# Section 12 Methods Performance

## 12.1 Materials Required

The following materials are required to operate the VetScan® Chemistry System according to the recommended procedure. The procedure is described in Section 3.5.

| Materials | Catalog Number |
|---|---|
| ***Provided with Purchase of Reagent Rotor Profiles*** | |
| ■ VetScan Reagent Profile | See Section 12.2 for description |
| ■ 100 mL Pipette | |
| ***Optional*** | |
| ■ Tips, disposable | Distributor |
| ■ Transfer pipettes (to dispense samples) (1.5 mL extended fine tip, small bulb recommended) | |
| ■ Compressed air, can | Computer store |

## 12.2 Intended Use and Description of Reagents

**Critical Care Profile (Abaxis Catalog Number 500-0017 – 10 Pack)**
The VetScan® Critical Care Profile reagent rotor used with the VetScan Chemistry Analyzer, utilizes dry and liquid reagents to provide in-vitro quantitative determinations of alanine aminotransferase (ALT), bicarbonate, creatinine, glucose, potassium, sodium, and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

Each VetScan Critical Care reagent rotor contains dry test-specific reagent beads. A dry sample blank reagent (comprised of buffer, surfactants, excipients, and preservatives) is included in each rotor for use in calculating concentrations of ALT, glucose, and urea nitrogen. Dedicated sample blank reagents are used to determine creatinine levels. Each reagent rotor also contains a diluent consisting of surfactants, excipients, and preservatives.

**Diagnostic Profile Plus (Abaxis Catalog Numbers 500-0002 – 10 Pack and 500-0052 – 25 Pack)**
The VetScan Diagnostic Profile Plus reagent rotor, used with the VetScan Chemistry Analyzer, utilizes dry and liquid reagents to provide in-vitro quantitative determinations of alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), amylase, calcium, cholesterol, creatinine, glucose, potassium, total bilirubin, total protein, and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

Each VetScan Diagnostic 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 rotor for use in calculating concentrations of ALT, albumin, ALP, amylase, calcium, glucose, potassium, and urea nitrogen. Dedicated sample blank reagents are used to determine cholesterol, creatinine, total bilirubin, and total protein levels. Each reagent rotor also contains a diluent consisting of surfactants, excipients, and preservatives.

**Comprehensive Diagnostic Profile (Abaxis Catalog Numbers 500- 0038-10 – 10 Pack, and 500-0038-25 – 25 Pack)**
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, alkaline phosphatase (ALP), amylase, calcium, creatinine, globulin (GLOB, calculated value), glucose, potassium, phosphorus, sodium, total bilirubin, total protein, and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

Each VetScan Diagnostic Profile II reagent rotor contains dry test-specific reagent beads. A dry sample blank reagent (comprised of buffer, surfactants, excipients, and preservatives) is included in each rotor for use in calculating concentrations of albumin, ALP, calcium, glucose and phosphorous. Dedicated sample blank reagents are used to determine creatinine, total bilirubin, and total protein levels. Each reagent rotor also contains a diluent consisting of surfactants, excipients, and preservatives.

**Equine Profile (Abaxis Catalog Number 500-0014 10 Pack)**
The VetScan Equine Profile reagent rotor, used with the VetScan Chemistry Analyzer, utilizes dry and liquid reagents to provide in-vitro quantitative determinations of albumin, aspartate aminotransferase (AST), calcium, creatine kinase (CK), creatinine, gamma glutamyl transferase (GGT), glucose, total bilirubin, total protein, and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

Each VetScan Equine Profile reagent rotor contains dry test-specific reagent beads. A dry sample blank reagent (consisting of buffer, surfactants, excipients, and preservatives) is included in each rotor for use in calculating concentrations of ALT, albumin, ALP, AST, and GGT. Dedicated sample blank reagents are used to determine total bilirubin and total protein levels. Each reagent rotor also contains a diluent consisting of surfactants, excipients, and preservatives. Print space constraints made it necessary to change the reporting format for values of creatine kinase equal to or greater than 10,000 U/L and up to 14,000 U/L (10K to 14K).

| Measured Value (U/L) | Reported Value |
|---|---|
| 10,145 | 10K |
| 11,450 | 11K |
| 11,550 | 12K |

A result equal to or greater than 14,000 U/L will be reported as >14K.

**Large Animal Profile (Abaxis Catalog Number 500-0023 – 10 Pack)**
The VetScan Large Animal Profile reagent rotor, used with the VetScan Chemistry Analyzer, utilizes dry and liquid reagents to provide in-vitro quantitative determinations of albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium, creatine kinase (CK), gamma glutamyl transferase (GGT), globulin (GLOB, calculated value), magnesium, phosphorus (PHOS), total protein, and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

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 rotor for use in calculating concentrations of albumin, ALP, and calcium. Dedicated sample blank reagents are used to determine total protein levels. Each reagent rotor also contains a diluent consisting of surfactants, excipients, and preservatives.

**Prep Profile II (Abaxis Catalog Numbers 500-0026 – 10 Pack and 500-0256 – 25 Pack)**
The VetScan Prep Profile II reagent rotor, used with the VetScan Chemistry Analyzer, utilizes dry and liquid reagents to provide in-vitro quantitative determinations of alkaline phosphate (ALP), alanine aminotransferase

(ALT), creatinine, glucose, total protein, and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

Each VetScan Prep Profile II reagent rotor contains dry test-specific reagent beads. A dry sample blank reagent (consisting of buffer, surfactants, excipients, and preservatives) is included in each rotor for use in calculating concentrations of ALT, glucose, and urea nitrogen. Dedicated sample blank reagents are used to determine creatinine and total protein levels. Each reagent rotor also contains a diluent consisting of surfactants, excipients, and preservatives.

**Thyroxine-Cholesterol ($T_4$-Chol) Test (Abaxis Catalog Numbers 500-0037 – 10 Pack)**
The VetScan $T_4$/Cholesterol reagent rotor, used with the VetScan Chemistry Analyzer, utilizes dry and liquid reagents to provide in-vitro quantitative determinations of thyroxine $T_4$ and cholesterol in heparinized whole blood, heparinized plasma, or serum.

Each VetScan $T_4$/Cholesterol reagent rotor contains dry test-specific reagent beads. A dry sample blank reagent (consisting of buffer, surfactants, excipients, and preservatives) is included in each rotor for use in calculating sample indices. Each reagent rotor also contains a diluent consisting of surfactants, excipients, 8-anilino-1-naphthalenesulfonic acid (ANS), barbital, and preservatives.

**Avian/Reptilian Profile (Abaxis Catalog Number 500-0022 – 10 Pack)**
The VetScan Avian/Reptilian reagent rotor used with the VetScan Chemistry Analyzer utilizes dry and liquid reagents to provide in-vitro quantitative determinations of albumin (ALB), aspartate aminotransferase (AST), total calcium (CA++), creatine kinase (CK), globulin (GLOB, calculated value), phosphorus (PHOS), potassium (K+), sodium (NA+), total protein (TP), urea nitrogen (BUN), and Uric Acid (UA) in heparinized whole blood, heparinized plasma, or serum.

Each VetScan Avian/Reptilian 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.

