia, hypoglycemia, diabetes and liver disease.
Phosphorus:	Kidney disease, hypoparathyroidism and nutritional disorders.
Potassium:	Malnutrition and renal disease. This electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms.
Sodium:	Dehydration, and diabetes. This electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms.
Total bilirubin:	Hepatic disorders.
Total protein:	Dehydration, kidney, liver disease, metabolic and nutritional disorders.
Blood Urea Nitrogen:	Liver and kidney 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

Alanine Aminotransferase

The method developed for use on the VetScan Chemistry Analyzer is a modification of the Wróblewski and LaDue procedure recommended by the International Federation of Clinical Chemistry (IFCC).[1,2]

In this reaction, ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+, as illustrated in the following reaction scheme.

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

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

Albumin

Dye binding techniques are the most frequently used methods for measuring albumin. Bromcresol green (BCG) is the most commonly used of the dye binding methods.[3]

$$\text{BCG} + \text{Albumin} \xrightarrow[\text{Acid pH}]{\text{Surfactants}} \text{BCG-Albumin Complex}$$

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.

Alkaline Phosphatase

The VetScan procedure is modified from the AACC and IFCC methods.[4] Alkaline phosphatase hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate. The use of *p*-nitrophenyl phosphate (*p*-NPP) increases the speed of the reaction.[5,6] The reliability of this technique is greatly increased by the use of a metal-ion buffer to maintain the concentration of magnesium and zinc ions in the reaction.[7] The American Association for Clinical Chemistry (AACC) reference method uses *p*-NPP as a substrate and a metal-ion buffer.[8]

$$p\text{-Nitrophenyl Phosphate} + H_2O \xrightarrow[Zn^{2+},\ Mg^{2+}]{\text{ALP}} p\text{-Nitrophenol} + \text{Phosphate}$$

The amount of ALP in the sample is proportional to the rate of increase in absorbance difference between 405 nm and 500 nm.

Amylase

Commonly used methods are the saccharogenic and chromolytic methods. The “classic” amylase measurement technique is a saccharogenic method, but it is difficult and time-consuming.[9] Chromolytic methods using *p*-nitrophenyl-glycosides as substrates have been recently developed.[10] These assays have a higher specificity for pancreatic amylase than for salivary amylase and are easily monitored.[11]

In the Abaxis method, the substrate 2-chloro-*p*-nitrophenyl-α-D-maltotrioside (CNPG3) reacts with α-amylase in the patient sample, releasing 2-chloro-*p*-nitrophenol (CNP). The release of CNP creates a change in color.

$$\text{CNPG3} \xrightarrow{\alpha\text{-Amylase}} \text{CNP} + \text{D-Maltotrioside}$$

The reaction is measured bichromatically at 405 nm and 500 nm. The change in absorbance due to the formation of CNP is directly proportional to α-amylase activity in the sample.

Total Calcium

The reference method for calcium is atomic absorption spectroscopy; however, this method is not suited for routine use.[12] Spectrophotometric methods using either *o*-cresolphthalein complexone (CPC) or arsenazo III metallochromic indicators are most commonly used.[13,14,15] Arsenazo III has a high affinity for calcium and is not as temperature dependent as CPC.

Calcium in the patient sample binds with arsenazo III to form a calcium-dye complex.

$$Ca^{2+} + \text{Arsenazo III} \longrightarrow Ca^{2+}\text{-Arsenazo III Complex}$$

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.

Creatinine

The Jaffe method, first introduced in 1886, is still a commonly used method of determining creatinine levels in blood. The current reference method combines the use of Fuller's earth (floridin) with the Jaffe technique to increase the specificity of the reaction.[16,17] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[18,19,20] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.[21]

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine Amidohyrolase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatine Amidinohydrolase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{Sarcosine Oxidase}} \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

$$H_2O_2 + \text{TBHBA} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine Dye} + H_2O$$

Two cuvettes are used to determine the concentration of creatinine in the sample. Endogenous creatine is measured in the blank cuvette, which is subtracted from the combined endogenous creatine and the creatine formed from the enzyme reactions in the test cuvette. Once the endogenous creatine is eliminated from the calculations, the concentration of creatinine is proportional to the intensity of the red color produced. The endpoint reaction is measured as the difference in absorbance between 550 nm and 600 nm.

