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

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## 1. Intended Use

The VetScan<sup>®</sup> Preventive Care Profile Plus reagent rotor used with the VetScan VS2 Chemistry Analyzer utilizes dry and liquid reagents to provide *in vitro* quantitative determination of alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), total calcium (CA), chloride (CL<sup>-</sup>), creatinine (CRE), globulin\* (GLOB), glucose (GLU), potassium (K<sup>+</sup>), sodium (NA<sup>+</sup>), total bilirubin (TBIL), total carbon dioxide (tCO<sub>2</sub>) and total protein (TP) in heparinized whole blood, heparinized plasma, or serum.

\* Calculated Value

#### 2. Summary and Explanation of Tests

The VetScan Preventive Care Profile Plus reagent rotor and the VetScan VS2 Chemistry Analyzer comprise an *in vitro* diagnostic system that aids the veterinarian in diagnosing the following disorders:

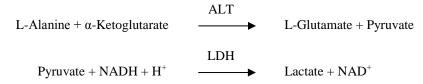
Alanine Aminotransferase (ALT)	Liver diseases, including viral hepatitis and cirrhosis; heart diseases
Albumin (ALB)	Liver and kidney diseases
Alkaline Phosphatase (ALP)	Liver, bone, parathyroid, and intestinal diseases
Aspartate Aminotransferase (AST)	Liver disease including hepatitis and viral jaundice; shock
Blood Urea Nitrogen (BUN)	Liver and kidney diseases
Calcium (CA)	Parathyroid, bone and chronic renal disease; tetany
Chloride (CL <sup>-</sup> )	Chronic diarrhea, chronic vomiting, renal disease, parathyroid disease, chronic respiratory acidosis or alkalosis, hyperadrenocorticism, hypoadrenocorticism, and thiazide therapy
Creatinine (CRE)	Renal disease
Globulin* (GLOB)	Globulin concentration will increase with dehydration and should also increase with antigenic stimulation
Glucose (GLU)	Diabetes, hyperglycemia, hypoglycemia, and liver disease
Potassium (K <sup>+</sup> )	Malnutrition and renal disease. This electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms
Sodium (NA <sup>+</sup> )	Dehydration, and diabetes. This electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms
Total Bilirubin (TBIL)	Hepatic disorders
Total Carbon Dioxide (tCO <sub>2</sub> )	Primary metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis
Total Protein (TP)	Dehydration, liver and kidney disease, metabolic and nutritional disorders

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 (ALT)

The method developed for use on the VetScan VS2 Chemistry Analyzer is a modification of the Wróblewski and LaDue procedure recommended by the International Federation of Clinical Chemistry (IFCC).<sup>1,2</sup> 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<sup>+</sup>, as illustrated in the following reaction scheme.



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

### Albumin (ALB)

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.<sup>3</sup>

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 (ALP)

The VetScan procedure is modified from the AACC and IFCC methods.<sup>4</sup> Alkaline phosphatase hydrolyzes p-NPP in a metalion buffer and forms p-nitrophenol and phosphate. The use of p-nitrophenyl phosphate (p-NPP) increases the speed of the reaction.<sup>5,6</sup> 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.<sup>7</sup> The American Association for Clinical Chemistry (AACC) reference method uses p-NPP as a substrate and a metal-ion buffer.<sup>8</sup>

*p*-Nitrophenyl Phosphate + H<sub>2</sub>O 
$$\xrightarrow{ALP}$$
 *p*-Nitrophenol + Phosphate  $Zn^{2+}, Mg^{2+}$ 

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

#### Aspartate Aminotransferase (AST)

The Abaxis AST method is a modification of the IFCC reference method.<sup>9,10</sup> This method catalyzes the reaction of L-aspartate and  $\alpha$ -ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD<sup>+</sup> by the enzyme malate dehydrogenase (MDH).

L-aspartate + 
$$\alpha$$
-Ketoglutarate  $\longrightarrow$  Oxaloacetate + L-glutamate   
Oxaloacetate + NADH + H<sup>+</sup>  $\longrightarrow$  Malate +NAD<sup>+</sup>

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

#### **Blood 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.<sup>11</sup> Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.<sup>12</sup> The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique<sup>13,14</sup> and coupled enzymatic reactions.<sup>15,16</sup> Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.<sup>17</sup> 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.<sup>18</sup>

In the coupled-enzyme reaction, urease hydrolyzes urea into ammonia and carbon dioxide. Upon combining ammonia with  $\alpha$ -ketoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD<sup>+</sup>.