## 12.3 Test Principles

**Alanine Aminotransferase (ALT)**
Alanine aminotransferase (ALT) has been measured by three methods. Two of these methods — the colorimetric dinitrophenylhydrazine coupling technique[1,2] and the fluorescent enzymatic assay — are rarely used[3]. An enzymatic method based on the work of Wróblewski and LaDue[4] is the most common technique for determining ALT concentrations in serum. A modified Wróblewski and LaDue procedure has been proposed as the recommended procedure of the International Federation of Clinical Chemistry (IFCC)[5].

The method used by Abaxis is a modification of the International Federation of Clinical Chemistry (IFCC) recommended procedure. In this reaction, ALT catalyzes the transfer of an amino group from L-alanine to $\alpha$-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.

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

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

The rate of change of the absorbance at 340 nm relative to the change at 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 (ALB)**
Early methods used to measure albumin include fractionation techniques[6-8] and tryptophan content of globulins[9-10]. These methods are unwieldy to perform and do not have a high specificity. Two immunochemical techniques are considered as reference methods, but are expensive and time consuming[11]. Dye-binding techniques are the most frequently used methods for measuring albumin. Bromcresol green (BCG)[12] is the most commonly used of the dye-binding methods. Measurement of albumin with BCG has been optimized[13]. The reliability of this method has been further optimized to reduce the impact of heparin[14].

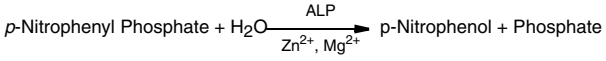
BCG that binds with albumin changes color from yellow to blue-green. The absorbance maximum changes with the color shift.

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

Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as the difference in absorbance between 630 nm and 500 nm.

**Alkaline Phosphatase (ALP)**
Techniques to measure alkaline phosphatase were first developed over 60 years ago. Several of these endpoint or two-point spectrophotometric methods[15,16] are now considered obsolete or too cumbersome. The use of *p*-nitrophenyl phosphate (*p*-NPP) increased the speed of the reaction[17,18]. The reliability of this technique was greatly increased by the use of a metal-ion buffer to maintain the concentration of magnesium and zinc ions in the reaction[19]. The American Association for Clinical Chemistry (AACC) reference method[20] uses *p*-NPP as a substrate and a metal-ion buffer.

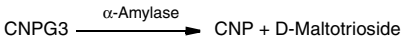
The Abaxis procedure is modified from the AACC and IFCC[21] methods. ALP hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate.




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

**Amylase (AMY)**
About 200 different tests have been developed to measure amylase. Most procedures use a buffered polysaccharide solution but employ different detection techniques. Viscosimetric methods are lacking in precision and accuracy[22], while turbidimetric and iodometric methods are difficult to standardize[23,24]. Commonly used are saccharogenic and chromolytic methods. The "classic" amylase measurement technique is a saccharogenic method[25], but is difficult and time-consuming[26]. Chromolytic methods using *p*-nitrophenylglycosides as substrates have been recently developed[27]. These assays have a higher specificity for pancreatic amylase than for salivary amylase and are easily monitored[27].

The substrate, 2-chloro-*p*-nitrophenyl 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.



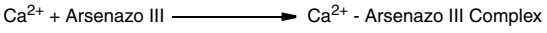

The reaction is measured bichromatically at 405 nm and 500 nm. The change in absorbance due to the formation of CNP is directly proportional to α-amylase activity in the sample.

**Aspartate Aminotransferase (AST)**
The aspartate aminotransferase test is based on the Karmen rate method[28] as modified by Bergmeyer[29]. The current IFCC reference method utilizes the Karmen/Bergmeyer technique of coupling malate dehydrogenase (xxH) and reduced nicotinamide dinucleotide (NADH) in the detection of AST in serum[29,30]. Lactate dehydrogenase (LDH) is added to the reaction to decrease interference caused by endogenous pyruvate.

AST 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 xxH.


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

**Calcium (Ca++)**
The first methods used to analyze calcium involved precipitating calcium with an excess of anions[31-33]. Precipitation methods are laborious and often imprecise. The reference method for calcium is atomic absorption spectroscopy; however, this method is not suited for routine use[34].Spectrophotometric methods using either *o*-cresolphthalein complexone (CPC) or arsenazo III metallochromic indicators are most commonly used[35-37]. Arsenazo III has a high affinity for calcium and is not temperature dependent as is CPC.



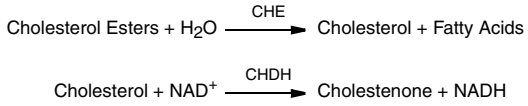

Calcium in the patient sample binds with arsenazo III to form a calcium-dye complex.

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.

**Cholesterol (CHOL)**
Total cholesterol assays measure both free and esterified cholesterol. The first cholesterol determinations were made using the Liebermann-Burchard method[38,39]. The Liebermann-Burchard reaction overestimates esterified cholesterol, giving artificially high total cholesterol levels. Abell, et al[40] developed a three-step method for the determination of free cholesterol. This method is labor intensive but is the current accepted reference method[41]. Iron-salt and *p*-toluene-sulfonic acid procedures have been developed[42,43] but are rarely used.

Today, the most common tests employ enzymatic endpoint reactions. These simple procedures typically use cholesterol esterase and cholesterol oxidase with a Trinder finish[44,45]. Abaxis has developed an enzymatic method that uses cholesterol dehydrogenase in place of cholesterol oxidase. The use of cholesterol dehydrogenase eliminates the Trinder reaction, thus avoiding interference from physiological analytes such as bilirubin and hemoglobin.

Cholesterol esterase (CHE) hydrolyzes cholesterol esters and $H_2O$ to form cholesterol and fatty acids. The cholesterol is oxidized by cholesterol dehydrogenase (CHDH) to cholestenone and the nicotinamide adenine dinucleotide ($NAD^+$) is reduced to NADH.




Absorbance is measured bichromatically at 340 nm and 405 nm. A dedicated sample blank is also measured to ensure no extraneous reactions interfere with the calculations of cholesterol levels. The production of NADH in this endpoint reaction is directly proportional to the amount of cholesterol present in the sample.

**Creatine Kinase (CK)**
Creatine kinase (CK) catalyzes the reversible phosphorylation of creatine by adenosine triphoshate (ATP). The phosphorylation reaction is favored by alkaline conditions (optimum at pH 9.0) and the dephosphorylation reaction is favored by acidic conditions (optimum at pH 6.5 at

37 ºC). Early CK measurement methods were based on the "forward reaction" with creatine phosphate and adenosine diphosphate (ADP) as the products[46-48].The sensitivity of these tests was shown to be low due to problems with interferences. The procedure of choice utilizes the reverse reaction coupled with a reaction to produce NADPH, which is directly related to CK levels[49-51].

The CK measurement procedure used by Abaxis is a modified version of the International Federation of Clinical Chemistry (IFCC) method[52]. Key modifications are sample volume fraction, buffer, and temperature. N-acetyl cysteine (NAC) has been added to reactivate the CK[53]. 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$^1$) 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 ATP from creatine phosphate and ADP at pH 6.7. With hexokinase as a catalyst, ATP reacts with D-glucose to form A D P and D-glucose-6-phosphate (G-6-P), which is reacted with nicotinamide adenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate (NADP in the presence of glucose-6-phosphate dehydrogenase [G-6-PDH]) to produce G-6-P and NADPH.

$$\text{Creatine Phosphate + ADP} \xrightarrow[Mg^{2+}]{CK} \text{Creatine + ATP}$$

$$\text{ATP + D-glucose} \xrightarrow[Mg^{2+}]{\text{Hexokinase}} \text{ADP + G-6-P}$$

$$\text{G-6-P + NADP} \xrightarrow{CHDH} \text{6-Phosphogluconate + NADPH + } H^+$$

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.