Glucose

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu and Somogyi-Nelson)[22,23,24] The lack of specificity in copper-reduction techniques led to the development of quantitative procedures using the enzymes hexokinase and glucose oxidase. The Abaxis glucose is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[25] 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.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{Hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{G-6-P} + NAD^+ \xrightarrow{\text{G-6-PDH}} \text{6-Phosphogluconate} + \text{NADH} + H^+$$

Phosphorus

The Abaxis phosphorus method uses sucrose phosphorylase (SP) coupled with the phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G-6-PDH) reactions.[26,27] 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.

$$\text{Sucrose} + P_i \xrightarrow{\text{SP}} \text{Glucose-1-Phoshpate (G-1-P)} + \text{Fructose}$$

$$\text{G-1-P} \xrightarrow{\text{PGM, } Mg^{2+}} \text{Glucose-6-Phosphate}$$

$$\text{Glucose-6-Phosphate} + NAD^+ \xrightarrow{\text{G-6-PDH}} \text{NADH} + \text{6-Phosphogluconate} + H^+$$

Potassium
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.[28,29,30] Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamate dehydrogenase respectively.[28]

In the coupled-enzyme reaction, PK dephosphorylates phosphoenolpyruvate (PEP) to form pyruvate. Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+. The rate of change in absorbance between 340 nm and 405 nm is due to the conversion of NADH to NAD^+, which is directly proportional to the amount of potassium in the sample.

$$\text{ADP + PEP} \xrightarrow{K^+,\ \text{PK}} \text{Pyruvate + ATP}$$

$$\text{Pyruvate + NADH + } H^+ \xrightarrow{\text{LDH}} \text{Lactate + } NAD^+$$

Sodium
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.[31,32,33] 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. The reaction rate between 405 nm and 500 nm is proportional to sodium concentration.

$$\text{ONPG+ } H_2O \xrightarrow[\beta\text{-Galactosidase}]{Na^+} \text{o-Nitrophenol + Galactose}$$

Total Bilirubin
Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.[34,35] A newer, more specific method has been developed using the enzyme bilirubin oxidase. [36,37,38] In addition to using the more specific total bilirubin test method, photodegradation of the analyte is minimized on the analyzer because the sample can be tested immediately after collection.

In the enzymatic procedure, bilirubin is oxidized by bilirubin oxidase into biliverdin. Bilirubin is quantitated as the difference in absorbance between 467 nm and 550 nm. The initial absorbance of this endpoint reaction is determined from the bilirubin blank cuvette and the final absorbance is obtained from the bilirubin test cuvette. The amount of bilirubin in the sample is proportional to the difference between the initial and final absorbance measurements.

$$\text{Bilirubin + } O_2 \xrightarrow{\text{Bilirubin Oxidase}} \text{Biliverdin + } H_2O$$

Total Protein
The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity. [39] It was originally developed by Riegler and modified by Weichselbaum, Doumas, et al. The biuret reaction is a candidate total protein reference method.[40,41,42]

In the biuret reaction, the protein solution is treated with cupric [Cu(II)] ions in a strong alkaline medium. Sodium potassium tartrate and potassium iodide are added to prevent the precipitation of copper hydroxide and the auto-reduction of copper, respectively. [41] The Cu(II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-Protein complex.

$$\text{Total Protein + Cu(II)} \xrightarrow{OH^-} \text{Cu-Protein Complex}$$

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
A coupled-enzymatic reaction is used by the Abaxis system. In this reaction, urease hydrolyzes urea into ammonia and carbon dioxide.[43] Upon combining ammonia with 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD^+.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} NH_3 + CO_2$$

$$NH_3 + \text{NADH} + H^+ + \text{2-Oxoglutarate} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + H_2O + NAD^+$$

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 Comprehensive Diagnostic 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 alanine aminotransferase, albumin, alkaline phosphatase, amylase, calcium, glucose, phosphorus, potassium, sodium, and urea nitrogen. Dedicated sample blanks are included in the rotor to calculate the concentration of creatinine, total bilirubin and total protein levels. Each reagent rotor also contains a diluent consisting of surfactants and preservatives.

Warnings and Precautions

- For Veterinary *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.

- Some Reagent beads 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 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). 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 Chemistry 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.

6. Instrument

See the VetScan 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 control. The reagent rotor sample chamber can contain up to 120 µL of sample.

