VETSCAN® Preventive Care Profile Plus

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

January 2023
PN: 51630200

1. Intended Use

The VetScan® 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^-), creatinine (CRE), globulin* (GLOB), glucose (GLU), potassium (K^+), sodium (NA^+), total bilirubin (TBIL), total carbon dioxide (tCO_2) 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^-)	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^+)	Malnutrition and renal disease. This electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms
Sodium (NA^+)	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_2)	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).[1,2] In this reaction, ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+, as illustrated in the following reaction scheme.

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

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

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

Albumin (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.[3]

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

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

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

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

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

Aspartate Aminotransferase (AST)
The Abaxis AST method is a modification of the IFCC reference method.[9,10] This method catalyzes the reaction of L-aspartate and α-ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD^+ by the enzyme malate dehydrogenase (MDH).

$$\text{L-aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-glutamate}$$

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

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

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.[11] Indirect methods measure ammonia created from the urea; the use of the

enzyme urease has increased the specificity of these tests.[12] The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique[13,14] and coupled enzymatic reactions.[15,16] Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.[17] 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.[18]

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

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

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

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.

Calcium (CA)

The reference method for total calcium is atomic absorption spectroscopy; however, this method is not suited for routine use.[19] Spectrophotometric methods using either o-cresolphthalein complexone (CPC) or arsenazo III metallochromic indicators are most commonly used.[20,21,22] Arsenazo III has a high affinity for calcium and is not as temperature dependent as CPC.

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

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

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

Chloride (Cl^-)

The method is based on the determination of chloride-dependent activation of α-amylase activity. Deactivated α-amylase is reactivated by addition of the chloride ion, allowing the calcium to re-associate with the enzyme. The reactivation of α-amylase activity is proportional to the concentration of chloride ions in the sample. The reactivated α-amylase converts the substrate, 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) to 2-chloro-p-nitrophenol (CNP) producing color and α-maltotriose (G3). The reaction is measured bichromatically and the increase in absorbance is directly proportional to the reactivated α-amylase activity and the concentration of chloride ion in the sample.[23]

$$\text{CNPG3} \xrightarrow[Cl^-,\ Ca^{2+}]{\alpha\text{-Amylase}} \text{CNP} + \text{G3}$$

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

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

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

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

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

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

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu and Somogyi-Nelson).[30,31,32] 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.[33] The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{HK}} \text{G-6-P} + \text{ADP}$$

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

Potassium (K^+)

Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. The Abaxis enzymatic method is based on the activation of pyruvate kinase (PK) with potassium and shows excellent linearity and negligible susceptibility to endogenous substances.[34,35,36] 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.

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

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

Sodium (NA^+)

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

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

Total Bilirubin (TBIL)

Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.[40,41] A newer, more specific method has been developed using the enzyme bilirubin oxidase.[42,43,44] In addition to using the more specific total bilirubin test

method, photodegradation of the analyte is minimized on the analyzer because the sample can be tested immediately after collection.

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

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

Total Carbon Dioxide (tCO_2)
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.[45,46] 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^+ and malate. The rate of change in absorbance due to the conversion of NADH to NAD^+ is directly proportional to the amount of tCO_2 in the sample.

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

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

Total Protein (TP)
The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity.[47] It was originally developed by Riegler and modified by Weichselbaum, Doumas, et al. The biuret reaction is a candidate total protein reference method.[48,49,50]

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

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

The amount of total protein present in the sample is directly proportional to the absorbance of the Cu-protein complex. The total protein test is an endpoint reaction and the absorbance is measured as the difference in absorbance between 550 nm and 850 nm.

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^-), glucose (GLU), potassium (K^+), sodium (NA^+), total carbon dioxide (tCO_2) 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˚C (36-46˚F). Do not expose opened or unopened rotors to direct sunlight or temperatures above 32˚C (90˚F). Do not allow the rotors sealed in their foil pouches to remain at room temperature longer than 48 hours prior to use. Open the pouch and remove the rotor just prior to use.

Indications of Reagent Rotor Instability or Deterioration

- All reagents contained in the reagent rotor, when stored as described above, are stable until the expiration date printed on the rotor pouch. Do not use a rotor after the expiration date. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on the VetScan 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 ~100 µL of heparinized whole blood, heparinized plasma, serum or control material. The reagent rotor sample chamber can contain up to 120 µL of sample.