**Creatinine (CRE)**
The Jaffé 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 Jaffé technique to increase the specificity of the reaction[54,55]. Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffé technique[56-58]. Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase[59].

In the coupled enzyme reactions, creatinine amidohydrolase hydrolyzes creatinine to creatine. A second enzyme, creatine amidinohydrolase, catalyzes the formation of sarcosine from creatine. Sarcosine oxidase causes the oxidation of sarcosine to glycine, formaldehyde and hydrogen peroxide ($H_2O_2$). In a Trinder finish, peroxidase catalyzes the reaction among the hydrogen peroxide, 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA), and 4-aminoantipyrine (4-AAP) into a red quinoneimine dye. Potassium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimize the potential interference of bilirubin and ascorbic acid, respectively.

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

$$\text{Creatinine + } H_2O \xrightarrow{\text{Creatinine Amidohydrolase}} \text{Sarcosine + Urea}$$

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

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

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 630 nm.

**Gamma Glutamyl Transferase (GGT)**
The first quantitative methods developed to measure gamma glutamyl transferase (GGT) involved a second reaction to form an azo dye that combined with a chromophore[60,61].The change to L-γ-glutamyl-*p*-nitroanilide as the substrate in the reaction eliminated the dye-formation step[62]. Due to the poor solubility and stability of L-γ-glutamyl-*p*-nitroanilide, this procedure was modified to use the substrate L-γ-glutamyl-3-carboxy-4-nitroanilide[63].The International Federation of Clinical Chemistry (IFCC) recommended GGT method is based on the latter substrate, with glycylglycine as the other substrate[64].

Abaxis has modified the IFCC method to react at 37 ºC. 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.

$$\text{L-γ-glutamyl-3-carboxy-4-nitroanilide + Gly-gly} \xrightarrow{GGT} \text{Glu-gly-gly + 3-carboxy-4-nitroaniline}$$

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

**Glucose (GLU)**
Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu[65] and Somogyi-Nelson[66,67]). 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 VetScan Diagnostic Profile Plus is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method[68].

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-phospho-gluconate and the reduction of nicotinamide adenine dinucleotide ($NAD^+$) to NADH.

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

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

The absorbance is measured bichromatically at 340 nm and 850 nm. The production of NADH is directly proportional to the amount of glucose present in the sample.

**Magnesium (MG)**

Atomic absorption spectrometry[117] is the reference method for magnesium in serum. Methods that are more adaptable to our instrumentation are the colorimetric methods xylidyl blue and calmagite, and the enzymatic methods phosphoglucomutase activation[118] and hexokinase activation[119]. We investigated these methods extensively and found that the hexokinase activation method is best fit for our system in terms of sensitivity, precision, and accuracy.

The enzymatic magnesium method can be written as follows:

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

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

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)**

Historically, the molybdenum blue method of Fiske and SubbaRow[120] and various modifications are widely used for inorganic phosphorus (Pi) determination. With proper blanking, this is considered a reliable method. Several enzymatic methods for phosphorus determination have been published[121,122,123,124,125]. Among these is a method that uses sucrose phosphorylase (SP) coupled through phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G-6-PDH)[125]. This method was the most applicable to the Abaxis system. In this enzymatic system, one mole of phosphate from the sample will produce one mole of NADH. The amount of NADH formed can be measured as an endpoint reaction at 340 nm. The method principle used is:

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

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

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

**Potassium (K+)**

Potassium is typically measured using flame atomic emission spectroscopy (FAES) and ion-selective electrode potentiometry (ISE). The FAES technique measures potassium concentration[69,70] and is the currently accepted reference method[71]. ISE methods quantitate potassium on activity[72,73] and have been adapted for use with whole blood on modified automated analyzers[74].Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. An enzymatic method based on the activation of pyruvate kinase with potassium shows excellent linearity and negligible susceptibility to endogenous substances [75-77]. Interference from sodium and ammonium ion are minimized with the addition of Krypotofix and glutamate dehydrogenase, respectively[75].

In the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to $NAD^+$.

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

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

The rate of change of 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 potassium in the sample.

**Sodium (Na+)**

Sodium can be measured using atomic absorption spectrophotometry (AAS), flame atomic emission spectroscopy (FAES), ion-selective electrode potentiometry (ISE), and absorbance spectrophotometry. The FAES technique measures sodium concentration[69,70] and is the currently accepted reference method[78]. ISE methods quantitate sodium ion activity[72,73] and have been adapted for use on most automated analyzers. Colorimetric[79,80] and enzymatic[81] methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation. In the enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of *o*-nitrophenyl-β-D-glactopyranoside (ONPG) to *o*-nitrophenol and galactose.

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

The rate of change of the absorbance difference between 405 nm and 550 nm, caused by the conversion of ONPG to *o*-nitrophenol, is proportional to the concentration of sodium in the sample.

**Thyroxine (T4)**

The first clinically feasible direct method to measure thyroxine was a competitive protein-binding assay (CPBA) developed by Murphy & Pattee in the early 1960s[82]. Radio immunoassay techniques, with higher sensitivity and specificity, largely replaced CPBA[83]. Concerns about radioactive waste and potential health hazards helped prompt the development of non-isotopic tests such as enzyme and fluorescence immunoassays. Enzyme immunoassays (EIAs) for thyroxine have been shown to have, at clinically important levels, accuracy and precision equivalent to automated RIA procedures[84].An isotope dilution-mass spectrometric procedure has been proposed as a reference method[85], but is very complicated and labor-intensive.

Abaxis has adapted a commercially available EIA method for use on the VetScan Chemistry Analyzer. In the reaction, barbital and 8-anilino-1-naphthalene sulfonic acid (ANS) causes the release of endogenous T4 from the binding proteins. The released endogenous T4 competes for antibody (Ab) binding sites with T4 labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH-T4 conjugate). G6PDH-T4 conjugate bound to antibody has lower activity than does unbound conjugate. As the binding of endogenous T4 increases, the amount of the unbound enzyme conjugate increases. The active enzyme reduces nicotinamide adenine dinucleotide ($NAD^+$) to NADH.

$$T_4 + T_4\text{ - G6PDH} + NAD^+ \xrightarrow{Ab} \text{Ab:T4} + \text{Ab:T4 - G6PDH} + \text{NADH}$$

The rate of change of the absorbance at 340 nm is due to the conversion of $NAD^+$ to NADH, and is directly proportional to the amount of endogenous $T_4$ in the sample.

**Total Bilirubin (TBIL)**

Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid[86,87]. A newer, more specific method has been developed using the enzyme bilirubin oxidase[88-90]. In addition to using the more specific total bilirubin test method, photodegradation of the analyte is minimized in the VetScan System because the sample can be tested immediately after collection.

In the enzyme procedure, bilirubin is oxidized by bilirubin oxidase into biliverdin. The final reaction is the conversion of biliverdin into various purple compounds.

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

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.