- Specimens 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 may 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.[44] 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.

- **Glucose** concentrations decrease approximately 5-12 mg/dL in 1 hour in uncentrifuged samples stored at room temperature.[45]

- Refrigerating whole blood samples can cause significant changes in concentrations of **glucose** and **creatinine**.[46]

- **Total bilirubin** results may be adversely affected by photodegradation.[47] Whole blood samples not run immediately should be stored in the dark for no longer than 60 minutes. If the sample can not be analyzed within that period, it should be separated into plasma or serum and stored in a capped sample tube in the dark at low temperatures.[48]

Known Interfering Substances

- The only anticoagulant recommended for use with the VetScan Chemistry Analyzer is lithium heparin. Sodium heparin must not be used when collecting blood samples for use with this panel. Abaxis has performed studies demonstrating that EDTA, fluoride, oxalate, and any anticoagulant containing ammonium ions will interfere with at least one chemistry in the VetScan Comprehensive Diagnostic Profile reagent rotor.

- 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 Chemistry 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.

- Hemolysis may cause erroneously high results in potassium assays. This problem may go undetected when analyzing whole blood (release of potassium from as few as 0.5% of the erythrocytes can increase the potassium serum level by 0.5 mmol/L). In particular, even unhemolyzed specimens that are not properly processed may have increased potassium levels due to intracellular potassium leakage.[49]
- Bilirubin may interfere with the peroxidase used in the creatinine reaction.[50] Creatinine results are lowered when bilirubin levels are > 10 mg/dL.

- 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.[51]

- Interference may be seen in the total protein test when analyzing samples with a 3 + lipemic index.[52] Samples with a triglyceride concentration >400 mg/dL may show an increased total protein level.[48] The VetScan Chemistry Analyzer suppresses any results that are affected by >10% interference from lipemia. "LIP" is printed on the result card in place of the result.

- 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 Comprehensive Diagnostic Profile Reagent Rotor

Materials Required but not Provided

- VetScan 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 Comprehensive Diagnostic Profile 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 Operator's Manual.

Calibration

The VetScan Chemistry 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 Operator's Manual.

Quality Control

Controls may be run periodically on the VetScan Chemistry 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 Operator's Manual to run controls.

9. Results

The VetScan Chemistry Analyzer automatically calculates and prints the analyte concentrations in the sample. Details of the endpoint and rate reaction calculations are found in the VetScan 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.**

- 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.

  Warning: Extensive testing of the VetScan Chemistry Analyzer 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

These normal intervals are provided only as a guideline. The most definitive reference intervals 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 Chemistry Analyzer for the "Other" bank, refer to your VetScan Operator's Manual under the Menu Key functions.

Table 1: Reference Intervals

	Canine	**Feline**	**Equine**
ALT	10-118 U/L (10-118 U/L)	20-100 U/L (20-100 U/L)	5-20 U/L (5-20 U/L)
ALB	2.5-4.4 g/dL (25-44 g/L)	2.2-4.4 g/dL (22-44 g/L)	2.2-3.7 g/dL (22-37 g/L)
ALP	20-150 U/L (20-150 U/L)	10-90 U/L (10-90 U/L)	50-170 U/L (50-170 U/L)
AMY	200-1200 U/L (200-1200 U/L)	300-1100 U/L (300-1100 U/L)	5-15 U/L (5-15 U/L)
CA^{++}	8.6-11.8 mg/dL (2.2-3.0 mmol/L)	8.0-11.8 mg/dL (2.0-3.0 mmol/L)	11.5-14.2 mg/dL (2.9-3.6 mmol/L)
CRE	0.3-1.4 mg/dL (27-124 µmol/L)	0.3-2.1 mg/dL (27-186 µmol/L)	0.6-2.2 mg/dL (53-194 µmol/L)
GLOB	2.3-5.2 g/dL (23-52 g/L)	1.5-5.7 g/dL (15-57 g/L)	2.7-5.0 g/dL (27-50 g/L)
GLU	60-110 mg/dL (3.3-6.1 mmol/L)	70-150 mg/dL (3.9-8.3 mmol/L)	65-110 mg/dL (3.6-6.1 mmol/L)
PHOS	2.9-6.6 mg/dL (0.94-2.13 mmol/L)	3.4-8.5 mg/dL (1.10-2.74 mmol/L)	1.9-4.3 mg/dL (0.61-1.39 mmol/L)
K^+	3.7-5.8 mmol/L (3.7-5.8 mmol/L)	3.7-5.8 mmol/L) (3.7-5.8 mmol/L)	2.5-5.2 mmol/L (2.5-5.2 mmol/L)
Na^+	138-160 mmol/L (138-160 mmol/L)	142-164 mmol/L (142-164 mmol/L)	126-146 mmol/L (126-146 mmol/L)
TBIL	0.1-0.6 mg/dL (2-10 µmol/L)	0. 1-0.6 mg/dL (2-10 µmol/L)	0.5-2.3 mg/dL (9-39 µmol/L)
TP	5.4-8.2 g/dL (54-82 g/L)	5.4-8.2 g/dL (54-82 g/L)	5.7-8.0 g/dL (57-80 g/L)
BUN	7-25 mg/dL (2.5-8.9 mmol/L)	10-30 mg/dL (3.6-10.7 mmol/L)	7-25 mg/dL (2.5-8.9 mmol/L)