Urea + H<sub>2</sub>O  $\xrightarrow{\text{Urease}}$  2NH<sub>3</sub> + CO<sub>2</sub> GLDHNH<sub>3</sub> +  $\alpha$ -Ketoglutarate + NADH + H<sup>+</sup>  $\xrightarrow{\text{L-Glutamate}}$  L-Glutamate + H<sub>2</sub>O + NAD<sup>+</sup>

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

#### Calcium (CA)

The reference method for total calcium is atomic absorption spectroscopy; however, this method is not suited for routine use.<sup>19</sup> Spectrophotometric methods using either o-cresolphthalein complexone (CPC) or arsenazo III metallochromic indicators are most commonly used.<sup>20,21,22</sup> 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.

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

#### Chloride (Cl<sup>-</sup>)

The method is based on the determination of chloride-dependent activation of  $\alpha$ -amylase activity. Deactivated  $\alpha$ -amylase is reactivated by addition of the chloride ion, allowing the calcium to re-associate with the enzyme. The reactivation of  $\alpha$ -amylase activity is proportional to the concentration of chloride ions in the sample. The reactivated  $\alpha$ -amylase converts the substrate, 2-chloro-p-nitrophenyl- $\alpha$ -D-maltotrioside (CNPG3) to 2-chloro-p-nitrophenol (CNP) producing color and  $\alpha$ -maltotriose (G3). The reaction is measured bichromatically and the increase in absorbance is directly proportional to the reactivated  $\alpha$ -amylase

CNPG3 
$$\xrightarrow{\alpha$$
-Amylase   
CNPG3  $\xrightarrow{\alpha}$  CNP + G3

#### Creatinine (CRE)

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.<sup>24,25</sup> Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.<sup>26,27,28</sup> Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.<sup>29</sup>

Creatinine 
$$+$$
 H<sub>2</sub>O

activity and the concentration of chloride ion in the sample.<sup>23</sup>

Creatine

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 (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu and Somogyi-Nelson).<sup>30,31,32</sup> 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.<sup>33</sup> 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<sup>+</sup>) to NADH.

Glucose + ATP 
$$\xrightarrow{HK}$$
 G-6-P + ADP  
G-6-P + NAD<sup>+</sup>  $\xrightarrow{G-6-PDH}$  6-Phosphogluconate + NADH + H<sup>+</sup>

#### Potassium (K<sup>+</sup>)

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.<sup>34,35,36</sup> Interference from sodium and ammonium ions are minimized with the addition of Kryptofix and glutamine synthetase respectively.

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  $NAD^+$  and is directly proportional to the amount of potassium in the sample.

ADP + PEP  $\xrightarrow{K^+, PK}$  Pyruvate + ATP Pyruvate + NADH + H<sup>+</sup>  $\xrightarrow{LDH}$  Lactate + NAD<sup>+</sup>

#### Sodium (NA<sup>+</sup>)

Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.<sup>37,38,39</sup> In the Abaxis enzymatic reaction,  $\beta$ -galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of o-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) to o-nitrophenol and galactose. The reaction rate between 405 nm and 500 nm is proportional to sodium concentration.

ONPG + H<sub>2</sub>O 
$$\xrightarrow{\text{Na}^+}$$
 o-Nitrophenol + Galactose  $\beta$ -Galactosidase

#### **Total Bilirubin (TBIL)**

Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.<sup>40,41</sup> A newer, more specific method has been developed using the enzyme bilirubin oxidase.<sup>42,43,44</sup> 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.

Bilirubin Oxidase   
Bilirubin + 
$$O_2$$
   
Biliverdin +  $H_2O$ 

#### **Total Carbon Dioxide (tCO<sub>2</sub>)**

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,  $CO_2$  electrode and spectrophotometric enzymatic methods, which all produce accurate and precise results.<sup>45,46</sup> The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

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 (MDH) catalyzes the reaction of oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH) to NAD<sup>+</sup> and malate. The rate of change in absorbance due to the conversion of NADH to NAD<sup>+</sup> is directly proportional to the amount of tCO<sub>2</sub> in the sample.

$PEP + HCO_3^{-1}$	PEPC	Oxaloacetate + Phosphate
Oxaloacetate + NADH + $H^+$	MDH	$NAD^+ + Malate$

#### **Total Protein (TP)**

The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity.<sup>47</sup> It was originally developed by Riegler and modified by Weichselbaum, Doumas, et al. The biuret reaction is a candidate total protein reference method.<sup>48,49,50</sup>

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.<sup>49</sup> The Cu(II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-Protein complex.

Total Protein + Cu(II)  $\longrightarrow$  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.

#### 4. Principle of Operation

See the VetScan VS2 Chemistry Analyzer Operator's Manual for the Principles and Limitations of the Procedure.