**Total Carbon Dioxide (tCO2)**

Total carbon dioxide in serum or plasma exists as dissolved carbon dioxide, carbamino derivatives of proteins, bicarbonate and carbonate ions, and carbonic acid. Total carbon dioxide can be measured by pH indicator[91], $CO_2$ electrode, and spectrophotometric enzymatic methods, which all produce accurate and precise results[92]. The reference method manometrically measures liberated $CO_2$ gas, but is rarely used[93]. The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

$$\text{PEP} + HCO_3^- \xrightarrow{\text{PEPC}} \text{Oxaloacetate} + \text{Phosphate}$$

$$\text{Oxaloacetate} + \text{NADH} \xrightarrow{\text{MDH}} NAD^+ + \text{Malate}$$

In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide ($CO_2$) toward bicarbonate ($HCO_3^-$). Phosphoenolpyruvate (PEP) and $HCO_3^-$ then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (xxH) catalyzes the reaction of oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH) to $NAD^+$ and malate.

**Total Protein (TP)**

The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity[94]. Originally developed by Riegler[95] and modified by Weichselbaum[96], Doumas, et al[97] proposed a biuret reaction as a candidate total protein reference method.

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 autoreduction of copper, respectively[96].The Cu(II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-protein complex.

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

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 (BUN)**

Urea can be measured both directly and indirectly. The diacetyl monoxime reaction, the only direct method to measure urea, is commonly used but employs dangerous reagents[98]. Indirect methods measure ammonia created

from the urea; the use of the enzyme urease has increased the specificity of these tests[99]. The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique[100,101], and coupled enzymatic reactions[102,103]. Catalyzed Berthelot procedures, however, are erratic when measuring ammonia[104]. Coupled-enzyme reactions are rapid, have a high specificity for ammonia, and are commonly used. One such reaction has been proposed as a candidate reference method[105].

In the coupled-enzyme reaction, urease hydrolyzes urea into ammonia and carbon dioxide. Upon combining ammonia with 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to $NAD^+$.

$$\text{Urea} + H_20 \xrightarrow{\text{Urease}} NH_3 + CO_2$$

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

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.

**Uric Acid**
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 (H202), 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.

$$\text{Uric Acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{DHBSA} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

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.

## 12.4 Warnings and Precautions

• 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.

• The reagent rotors are plastic and may crack or chip if dropped. Never use a dropped rotor, as it may spray biohazardous material throughout the interior of the analyzer.

• 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.

• Calcium reagent beads contain arsenazo III sodium, a possible carcinogen. The operator does not come into contact with the reagent beads when following the recommended procedures. If the operator does come into contact with the beads, immediately flush eyes or skin with copious amounts of water for 15 minutes. If inhaled, remove to fresh air. If swallowed, wash out mouth with water. Call a physician.

## 12.5 Storage

Store reagent rotors in their sealed pouches at 2–8 ºC (36–46 ºF). To use reagent rotors, remove the rotors in their sealed foil pouches from the refrigerator. Open the pouch, and remove the rotor just prior to running the test.

Ensure that the total time rotors are at room temperature does not exceed 48 hours. Open the pouch and remove the rotor just prior to running the test.

Do not expose rotors, in or out of the foil pouches, to direct sunlight or to temperatures above 32 ºC (90 ºF). Rotors must be used within 20 minutes of opening the pouches; rotors in opened pouches cannot be returned to the refrigerator for later use.

## 12.6 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.

## 12.7 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 collection tubes should be filled at least halfway to prevent an excessive concentration of anti-coagulant in the patient sample.

• Samples 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 homogeneous before transferring a sample to the reagent rotor. Gently invert the collection tube several times just prior to sample transfer. Do **not** shake the collection tube; shaking can cause hemolysis.

• The test must be begun **within 10 minutes** of transferring the sample into the reagent rotor.

• Whole blood venipuncture samples should be run within 60 minutes of collection[106]. **Glucose** concentrations decrease approximately 5–12 mg/dL in 1 hour in uncentrifuged samples stored at room temperature[107]. **AST** samples may be artificially high due to in-vitro hemolysis in stored whole blood[108]. The sample may be separated into plasma or serum and stored in capped sample tubes at 2–8 ºC (36–46 ºF) if the sample can not be run within 60 minutes.

• **Total protein** and **albumin** results from hay-eating horses may be altered if the sample has been taken within a few hours of the last meal[109].

• **Total bilirubin** results may be adversely affected by photodegradation[110]. 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[111].

• **Glucose** concentrations are affected by the length of time since the patient has last eaten and by the type of sample collected from the patient. To accurately interpret glucose results, samples should be obtained from a patient who has been fasting for at least 12 hours. Glucose concentrations in plasma and serum are typically higher than glucose levels in whole blood[109].

## 12.8 Known Interfering Substances

• 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. If the presence of one or more interferents causes a significant change in the concentration of a particular analyte, that result is suppressed. See Section 7, “Troubleshooting” for an explanation of sample indices and suppressed results.

• Amylase activity may be elevated due to contamination of the sample with human salivary amylase. Salivary amylase is found in human saliva, sweat glands, and lungs[112].

• Potassium levels are affected by the presence of high levels of platelets (>1,000,000/µL) or white blood cells (>200,000/µL). Potassium may be released from these blood constituents when the blood is allowed to clot. This effect may also be observed when abnormal cells are present[113].

• Potassium levels in canines, felines, and equines may be artificially increased due to potassium released from platelets during clotting[108,114]. Normal canine and feline red blood cells do not contain significant levels of potassium, with the exception of the Akita breed[114]. Potassium levels in equines will be artificially increased by hemolysis.

• Sodium results in canines, felines, and equines may be artificially depressed due to hyperlipidemia or severe hyperproteinemia[108,114].

## 12.9 Test Parameters

The VetScan® Chemistry Analyzer operates at ambient temperatures between 15–32 ºC (59–90 ºF). The analysis time for all of the VetScan Rotors is <15 minutes. The analyzer maintains the reagent rotor at a temperature of 37 ºC (98.6 ºF) over the measurement interval.

## 12.10 Test Procedure

***Note: Complete step-by-step operating procedures are detailed in Section 3.5.***

1. Remove a reagent rotor from the refrigerator. (The rotor can be used directly from the refrigerator. There is no need to warm up the rotor.)

2. Collect the specimen.

3. Remove the reagent rotor from its pouch. Expel any air or air bubbles from the tip of the sample transfer device before dispensing 90–120 µL of sample or control into the rotor through the sample port. A 90 µL sample forms a line between the two arrows etched on the rotor. If there are air bubbles in the chamber, add more sample (up to a total of 120 µL).

***WARNING: Do not tap the rotor on the table or work bench to empty the sample port, as this may damage the rotor.***

4. Press **OPEN** to open the drawer. Place the rotor in the round cavity of the drawer and press **CLOSE**. There is no need to orient the rotor in a particular direction in the drawer. The analysis begins when the drawer closes.

5. Input patient and operator identification numbers as directed by the messages on the analyzer display. The analyzer processes the sample with no further input from the operator. The entire analysis takes

<15 minutes and is complete when the “analysis complete” message appears on the display.

6. The results are stored in the analyzer. Print results on the result card provided, transmit to a computer, or press **OPEN** to bypass this step. To print, insert the result card in the result card slot and remove it when directed to by the message on the display. To transmit, follow the instructions in Section 10, “Connecting to an External Computer” to set up the computer.

7. The drawer automatically opens. Remove the rotor from the drawer and follow standard hazardous waste disposal procedures when disposing the rotor. The rotor may be placed back into the pouch before disposal. Press **CLOSE** if another reagent rotor is not to be run immediately.