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 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 2: VetScan Dynamic Ranges

Analyte	Dynamic Ranges Common Units	SI Units
ALT	5-2000 U/L	5-2000 U/L
ALB	1-6.5 g/dL	10-65 g/L
ALP	5-2400 U/L	5-2400 U/L
AMY	5-4000 U/L	5-4000 U/L
CA++	4-16 mg/dL	1.0-4.0 mmol/L
CRE	0.2-20 mg/dL	18-1768µmol/L
GLOB*	1-11 g/dL	10-110 g/L
GLU	10-700 mg/dL	0.6-39mg/dL
PHOS	0-20 mg/dL	0-6.46 mmol/L
K+	1.5-8.5 mmol/L	1.5-8.5 mmol/L
NA+	110-170 mmol/L	110-170 mmol/L
TBIL	0.1-30 mg/dL	1.7-513 µmol/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.[52]Guidelines with modifications based on NCCLS EP18-P[53] for unit-use devices. Results for within-run and total precision were determined by testing bi-level controls.

Table 3: Precision

Analyte	Sample Size	Within-Run	Total
Alanine Aminotransferase (U/L)	n=80		
Control 1			
Mean		21	21
SD		2.76	2.79
%CV		13.1	13.3
Control 2			
Mean		52	52
SD		2.70	3.25
%CV		5.2	6.3
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

Table 3: Precision (Continued)

Analyte	Sample Size	Within-Run	Total
Alkaline Phosphatase (U/L)	n=80		
Control 1			
Mean		39	39
SD		1.81	2.29
%CV		4.6	5.9
Control 2			
Mean		281	281
SD		4.08	8.75
%CV		1.5	3.1
Amylase (U/L)	n=80		
Control 1			
Mean		46	46
SD		2.40	2.63
%CV		5.2	5.7
Control 2			
Mean		300	300
SD		11.15	11.50
%CV		3.7	3.8
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
Creatinine (mg/dL)	n=80		
Control 1			
Mean		1.1	1.1
SD		0.14	0.14
%CV		12.7	12.7
Control 2			
Mean		5.2	5.2
SD		0.23	0.27
%CV		4.4	5.2
Glucose (mg/dL)	n=80		
Control 1			
Mean		66	66
SD		0.76	1.03
%CV		1.2	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		0.2	0.2
%CV		2.2	2.6
Control 2			
Mean		3.4	3.4
SD		0.1	0.2
%CV		4.1	4.9

Table 3: Precision (Continued)

Analyte	Sample Size	Within-Run	Total
Potassium (mmol/L)	n=120		
Control 1			
Mean		6.7	6.7
SD		0.26	0.26
%CV		3.9	3.9
Control 2			
Mean		4.3	4.3
SD		0.22	0.22
%CV		5.1	5.1
Sodium (mmol/L)	n=80		
Control 1			
Mean		148	148
SD		5.1	5.1
%CV		3.4	3.4
Control 2			
Mean		118	118
SD		3.2	3.2
%CV		2.7	2.7
Total Bilirubin (mg/dL)	n=80		
Control 1			
Mean		0.8	0.8
SD		0.06	0.07
%CV		7.5	8.8
Control 2			
Mean		5.2	5.2
SD		0.09	0.15
%CV		1.7	2.9
Total Protein (g/dL)	n=80		
Control 1			
Mean		6.8	6.8
SD		0.05	0.08
%CV		0.7	1.2
Control 2			
Mean		4.7	4.7
SD		0.09	0.09
%CV		1.9	1.9
Urea Nitrogen (mg/dL)	n=120		
Control 1			
Mean		19	19
SD		0.35	0.40
%CV		1.8	2.1
Control 2			
Mean		65	65
SD		1.06	1.18
%CV		1.6	1.8