## 5. Description of Reagents

### Reagents

Each VetScan Preventive Care Profile Plus reagent rotor contains dry test specific reagent beads. A dry sample blank reagent (comprised of buffer, surfactants, excipients and preservatives) is included in each reagent rotor for use in calculating concentrations of alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), total calcium (CA), chloride (CL<sup>-</sup>), glucose (GLU), potassium (K<sup>+</sup>), sodium (NA<sup>+</sup>), total carbon dioxide (tCO<sub>2</sub>) and total protein (TP). Dedicated sample blanks are included in the rotor to calculate the concentration of creatinine (CRE) and total bilirubin (TBIL). Each reagent rotor also contains a diluent consisting of surfactants and preservatives.

#### Warnings and Precautions For *In vitro* Diagnostic Use

- The diluent container in the reagent rotor is automatically opened when the analyzer drawer closes. A rotor with an opened diluent container cannot be re-used. Ensure that the sample or control has been placed into the rotor before closing the drawer.
- The reagent rotors are plastic and may crack or chip if dropped. Never use a dropped rotor.
- 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 VS2 Operator's Manual. A rotor not used within 20 minutes of opening the pouch should be discarded. Rotors in opened pouches cannot be placed back in the refrigerator for use at a later time.

#### Storage

Store reagent rotors in their sealed pouches at  $2-8^{\circ}C$  (36-46°F). Do not expose opened or unopened rotors to direct sunlight or temperatures above  $32^{\circ}C$  (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 VS2 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 VS2 Operator's Manual for complete information on use of the analyzer.

## 7. Sample Collection and Preparation

Sample collection techniques are described in the "Sample Collection" section of the VetScan VS2 Chemistry Analyzer Operator's Manual.

- The minimum required sample size is  $\sim 100 \ \mu$ L of heparinized whole blood, heparinized plasma, serum or control material. The reagent rotor sample chamber can contain up to 120  $\mu$ L of sample.
- Use only lithium heparin (green stopper) evacuated specimen collection tubes for whole blood or plasma samples. Use noadditive (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.
- 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.<sup>51</sup> 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.
- The test must be started within 10 minutes of transferring the sample into the reagent rotor.
- Glucose concentrations decrease approximately 5-12 mg/dL in 1 hour in uncentrifuged samples stored at room temperature.<sup>52</sup>
- Refrigerating whole blood samples can cause significant changes in concentrations of **aspartate aminotransferase**, **glucose** and **creatinine**.<sup>53</sup>
- **Total bilirubin** results may be adversely affected by photodegradation.<sup>54</sup> Whole blood samples not run immediately should be stored in the dark for no longer than 60 minutes. If the sample cannot 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.<sup>55</sup>
- The concentration of **total carbon dioxide** is most accurately determined when the assay is done immediately after opening the tube and as promptly as possible after collection and processing of the blood in the unopened tube. Ambient air contains far less carbon dioxide than does plasma, and gaseous dissolved carbon dioxide will escape from the specimen into the air, with a consequent decrease in carbon dioxide value of up to 6 mmol/L in the course of 1 hour.<sup>56</sup>

#### **Known Interfering Substances**

- The only anticoagulant recommended for use with the VetScan VS2 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 Preventive Care Profile Plus 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 results print-out to inform the operator about the levels of interferents present in each sample. The VetScan VS2 Chemistry Analyzer suppresses any results that are affected by >10% interference from hemolysis, lipemia, or icterus. "HEM", "LIP", "ICT" is printed on the results print-out 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.<sup>57</sup>
- 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.

- Bilirubin may interfere with the peroxidase used in the **creatinine** reaction.<sup>58</sup> 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.<sup>59</sup>
- Interference may be seen in the **total protein** test when analyzing samples with a 3 + lipemic index.<sup>60</sup> Samples with a triglyceride concentration >400 mg/dL may show an increased total protein level.<sup>55</sup> The VetScan VS2 Chemistry Analyzer suppresses any results that are affected by >10% interference from lipemia. "LIP" is printed on the results print-out in place of the result.
- Extremely elevated amylase levels (>9,000 U/L) will have a significant effect, > 10% increase, on the **chloride** result. The concentration of amylase is not evaluated by the VetScan system for each specimen.

## 8. Procedure

#### **Materials Provided**

• One VetScan Preventive Care Profile Plus Reagent Rotor PN: 500-1047 (a box of 12 rotors PN: 500-0047-12)

### Materials Required but not Provided

• VetScan VS2 Chemistry Analyzer

#### **Test Parameters**

The VetScan System operates at ambient temperatures between  $15^{\circ}$ C and  $32^{\circ}$ C (59-90°F). The analysis time for each VetScan Preventive Care Profile Plus Reagent Rotor is approximately 12 minutes. The analyzer maintains the reagent rotor at a temperature of  $37^{\circ}$ 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 VS2 Operator's Manual.