## 12.11 Timing Considerations

• Analyze whole blood samples collected by venipuncture within 60 minutes of collection.

• Run the reagent rotor within 10 minutes of applying sample or control to the rotor.

• Dispense samples collected in micropipettes into the rotor immediately after collection.

## 12.12 Quality Control

Although elaborate quality controls are integrated into the VetScan Chemistry Analyzer and Reagent Profile Rotors, performance of the VetScan may also be verified by running external controls. This can be accomplished by testing an aliquoted sample with known values or by means of a serum-based commercially available control. See Section 4.11 to run controls.

## 12.13 Calibration

The VetScan Chemistry Analyzer is calibrated by the manufacturer before shipment. The bar code printed on the bar code ring provides the analyzer with rotor-specific calibration data. See Section 6 for details.

## 12.14 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 Section 8. Results are printed onto result cards supplied by Abaxis. The result cards have an adhesive backing for easy placement in the patients file.

The VetScan Chemistry Analyzer calculates the globulin concentration using the total protein and albumin concentrations determined by the chemical analyses. If either the total protein or albumin results are out of range or suppressed, the globulin concentration can not be reliably calculated and is not reported.

## 12.15 Messages Printed on the Result Card

• Results outside the reference range are indicated on the result card by an asterisk ( * ) printed next to the analyte concentration.

• Results outside the dynamic range are indicated on the result card by a greater than symbol ( > ) printed next to the highest value of the dynamic range or a less than symbol ( < ) printed next to the lowest value of the dynamic range. (For example, concentrations outside an analyte dynamic range are printed as >700 or <10, respectively.)

• A row of diamonds (◆◆◆) is printed in place of numbers when a result can not be determined. A result may be suppressed due to improper mixing of a reagent bead with diluted heparinized plasma, a nonlinear reaction, an endpoint of a particular reaction not reached, or a concentration outside the capabilities of the system.

• “HEM”, “LIP”, or “ICT” is printed in place of the analyte concentration if hemolysis (HEM), lipemia (LIP), or icterus (ICT) adversely affects the results for that analyte. LIP is also printed if both lipemia and icterus affect a result. HEM is also printed if hemolysis and icterus, hemolysis and lipemia, or hemolysis, lipemia, and icterus affect a particular analyte. Examine the sample indices to determine if more than one interferent is affecting a particular result.

• The sample indices are printed on the bottom of the result card. The indices indicate the degree of hemolysis, icterus, and lipemia found in the sample. Hemolysis, icterus, and lipemia are measured on a scale of 0 (clear), 1+ (slight), 2+ (moderate), and 3+ (gross).

## 12.16 Limitations of Procedure

• Lithium heparin is the only anticoagulant recommended for use with the VetScan Chemistry Analyzer.

• 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.

• If sample or reagent rotor errors are indicated on the analyzer display or printed out on the result card, consult Section 7 for an explanation of the error message.

• Samples with hematocrits in excess of 62–64% packed red cell volume (a volume fraction of 0.62–0.64) or that are hemolytic, lipemic, or icteric may give inaccurate results. Samples with high hematocrits may be reported on the result card as being hemolyzed. If hemolysis, lipemia, or icterus adversely affect results, a message will be printed on the result card in place of the analyte concentration (see Section 7).

## 12.17 Expected Values

These reference ranges are provided as a guideline. The most definitive reference ranges are those established for the patient population. Test results should be interpreted in conjunction with the patient's clinical signs. Potassium and total protein levels determined in plasma may differ from the ranges given below[102, 83].

**Table 1: Canine, Feline, Equine and Bovine Ranges**

| Analyte | Canine | Feline | Equine | Bovine |
|---|---|---|---|---|
| **ALT** | 10 – 118 U/L | 20 – 100 U/L | 5 – 20 U/L | N/A |
| **Albumin (ALB)** | 2.5 – 4.4 g/dL* (25 – 44 g/L) | 2.7 – 4.5 g/dL* (27 – 45 g/L) | 2.2 – 3.7 g/dL (22 – 37 g/L) | 2.5 – 3.8 g/dL (25 – 38 g/L) |
| **ALP** | 20 – 150 U/L | 10 – 90 U/L | 50 – 170 U/L | 23 – 135 U/L |
| **Amylase (AMY)** | 200 – 1200 U/L* | 300 – 1100 U/L* | 5 – 15 (U/L) | N/A |
| **AST** | 14 – 45 U/L | 12 – 43 U/L | 175 – 340 U/L | 66 – 211 U/L |
| **Calcium (Ca++)** | 8.6 – 11.8 mg/dL* (2.15 – 2.95 mmol/L) | 8.0 – 11.8 mg/dL* (2.00 – 2.95 mmol/L) | 11.5 – 14.2 mg/dL (2.8 – 3.55 mmol/L) | 7.9 – 9.6 mg/dL (1.97 – 2.39 mmol/L) |
| **Cholesterol (CHOL)** | 125 – 270 mg/dL (3.2 – 7.0 mmol/L) | 90 – 205 mg/dL (2.3 – 5.3 mmol/L) | 50 – 140 mg/dL (1.3 – 3.6 mmol/L) | N/A |
| **Creatine Kinase (CK)** | 20 – 200 U/L | 50 – 450 U/L | 120 – 470 U/L | 83 – 688 U/L |
| **Creatinine (CRE)** | 0.3 – 1.4 mg/dL** (27 – 124 µmol/L) | 0.3 – 2.1 mg/dL* (27 – 186 µmol/L) | 0.4 – 1.7 mg/dL (35 – 150 µmol/L) | N/A |
| **GGT** | 0 – 7.0 U/L | 0 – 2.0 U/L | 5 – 24 U/L | 12 – 48 U/L |
| **Globulin†** | 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) | 4.4 – 5.5 g/dL (44 – 55 g/L) |
| **Glucose (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) | N/A |
| **Magnesium (MG)** | 1.8 – 2.4 mg/dl (0.74 – 0.99 mmol/L) | 2.0 – 2.5 mg/dL (0.82 – 1.03 mmol/L) | 1.7 – 2.9 mg/dL (0.70 – 1.19 mmol/L) | 1.7 – 2.9 mg/dL (0.70 – 1.19 mmol/L) |
| **Phosphorus** | 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) | 4.1 – 9.2 mg/dL (1.32 – 2.97 mmol/L) |
| **Potassium (K+)** | 3.7 – 5.8 mmol/L | 3.7 – 5.8 mmol/L | 2.5 – 5.2 mmol/L | N/A |
| **Sodium (Na+)** | 138 – 160 mmol/L | 142 – 164 mmol/L | 126 – 146 mmol/L | N/A |
| **Thyroxine (T4)** | 1.1 – 4.0 g/dL (14 – 52 nmol/L) | 1.5 – 4.8 µg/dL (19 – 62 nmol/L) | 1.0 – 2.8 g/dL (13 – 36 nmol/L) | N/A |
| **Total Bilirubin (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 (8.6 – 39.3 µmol/L) | N/A |
| **Total Carbon Dioxide (TCO2)** | 12 – 27 mmol/L | 15 – 24 mmol/L | 20 – 33 mmol/L | N/A |
| **Total Protein (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) | 6.6 – 9.3 g/dL (66 – 93 g/L) |
| **Urea Nitrogen (BUN)** | 7 – 25 mg/dL* (2.5 – 8.9 mmol urea/L) | 10 – 30 mg/dL (3.6 – 10.7 mmol urea/L) | 7 – 25 U/L (2.5 – 8.9 mmol urea/L) | 6 – 20 mg/dL (2.14 – 7.14 mmol urea/L) |
| **Uric Acid (UA)** | 1.0 – 9.0 mg/dL (60 – 536 µmol/L) | 1.0 – 9.0 mg/dL (60 – 536 µmol/L) | 1.0 – 9.0 mg/dL (60 – 536 µmol/L) | N/A |

* The ranges cited by Meyer, et al[115] for dogs and cats have been adjusted to reflect field data collected by Abaxis on the VetScan® Chemistry Analyzer.