Correlation
Field studies were conducted at a veterinary teaching hospital. Serum samples were analyzed by the VetScan Chemistry Analyzer and a comparative method. Representative correlation statistics are shown in Table 4.

Table 4: Correlation of the VetScan Chemistry Analyzer with Comparative Method(s)

		Correlation Coefficient	Slope	Intercept	N	Sample Range
Alanine Aminotransferase (U/L)	Canine	1.00	0.95	0	22-180	10-1549
	Feline	0.98	0.92	0	21-55	27-99
	Equine	0.97	0.94	6	7-101	11-30
Albumin (g/dL)	Canine	0.96	0.99	0.1	22-180	1.3-4.6
	Feline	0.75	1.02	0	21-55	2.1-4.8
	Equine	0.89	0.99	-0.6	7-101	1.2-3.2
Alkaline Phosphatase (U/L)	Canine	1.00	0.89	-5	22-180	15-1722
	Feline	0.97	0.81	1	21-55	6-54
	Equine	1.00	0.90	-4	7-101	119-1476
Amylase (U/L)	Canine	0.96	0.67	-34	22-180	366-1991
	Feline	1.0	0.74	117	21-55	473-3474
	Equine	N/A	N/A	N/A	7-101	N/A
Calcium (mg/dL)	Canine	0.84	1.24	-1.9	22-180	7.3-13.0
	Feline	0.77	1.24	-2.1	21-55	6.3-12.4
	Equine	0.94	1.18	-0.8	7-101	7.2-15.1
Creatinine (mg/dL)	Canine	0.99	1.00	0.0	22-180	0.6-10.6
	Feline	1.00	1.01	-0.1	21-55	0.3-13.6
	Equine	0.95	1.00	-0.4	7-101	0.3-6.2
Glucose (mg/dL)	Canine	0.96	1.01	-6	22-180	28-348
	Feline	1.00	0.97	3	21-55	52-607
	Equine	0.97	0.94	16	7-101	36-353
Phosphorus (mg/dL)	Canine	0.994	1.09	-0.19	22-180	0.8-87
	Feline	0.916	0.80	0.81	21-55	2.4-6.9
	Equine	0.971	0.991	-0.06	7-101	0.8-7.8
Potassium (mmol/L)	Canine	0.96	0.92	0.4	22-180	3.2-6.9
	Feline	0.91	0.92	0.5	21-55	2.7-5.3
	Equine	0.84	0.97	0.1	7-101	1.8-4.6
Sodium (mmol/L)	Canine	0.89	0.97	4.8	22-180	118-183
	Feline	0.86	1.08	-12.2	21-55	122-166
	Equine	0.86	1.00	-0.01	7-101	110-166
Total Bilirubin (mg/dL)	Canine	0.87	0.84	0.1	22-180	0.1-3.2
	Feline	1.00	0.92	-0.3	21-55	0.4-15.0
	Equine	1.00	0.90	0.1	7-101	0.6-26.1
Total Protein (g/dL)	Canine	0.98	1.03	0.1	22-180	2.6-10.7
	Feline	0.97	0.96	0.4	21-55	4.8-8.5
	Equine	0.99	0.97	0.3	7-101	3.0-9.5
Urea Nitrogen (mg/dL)	Canine	1.00	0.98	-2	22-180	4-117
	Feline	1.00	1.07	-5	21-55	14-165
	Equine	1.00	0.95	-1	7-101	3-64