#### Calibration

The VetScan VS2 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 VS2 Operator's Manual.

#### **Quality Control**

Controls may be run periodically on the VetScan VS2 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 VS2 Operator's Manual to run controls.

### 9. Results

The VetScan VS2 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 VS2 Operator's Manual.

### **10. Limitations of Procedure**

General procedural limitations are discussed in the VetScan VS2 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 62% packed red cell volume may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down to get plasma then re-run in a new reagent rotor.

**Warning:** Extensive testing of the VetScan VS2 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 the established reference ranges. The sample may be re-run using a new reagent rotor.

# 11. Expected Values

These reference ranges 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 VS2 Chemistry Analyzer for the "Other" bank, refer to your VetScan VS2 Operator's Manual.

Table 1: Reference Intervals				
Analyte	Units	Canine	Feline	Equine
Alanine Aminotransferase (ALT)	U/L	10 - 118	20 - 100	5 - 20
Albumin (ALB)	g/dL g/L	2.5 – 4.4 25 – 44	2.2 - 4.4 22 - 44	2.2 - 3.7 22 - 37
Alkaline Phosphatase (ALP)	U/L	20 - 150	10 - 90	50 - 170
Aspartate Aminotransferase (AST)	U/L	14 - 45	12-43	175 - 340
Blood Urea Nitrogen (BUN)	mg/dL mmol urea/L	7 – 25 2.5 – 8.9	10 - 30 3.6 - 10.7	7 – 25 2.5 – 8.9
Calcium (CA)	mg/dL mmol/L	8.6 – 11.8 2.15 – 2.95	8.0 - 11.8 2.00 - 2.95	11.5 - 14.2 2.88 - 3.55
Chloride (CL <sup>-</sup> )	mmol/L	95 – 119	99 – 122*	92 - 104
Creatinine (CRE)	mg/dL µmol/L	0.3 – 1.4 27 – 124	0.3 - 2.1 27 - 186	0.6 - 2.2 53 - 194
Globulin (GLOB)	g/dL g/L	2.3 - 5.2 23 - 52	1.5 – 5.7 15 – 57	2.7 - 5.0 27 - 50
Glucose (GLU)	mg/dL mmol/L	60 – 110 3.3 – 6.1	70 – 150 3.9 – 8.3	65 - 110 3.6 - 6.1
Potassium (K <sup>+</sup> )	mmol/L	3.7 - 5.8	3.7 - 5.8	2.5 - 5.2
Sodium (NA <sup>+</sup> )	mmol/L	138 – 160	142 – 164	126 – 146
Total Bilirubin (TBIL)	mg/dL µmol/L	0.1 - 0.6 2 - 10	0.1 - 0.6 2 - 10	0.5 - 2.3 9 - 39
Total Carbon Dioxide (tCO <sub>2</sub> )	mmol/L	12 – 27	15 – 24	20-33
Total Protein (TP)	g/dL g/L	5.4 - 8.2 54 - 82	5.4 - 8.2 54 - 82	5.7 - 8.0 57 - 80

\*Feline reference interval is for adult cats only; kittens (cats younger than 6 months) may have lower chloride levels.

## **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 (refer to the VetScan VS2 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.** 

Analyte	Common	Units	SI Un	its
Alanine Aminotransferase (ALT)	5 - 2000	U/L	5 - 2000	U/L
Albumin (ALB)	1 - 6.5	g/dL	10-65	g/L
Alkaline Phosphatase (ALP)	5 - 2400	U/L	5 - 2400	U/L
Aspartate Aminotransferase (AST)	5 - 2000	U/L	5 - 2000	U/L
Blood Urea Nitrogen (BUN)	2 - 180	mg/dL	0.7 - 64.3	mmol urea/L
Calcium (CA)	4 – 16	mg/dL	1.0 - 4.0	mmol/L
Chloride (CL <sup>-</sup> )	80 - 135	mmol/L	80 - 135	mmol/L
Creatinine (CRE)	0.2 – 20	mg/dL	18 – 1768	µmol/L
Globulin* (GLOB)	1 – 11	g/dL	10 - 110	g/L
Glucose (GLU)	10 - 700	g/dL	0.6 - 38.9	mmol/L
Potassium (K <sup>+</sup> )	1.5 - 8.5	mmol/L	1.5 - 8.5	mmol/L
Sodium (NA <sup>+</sup> )	110 - 170	mmol/L	110 - 170	mmol/L
Total Bilirubin (TBIL)	0.1 – 30	mg/dL	1.7 – 513	µmol/L
Total Carbon Dioxide (tCO <sub>2</sub> )	5-40	mmol/L	5 - 40	mmol/L
Total Protein (TP)	2 - 14	g/dL	20 - 140	g/L