** Creatinine values have been lowered by 0.2 mg/dL from those given in Meyer, et al[115]. The Jaffé method measures creatinine as well as "quasi-creatinine" compounds that are not measured in the enzymatic method used in the VetScan Diagnostic Profile Plus[59].

† Calculated value.

## 12.18 Performance Characteristics

**Linearity**
The chemistry for each analyte is linear over the dynamic range listed in Table 2 when the VetScan® System is operated according to the recommended procedure (see Section 3).

**Table 2: VetScan Dynamic Ranges**

| | Dynamic Range | |
|---|---|---|
| **Analyte** | **Common Units** | **SI Units** |
| **ALT** | 5 – 2000 U/L | 5 – 2000 U/L |
| **Albumin (ALB)** | 1.0 – 6.5 g/dL | 10 – 65 g/L |
| **ALP** | 5 – 2400 U/L | 5 – 2400 U/L |
| **Amylase (AMY)** | 5 – 4000 U/L | 5 – 4000 U/L |
| **AST** | 5 – 2000 U/L | 5 – 2000 U/L |
| **Calcium (Ca++)** | 4.0 – 16.0 mg/dL | 1.0 – 4.0 mmol/L |
| **Cholesterol (CHOL)** | 20 – 520 mg/dL | 0.5 – 13.5 mmol/L |
| **Creatine Kinase (CK)** | 5 – 14000 U/L (14K) | 5 – 14000 U/L (14K) |
| **Creatinine (CRE)** | 0.2 – 20.0 mg/dL | 18 – 1768 µmol/L |
| **GGT** | 5 – 3000 U/L | 5 – 3000 U/L |
| **Glucose (GLU)** | 10 – 700 mg/dL | 0.6 – 38.9 mmol/L |
| **Magnesium (MG)** | 0 – 8 mg/dL | 0 – 3.29 mmol/L |
| **Phosphorus (PHOS)** | 0.2 – 20 mg/dL | 0.06 – 6.46 mmol/L |
| **Potassium (K+)** | 1.5 – 8.5 mmol/L | 1.5 – 8.5 mmol/L |
| **Sodium (Na+)** | 110 – 180 mmol/L | 110 – 180 mmol/L |
| **Thyroxine (T4)** | 0.5 – 8.0 µg/dL | 6 – 103 nmol/L |
| **Total Bilirubin (TBIL)** | 0.1 – 30.0 mg/dL | 2 – 513 µmol/L |
| **Total Carbon Dioxide (TCO2)** | 10 – 40 mmol/L | 10 – 40 mmol/L |
| **Total Protein (TP)** | 2.0 – 14.0 g/dL | 20 – 140 g/L |
| **Urea Nitrogen (BUN)** | 2 – 180 mg/dL | 0.7 – 64.3 mmol urea/L |
| **Uric Acid (UA)** | 0.3 – 25 mg/dL | 18 – 1488 mmol/L |

**Table 3: VetScan® System Ranges**

| | System Ranges | |
|---|---|---|
| **Analyte** | **Common Units** | **SI Units** |
| **ALT** | -15 – 5000 U/L | -15 – 5000 U/L |
| **Albumin (ALB)** | -0.2 – 10.0 g/dL | -2 – 100 g/L |
| **ALP** | -20 – 4000 U/L | -20 – 4000 U/L |
| **Amylase (AMY)** | -5 – 5500 U/L | -5 – 5500 U/L |
| **AST** | -15 – 5000 U/L | -15 – 5000 U/L |
| **Calcium (Ca++)** | -4.0 – 20.0 mg/dL | -1.0 – 5.0 mmol/L |
| **Cholesterol (CHOL)** | -10 – 800 mg/dL | -0.3 – 20.7 mmol/L |
| **Creatine Kinase (CK)** | -5 – 15000 U/L (15K) | -5 – 15000 U/L (15K) |
| **Creatinine (CRE)** | -0.5 – 30.0 mg/dL | -44 – 2652 µmol/L |
| **GGT** | -5 – 5500 U/L | -5 – 5500 U/L |
| **Glucose (GLU)** | -0.5 – 1200 mg/dL | 0.0 – 66.6 mmol/L |
| **Magnesium (MG)** | -2.0 – 10.0 mg/dL | -0.82 – 4.11 mmol/L |
| **Phosphorus (PHOS)** | -0.5 – 30.0 mg/dL | -0.16 – 9.69 mmol/L |
| **Potassium (K)** | 0.0 – 12.0 mmol/L | 1.5 – 8.5 mmol/L |
| **Sodium ($Na^+$)** | 100 – 180 mmol/L | 100 – 180 mmol/L |
| **Thyroxine (T4)** | -1.0 – 10.0 µg/dL | -13 – 129 nmol/L |
| **Total Bilirubin (TBIL)** | -1.5 – 35.0 mg/dL | -26 – 599 µmol/L |
| **Total Carbon Dioxide (TCO2)** | 2 – 45 mmol/L | 2 – 45 mmol/L |
| **Total Protein (TP)** | -0.5 – 15.0 g/dL | -5 – 150 g/L |
| **Urea Nitrogen (BUN)** | -2 – 600 mg/dL | -0.7 – 214.2 mmol urea/L |
| **Uric Acid (UA)** | -0.3 – 50 mg/dL | -18 – 1488 mmol/L |

**Note:** The analyzer does not print negative values. If the recovered value is less than zero, then “0*”, “0.0*”, or “0.00*” is printed. The analyzer does not print values outside of the system range — for example, if the analyzer calculated a T4 value of 12 µg/dL, it would print “>10 µg/dL”.

**Precision**

Results for within-run and total precision were determined by field testing two controls using NCCLS EPS-T2 guidelines[126]. The controls were chosen for their analyte concentrations relative to the reference ranges and dynamic ranges. Moni-trol controls are from Dade International, Inc., PAR controls are from Medical Analisys System, Inc., and Precitrol controls are from Boheringer Manheim.