13. Bibliography

1. Wróbleski F and LaDue. Serum glutamic-pyruvic transminase in cardiac and hepatic disease. Proc Soc Exp Biol Med. 1956;91:569-71.
2. Bergmeyer HU and Horder M. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J. Clin Chem Clin Biochem 1980;18:521-34.
3. Webster D, et al. An assessment on the suitability of bromocresol green for the determination of serum albumin. Clin Chim Acta 1974;53:101-8.
4. Bowers GN, et al. IFCC methods for the measurement of catalytic concentration of enzymes. Part 1. General considerations concerning the determination of the catalytic concentration of an enzyme in the blood serum or plasma of man. Clin Chim Acta 1979;98:163F-74F.
5. Ohmori Y. Uber die Phosphomomesterase. Enzymologia 1937;4:217-31.
6. Fujita H. Uber die Mikrobestimmung der Blutphosphatase. J Biochem, Japan. 1937;30:69-87.
7. Petitclerc C, et al. Mechanism of action of Mg^{2+} and Zn^{2+} on rat placental alkaline phosphatase. I. Studies on the soluble Zn^{2+} and Mg^{2+} alkaline phosphatase. Can J Biochem 1975;53:1089-1100.
8. Tietz NW, et al. A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem 1983;29:751-61.
9. Somogyi M. Modifications of two methods for the assay of amylase. Clin Chem 1960;6:23-35.
10. Gillard BK, Markman HC and Feig SA. Direct spectrophotometric determination of α-amylase activity in saliva with *p*-nitrophenyl α-maltoside as substrate. Clin Chem 1977;23:2279-82.
11. Wallenfels K, et al. The enzymic synthesis, by transglucosylation of a homologous series of glycosidically substituted malto- ologosaccharides, and their use as amylase substrateds. Carbohydrate Res 1978;61:359-68.
12. Cali JP, Bowers GN, Young DS, et al. A reference method for the determination of total calcium in serum. In: GR Cooper, ed., Selected methods of Clinical Chemistry. Washington, DC: American Association for Clinical Chemistry; 1977;8:3-8.
13. Kessler G, M Wolfman. An Automated procedure for the simultaneous determination of calcium and phosphorus. Clin Chem 1964;10: 686-703.
14. Michaylova V, P Ilkova. Photometric determination of micro amounts of calcium with arsenazo III. Anal Chim Acta 1971;53: 194-8.
15. Scarpa A, et al. Metallochromic indicators of ionized calcium. Ann NY Acad Sci 1978;307:86-112.
16. Knoll VE, et al. Spezifische Kreatininbetimmung Im Serum. Z Klin Chemi Clin Biochem. 1970;8:582-587.
17. Haeckel R, et al. Simplified Determinations of the “True” Creatinine Concentration In Serum And Urine. J Cklin Chem Clin Biochem. 1980;18:385-394.
18. Moss GA, et al. Kinetic Enzymatic Method For Determining Serum Creatinine. Clin Chem 1975;21:1422-1426.
19. Jaynes PK, et al. An Enzymatic, Reaction-Rate Assay For Serum Creatinine With a Centrifugal Analyzer. Clin Chem 1982;28:114-117.
20. Fossati P, et al. Enzymatic Creatinine Assay: A New Colorimetric Method Based on Hydrogen Peroxide Measurement. Clin Chem 1983;29:1494-1496.
21. Whelton A, et al. Nitrogen Metabolites and Renal Function. In:CA Burtis and ER Ashwood, Eds., Tietz Textbook of Clinical Chemistry, 3rd Ed. Philadelphia: W.B. Saunders Company. 1999;1513-1575.
22. Folin O, and Wu H. A System of blood analysis. J Biol Chem 1919;38: 81-110.
23. Somogyi M. A reagent for the copper-idiometric determination of very small amounts of sugar. J Biol Chem 1937;117: 771-776.
24. Nelson N. A photometric adaption of the Somogyi method for the determination of glucose. J Biol 1944; 153: 375-380.
25. Kaplan LA. Glucose. In:LA Kaplan and AJ Pesce, eds., Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed St. Louis: The C.V. Mosby Company; 1989;850-856.
26. Schulz DW, et al. An Enzymic Method for the Measurement of Inorganic Phosphate Determination Anal Biochem 1967;19:300-14.
27. Tedokon, M Suzuki, et al. Enzymatic Assay of Inorganic Phosphate with Use of Sucrose Phosphorylase and Phosphoglucomutase. Clin Chem 1992;38:512-5.
28. Berry MN, et al. Enzymatic determination of potassium in serum. Clin Chem 1989;35:817-20.
29. Van Pelt J. Enzymatic determination of sodium, potassium and chloride in serum compared with determination by flame photometry, coulometry and ion selective electrodes. Clin Chem 1994;40:846-7.
30. Hubl W, et al. Enzymatic determination of sodium, potassium and chloride in abnormal (hemolyzed, icteric, lipemic, paraprteinemic, or uremic) serum samples compared with indirect determination with ion selective electrodes. Clin Chem 1994;40:1528-31.
31. Helgerson RC, et al. Host-guest Complexation. 50. Potassium and sodium ion-selective chromogenic ionophores. J Amer Chem Soc 1989;111:6339-50.
32. Kumar A, et al. Chromogenic ionophere-based methods for spectrophotometric assay of sodium and potassium in serum and plasma. Clin Chem 1988;34:1709-12.