\* Calculated Value

### Precision

Precision studies were conducted using the Clinical and Laboratory Standards Institute (CLSI) EP5-A.<sup>61</sup> Guidelines with modifications based on CLSI EP18-P<sup>62</sup> for unit-use devices. Results for within-run and total precision were determined by testing bi-level controls.

Tuble 5. Treelston				
Analyte	Sample Size	Within-Run	Total	
Alanine Aminotransferase (U/L)				
Control 1	N=80			
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	

#### **Table 3: Precision**

Table 3: Precision (continued)

Analyte	Sample Size	Within-Run	Total	
Albumin (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	
/0 0 1		5.9	1.5	
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	
/0 <b>C</b> /		1.0	5.1	
Aspartate Aminotransferase (U/	<b>L</b> ) N=80			
Control 1	,			
Mean		47	47	
SD		0.98	1.84	
%CV		2.1	3.9	
Control 2				
Mean		145	145	
SD		1.83	4.62	
%CV		1.3	3.2	
/0 0 1		1.5	5.2	
Blood 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	
		110	1.0	
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	
/		0.0		

Table 3: Precision (continued)

Analyte	Sample Size	Within-Run	Total	
Chloride (mmol/L)				
Control 1	N=160			
Mean		97.8	97.8	
SD		1.63	1.74	
%CV		1.7	1.7	
Control 2				
Mean		113.6	113.6	
SD		1.97	2.22	
%CV		1.7	2.0	
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	
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		110	110	
Mean		118	118	
SD		3.2	3.2	
%CV		2.7	2.7	

Table 3: Precision (continued)

Analyte	Sample Size	Within-Run	Total	
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 Carbon Dioxide (mmol/L)	N=80			
Control 1				
Mean		19	19	
SD		1.39	1.39	
%CV		7.3	7.3	
Control 2				
Mean		9	9	
SD		0.60	0.60	
%CV		6.8	6.8	
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	

### Correlation

Field studies were conducted at a veterinary teaching hospital. Serum samples were analyzed by the VetScan VS2 Chemistry Analyzer and a comparative method. Representative correlation statistics are shown in Table 4.

			Correlation				Sample
Analyte	Units	Species	Coefficient	Slope	Intercept	Ν	Range
Alanine Aminotransferase (ALT)	UL	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 (ALB)	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 (ALP)	UL	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
Aspartate Aminotransferase (AST	Г) UL	Canine	1.00	1.02	1	22 - 180	18 – 176
		Feline	1.00	1.03	1	21 - 55	18 – 125
		Equine	1.00	0.94	16	7 – 101	107 - 1787
Blood Urea Nitrogen (BUN)	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
Calcium (CA)	mg/dL	Canine	0.84	1.24	-1.9	22 - 180	7.3 - 13.0
	•	Feline	0.74	1.24	-2.1	21 - 55	6.3 – 12.4
		Equine	0.94	1.18	-0.8	7 - 101	7.2 – 15.1
Chloride (CL <sup>-</sup> )	mmol/L	Canine	0.94	0.88	15	38	78 – 132
	innioi, E	Feline	0.98	0.88	12	20	86 - 123
		Equine	NA	NA	NA	NA	NA
Creatinine (CRE)	mg/dL	Canine	0.99	1.00	0.0	22 - 180	0.6 - 10.6
	0	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 (GLU)	mg/dL	Canine	0.96	1.01	-6	22 - 180	28 - 348
	U	Feline	1.00	0.97	3	21 - 55	52 - 607
		Equine	0.97	094	16	7 - 101	36 - 353
Potassium (K <sup>+</sup> )	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 (NA <sup>+</sup> )	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 (TBIL)	mg/dL	Canine	0.87	0.84	0.1	22 - 180	0.13.2
		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 Carbon Dioxide (tCO <sub>2</sub> )	mmol/L	Canine	0.81	0.86	3.5	22 - 180	6 – 23
		Feline	0.93	0.90	2.4	21 - 55	7 – 31
		Equine	0.97	0.93	2.1	7 – 101	9 - 39
Total Protein (TP)	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

Table 4: Correlation of the VetScan VS2 Chemistry Analyzer with Comparative Method(s)

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