**Table 4: Precision (N = 80)**

| Analyte | | Within-Run (n = 80) | Total (n = 80) |
|---|---|---|---|
| **Alanine Amino-transferase (U/L)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 21 | 21 |
| | SD | 2.76 | 2.79 |
| | %CV | 13.1 | 13.3 |
| Moni-Trol 2 | | | |
| | Mean | 52 | 52 |
| | SD | 2.70 | 3.25 |
| | %CV | 5.2 | 6.3 |
| **Albumin (g/dL)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 3.9 | 3.9 |
| | SD | 0.13 | 0.14 |
| | %CV | 3.3 | 3.6 |
| Moni-Trol 2 | | | |
| | Mean | 2.3 | 2.3 |
| | SD | 0.09 | 0.10 |
| | %CV | 3.9 | 4.3 |
| **Alkaline Phosphatase (ALP) (U/L)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 39 | 39 |
| | SD | 1.81 | 2.29 |
| | %CV | 4.6 | 5.9 |
| Moni-Trol 2 | | | |
| | Mean | 281 | 281 |
| | SD | 4.08 | 8.75 |
| | %CV | 1.5 | 3.1 |
| **Amylase (U/L)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 46 | 46 |
| | SD | 2.40 | 2.63 |
| | %CV | 5.2 | 5.7 |
| Moni-Trol 2 | | | |
| | Mean | 300 | 300 |
| | SD | 11.15 | 11.50 |
| | %CV | 3.7 | 3.8 |
| **AST (U/L)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 47 | 47 |
| | SD | 0.98 | 0.92 |
| | %CV | 2.1 | 2.0 |
| Moni-Trol 2 | | | |
| | Mean | 145 | 145 |
| | SD | 1.83 | 1.70 |
| | %CV | 1.3 | 1.2 |

**Table 4: Precision (N = 80) (Continued)**

| Analyte | | Within-Run (n = 80) | Total (n = 80) |
|---|---|---|---|
| **Calcium (mg/dL)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 8.6 | 8.6 |
| | SD | 0.21 | 0.25 |
| | %CV | 2.4 | 2.9 |
| Moni-Trol 2 | | | |
| | Mean | 11.8 | 11.8 |
| | SD | 0.39 | 0.40 |
| | %CV | 3.3 | 3.4 |
| **Cholesterol (mg/dL)** | | | |
| PAR 2 | | | |
| | Mean | 204 | 204 |
| | SD | 6.64 | 6.84 |
| | %CV | 3.3 | 3.4 |
| PAR 3 | | | |
| | Mean | 275 | 275 |
| | SD | 6.46 | 8.00 |
| | %CV | 2.3 | 2.9 |
| **Creatine Kinase (U/L)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 105 | 105 |
| | SD | 2.89 | 3.74 |
| | %CV | 2.8 | 3.6 |
| Moni-Trol 2 | | | |
| | Mean | 469 | 469 |
| | SD | 12.23 | 28.32 |
| | %CV | 2.6 | 6.0 |
| **Creatinine (mg/dL)** | | | |
| Precitrol-N | | | |
| | Mean | 1.1 | 1.1 |
| | SD | 0.14 | 0.14 |
| | %CV | 12.7 | 12.7 |
| Precitrol-A | | | |
| | Mean | 5.2 | 5.2 |
| | SD | 0.23 | 0.27 |
| | %CV | 4.4 | 5.2 |
| **GGT (U/L)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 25 | 25 |
| | SD | 0.59 | 0.74 |
| | %CV | 2.4 | 3.0 |
| PAR 3 | | | |
| | Mean | 106 | 106 |
| | SD | 1.52 | 2.29 |
| | %CV | 1.4 | 2.2 |

**Table 4: Precision (N = 80) (Continued)**

| Analyte | | Within-Run (n = 80) | Total (n = 80) |
|---|---|---|---|
| **Glucose (mg/dL)** | | | |
| Precitrol-N | | | |
| | Mean | 66 | 66 |
| | SD | 0.76 | 1.03 |
| | %CV | 1.2 | 1.6 |
| Precitrol-A | | | |
| | Mean | 278 | 278 |
| | SD | 2.47 | 3.84 |
| | %CV | 0.9 | 1.4 |
| **Magnesium (mg/dL)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 4.9 | 4.9 |
| | SD | 0.07 | 0.07 |
| | %CV | 1.4 | 1.4 |
| Moni-Trol 2 | | | |
| | Mean | 2.0 | 2.0 |
| | SD | 0.04 | 0.04 |
| | %CV | 2.0 | 2.1 |
| **Phosphorus (mg/dL)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 6.9 | 6.9 |
| | SD | 0.2 | 0.2 |
| | %CV | 2.2 | 2.6 |
| Moni-Trol 2 | | | |
| | Mean | 3.4 | 3.4 |
| | SD | 0.1 | 0.2 |
| | %CV | 4.1 | 4.9 |
| **Potassium (mmol/L)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 6.7 | 6.7 |
| | SD | 0.26 | 0.26 |
| | %CV | 3.9 | 3.9 |
| Moni-Trol 2 | | | |
| | Mean | 4.3 | 4.3 |
| | SD | 0.22 | 0.22 |
| | %CV | 5.1 | 5.1 |
| **Sodium (Na+) (mmol/L)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 148 | 148 |
| | SD | 5.1 | 5.1 |
| | %CV | 3.4 | 3.4 |
| Moni-Trol 2 | | | |
| | Mean | 118 | 118 |
| | SD | 3.2 | 3.2 |
| | %CV | 2.7 | 2.7 |
| **Total Bilrubin (mg/dL)** | | | |
| Precitrol-N | | | |
| | Mean | 0.8 | 0.8 |
| | SD | 0.06 | 0.07 |
| | %CV | 7.5 | 8.8 |
| Precitrol-A | | | |
| | Mean | 5.2 | 5.2 |
| | SD | 0.09 | 0.15 |
| | %CV | 1.7 | 2.9 |

**Table 4: Precision (N = 80) (Continued)**

| Analyte | | Within-Run (n = 80) | Total (n = 80) |
|---|---|---|---|
| **Total Carbon Dioxide (TC02)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 19 | 19 |
| | SD | 1.39 | 1.39 |
| | %CV | 7.3 | 7.3 |
| Moni-Trol 2 | | | |
| | Mean | 9 | 9 |
| | SD | 0.6 | 0.6 |
| | %CV | 6.8 | 6.8 |
| **Total Protein (g/dL)** | | | |
| Precitrol-N | | | |
| | Mean | 6.8 | 6.8 |
| | SD | 0.05 | 0.08 |
| | %CV | 0.7 | 1.2 |
| Precitrol-A | | | |
| | Mean | 4.7 | 4.7 |
| | SD | 0.09 | 0.09 |
| | %CV | 1.9 | 1.9 |
| **Urea Nitrogen (mg/dL)** | | | |
| Precitrol-N | | | |
| | Mean | 19 | 19 |
| | SD | 0.35 | 0.40 |
| | %CV | 1.8 | 2.1 |
| Precitrol-A | | | |
| | Mean | 65 | 65 |
| | SD | 1.06 | 1.18 |
| | %CV | 1.6 | 1.8 |
| **Uric Acid (mg/dL)** | | | |
| Moni-Trol 1 | | | |
| | Mean | 3.8 | 3.8 |
| | SD | 0.15 | 0.18 |
| | %CV | 4.0 | 4.8 |
| Moni-Trol 2 | | | |
| | Mean | 7.5 | 7.5 |
| | SD | 0.24 | 0.29 |
| | %CV | 3.2 | 3.9 |

| Analyte | | Within-Run | Total |
|---|---|---|---|
| **Thyroxine (T4) (µg/dL)** | | | |
| Level 1 | | | |
| | Mean | 2.4 | 2.4 |
| | SD | 0.28 | 0.39 |
| | %CV | 11.7 | 16.3 |
| Level 2 | | | |
| | Mean | 7.7 | 7.7 |
| | SD | 0.37 | 0.47 |
| | %CV | 4.8 | 6.1 |