13. Bibliography (Continued)

33. Berry MN, et al. Enzymatic determination of sodium in serum. Clin Chem 1988;34:2295-8.
34. Malloy HT, and Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J biol Chem 1937; 119:481-90.
35. Meites S. Bilirubin, directing reacting and total, modified Mally-Evelyn method. *In*: WR Faulkner and S Meites, eds., Selected Methods of Clinical Chemistry, Washington, DC: American Association for Clinical Chemistry; 1982;9:119-24.
36. Murao S and Tanaka N. A new enzyme "bilirubin oxidase" produced by *Myrothecium* verrucaria MT-1. Agric Biol Chem 1981;45:2383-4.
37. Osaki, S and S Anderson. Enzymatic determination of bilirubin. Clin Chem 1982;30:971. (Abstract)
38. Perry B, et al. Measurement of total bilirubin by use of bilirubin oxidase. Clin Chem 1986;32:329-32.
39. Koller A and Kaplan LA. Total serum protein. *In:* LA Kaplan and AJ Pesce, eds., Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. St Louis: The C.V. Mosby Company; 1989:1057-60.
40. Reigler E. Eine kolorimetrische Bestimmungsmethods des Eiweisses. Z Anal Chem 1914;53:242-5.
41. Weicheselbaum TE. An accurate and rapid method for determination of proteins in small amounts of blood serum and plasma. Am J Clin Path 1946;16:40-9.
42. Doumas BT, et al. A candidate reference method for determination of total protein in serum. I. Development and validation. Clin Chem 1981;27:1642-50.
43. Sampson, EJ MA Baird, CA Burtis, EM Smith, DL Witte, and DD Bayse. A coupled-enzyme equilibrium method for measuring urea in serum: optimization and evaluation of the AACC study group on urea candidate reference method. Clin Chem 1980;26: 816-826.
44. National Committee for Clinical Laboratory Standards (NCCLS). Procedures for Handling and Processing of Blood Specimens; tentative standard. NCCLS document H18-A2. Wayne, PA: NCCLS, 1999.
45. Overfield CV, Savory J, and Heintges MG. Glycosis: a re-evealuation of the effect on blood glucose. Clin Chim Acta 1972;39:35-40.
46. Rehak NN and Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem 1988;34:2111-14.
47. Sherwin JE and Obernolte R. Bilirubin. *In:*LA Kaplan and AJ Pesce, eds. Clinical Chemistry: Theory, Analysis and Correlation, 2nd ed. St. Louis: The C.V. Mosby Company; 1989;1009-1015.
48. Henry RJ, Canon DC and Winkelman. Clinical Chemistry Principles and Technics, 2nd ed. New York: Harper and Row; 1974;417-21; 127-8.
49. Scott MG, Electrolytes and Blood Gases. *In:* Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders Company, 1999:617-721.
50. Witte DL, Brown LF, and RL Williams. Effects of bilirubin on detection of hydrogen peroxide by use of peroxidase. Clin Chem 1978;24:1778-82.
51. Melnik J and Potter JL. Variance in capillary and venous glucose levels during glucose tolerance test. Am J Med Tech 1982;48:543-5.
52. National Committee for Clinical Laboratory Standards (NCCLS). Evaluation of precision performance of clinical chemistry devices; approved guideline NCCLS Document EP5-A. Wayne, PA: NCCLS, 1999.
53. National Committee for Clinical Laboratory Standards (NCCLS). Quality management for unit-use testing; proposed guideline. NCCLS Document EP18-P. Wayne, PA: NCCLS, 1999.