**Table 5: Correlation of VetScan® System with Comparative Method**

| Analyte | | Canine (n = 22–180) | Feline (n = 21–55) | Equine (n = 7–101) | Bovine (n = 126) |
|---|---|---|---|---|---|
| **ALT (U/L)** | | | | | |
| | Correlation (r) | 1.00 | 0.98 | 0.97 | N/A |
| | Slope | 0.95 | 0.92 | 0.94 | |
| | Intercept | 0 | 0 | 6 | |
| | Sample range | 10–1549 | 27–99 | 11–30 | |
| **Albumin (g/dL)** | | | | | |
| | Correlation (r) | 0.96 | 0.75 | 0.89 | 0.97 |
| | Slope | 0.99 | 1.02 | 0.99 | 0.83 |
| | Intercept | 0.1 | 0 | -0.6 | 7 |
| | Sample range | 1.3–4.6 | 2.1–4.8 | 1.2–3.2 | 2.4–4.0 |
| **ALP (U/L)** | | | | | |
| | Correlation (r) | 1.00 | 0.97 | 1.00 | 0.97 |
| | Slope | 0.89 | 0.81 | 0.90 | 0.83 |
| | Intercept | -5 | 1 | -4 | 7 |
| | Sample range | 15–1722 | 6–54 | 119–1476 | 13–136 |
| **Amylase (U/L)** | | | | | |
| | Correlation (r) | 0.96 | 1.00 | N/A | N/A |
| | Slope | 0.67 | 0.74 | | |
| | Intercept | -34 | 117 | | |
| | Sample range | 366–1991 | 473–3474 | | |
| **AST (U/L)** | | | | | |
| | Correlation (r) | 1.00 | 1.00 | 1.00 | 0.94 |
| | Slope | 1.02 | 1.03 | 0.94 | 0.89 |
| | Intercept | 1 | 1 | 16 | -0.58 |
| | Sample range | 18–176 | 18–125 | 107–1787 | 68–262 |
| **Calcium (mg/dL)** | | | | | |
| | Correlation (r) | 0.84 | 0.77 | 0.94 | 0.89 |
| | Slope | 1.24 | 1.24 | 1.18 | 0.78 |
| | Intercept | -1.9 | -2.1 | -0.8 | 0.66 |
| | Sample range | 7.3–13.0 | 6.3–12.4 | 7.2–15.1 | 5.2–9.8 |
| **Cholesterol (mg/dL)** | | | | | |
| | Correlation (r) | 0.99 | 0.99 | N/A | N/A |
| | Slope | 0.99 | 1.06 | | |
| | Intercept | 6 | -3 | | |
| | Sample range | 103–450 | 63–257 | | |
| **Creatine Kinase (U/L)** | | | | | |
| | Correlation (r) | N/A | N/A | 1.00 | 0.99 |
| | Slope | | | 0.97 | 0.95 |
| | Intercept | | | -2 | -36.2 |
| | Sample range | | | 69–14000 | 79–2047 |
| **Creatinine (mg/dL)** | | | | | |
| | Correlation (r) | 0.99 | 1.00 | 0.95 | N/A |
| | Slope | 1.00 | 1.01 | 1.00 | |
| | Intercept | 0.0 | -0.1 | -0.4 | |
| | Sample range | 0.6–10.6 | 0.3–13.6 | 0.3–6.2 | |
| **GGT (U/L)** | | | | | |
| | Correlation (r) | 1.00 | N/A | 0.99 | 0.97 |
| | Slope | 0.96 | | 1.11 | 1.13 |
| | Intercept | 2 | | 0 | 0.67 |
| | Sample range | 5–65 | | 5–317 | 7–54 |
| **Glucose (mg/dL)** | | | | | |
| | Correlation (r) | 0.96 | 1.00 | 0.97 | N/A |
| | Slope | 1.01 | 0.97 | 0.94 | |
| | Intercept | -6 | 3 | 16 | |
| | Sample range | 28–348 | 52–607 | 36–353 | |

**Table 5: Correlation of VetScan® System with Comparative Method (Continued)**

| Analyte | | Canine (n = 22–180) | Feline (n = 21–55) | Equine (n = 7–101) | Bovine (n = 126) |
|---|---|---|---|---|---|
| **Magnesium (mg/dL)** | | | | | |
| | Correlation (r) | N/A | N/A | N/A | 0.98 |
| | Slope | | | | 1.09 |
| | Intercept | | | | -0.1 |
| | Sample range | | | | 1.2–4.2 |
| **Phosphorus (mg/dL)** | | | | | |
| | Correlation (r) | 0.994 | 0.916 | 0.971 | 0.99 |
| | Slope | 1.09 | 0.80 | 0.991 | 1.06 |
| | Intercept | -0.19 | 0.81 | -0.06 | -0.5 |
| | Sample range | 0.8–87 | 2.4–6.9 | 0.8–7.8 | 1.9–9.7 |
| **Potassium (mmol/L)** | | | | | |
| | Correlation (r) | 0.96 | 0.91 | 0.84 | N/A |
| | Slope | 0.92 | 0.92 | 0.97 | |
| | Intercept | 0.4 | 0.5 | 0.1 | |
| | Sample range | 3.2–6.9 | 2.7–5.3 | 1.8–4.6 | |
| **Sodium (mg/dL)** | | | | | |
| | Correlation (r) | 0.89 | 0.86 | 0.86 | N/A |
| | Slope | 0.97 | 1.08 | 1.00 | |
| | Intercept | 4.8 | -12.2 | -0.01 | |
| | Sample range | 118–183 | 122–166 | 110–166 | |
| **Thyroxine* (µg/dL)** | | | | | |
| | Correlation (r) | 0.92 | 0.86 | N/A | N/A |
| | Slope | 1.01 | 1.49 | | |
| | Intercept | -0.1 | -0.4 | | |
| | Sample range | 0.5–8.1 | 0.5–4.5 | | |
| **Total Bilirubin (mg/dL)** | | | | | |
| | Correlation (r) | 0.87 | 1.00 | 1.00 | N/A |
| | Slope | 0.84 | 0.92 | 0.90 | |
| | Intercept | 0.1 | -0.3 | 0.1 | |
| | Sample range | 0.1–3.2 | 0.4–15.0 | 0.6–26.1 | |
| **Total Protein (g/dL)** | | | | | |
| | Correlation (r) | 0.98 | 0.97 | 0.99 | 0.98 |
| | Slope | 1.03 | 0.96 | 0.97 | 1 |
| | Intercept | 0.1 | 0.4 | 0.3 | 0.47 |
| | Sample range | 2.6–10.7 | 4.8–8.5 | 3.0–9.5 | 6–10 |
| **Total Carbon Dioxide (mmol/L)** | Correlation (r) | 0.81 | 0.93 | 0.97 | N/A |
| | Slope | 0.86 | 0.90 | 0.93 | |
| | Intercept | 3.5 | 2.4 | 2.1 | |
| | Sample range | 6–23 | 7–31 | 9–39 | |
| **Urea Nitrogen (mg/dL)** | | | | | |
| | Correlation (r) | 1.00 | 1.00 | 1.00 | 0.98 |
| | Slope | 0.98 | 1.07 | 0.95 | 0.99 |
| | Intercept | -2 | -5 | -1 | 1.4 |
| | Sample range | 4–117 | 14–165 | 3–64 | 6–25 |

Comparative Method: Hitachi 911

* Comparative Method: RIA