- Use only lithium heparin (green stopper) evacuated specimen collection tubes for whole blood or plasma samples. Use no-additive (red stopper) evacuated specimen collection tubes or serum separator tubes (red or red/black stopper) for serum samples.

- Whole blood samples obtained by venipuncture must be homogenous before transferring a sample to the reagent rotor. Gently invert the collection tubes several times just prior to sample transfer. Do **not** shake the collection tube; shaking may cause hemolysis.

- 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.[51] 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.[52]

- Refrigerating whole blood samples can cause significant changes in concentrations of **aspartate aminotransferase**, **glucose** and **creatinine**.[53]

- **Total bilirubin** results may be adversely affected by photodegradation.[54] 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.[55]

- 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.[56]

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 Whole Blood Analyzer suppresses any results that are affected by significant 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.[57]

- 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.[58] 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.[59]

- Interference may be seen in the **total protein** test when analyzing samples with a 3 + lipemic index.[60] Samples with a triglyceride concentration >400 mg/dL may show an increased total protein level.[55] 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°C and 32°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°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. 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.**

Table 2: VetScan Dynamic Ranges

Analyte	Common Units		SI Units	
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^-)	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	mg/dL	0.6 – 38.9	mmol/L
Potassium (K^+)	1.5 – 8.5	mmol/L	1.5 – 8.5	mmol/L
Sodium (NA^+)	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_2)	5 – 40	mmol/L	5 – 40	mmol/L
Total Protein (TP)	2 – 14	g/dL	20 – 140	g/L

* Calculated Value

12. Bibliography

1. Wróbleski F, LaDue JS. Serum glutamic-pyruvic transaminase in cardiac and hepatic disease. Proc Soc Exp Biol Med 1956; 91: 569-71.
2. Bergmeyer HU, Horder M. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC Method for alanine aminotransferase. J. Clin Chem Clin Biochem 1980; 18: 521-34.
3. Webster D, et al. An assessment on the suitability of bromocresol green for the determination of serum albumin. Clin Chim Acta 1974; 53:101-8.
4. Bowers GN, et al. IFCC methods for the measurement of catalytic concentration of enzymes. Part 1. General considerations concerning the determination of the catalytic concentration of an enzyme in the blood serum or plasma of man. Clin Chim Acta 1979; 98:163F-74F.
5. Ohmori Y. Uber die Phosphomonoesterase. Enzymologia 1937; 4:217-31.
6. Fujita H. Uber die Mikrobestimmung der Blutphosphatase. J Biochem, Japan. 1937; 30: 69-87.
7. Petitclerc C, et al. Mechanism of action of Mg2+ and Zn2+ on rat placental alkaline phosphatase. I. Studies on the soluble Zn2+ and Mg2+ alkaline phosphatase. Can J Biochem 1975; 53: 1089-1100.
8. Tietz NW, et al. A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem 1983; 29: 751-61.

9. Bergmeyer HU, et al. Provisional recommendations on IFCC methods of catalytic concentrations of enzymes, Part 2. IFCC method for aspartate aminotransferase. Clin Chem 1977; 23: 887-99.
10. Bergmeyer HU, et al. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. Revised IFCC method for aspartate aminotransferase. Clin Chem 1978; 24: 720-1.
11. Fales FW. Urea in serum, direct diacetyl monoxime method. In: Selected Methods of Clinical Chemistry, Vol 9. Faulkner WR, Meites S, eds. Washington, DC.: American Association for Clinical Chemistry. 1982: 365-373.
12. Van Slyke, et al. A permanent preparation of urease, and its use in the determination of urea. J Biol Chem 1914; 19: 11-228.
13. Fawcett JK, et al. A rapid and precise method for the determination of urea. J Clin Pathol 1960; 13: 156-159.
14. Chaney, et al. Urea and ammonia determinations. Clin Chem 1962; 8: 130-132.
15. Talke H, et al. Enzymatische harnstoffbestimmung in blut and serum im optischen Test nach Warburg. Klin Wochensch, 1965; 43: 174-175.
16. Hallett, et al. Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. Clin Chim Acta 1971; 35: 33-37.
17. Patton, et al. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal Chem 1977; 49: 464-469.
18. Sampson EJ, et al. A coupled-enzyme equilibrium method for the measuring urea in serum: optimization and evaluation of the AACC study group on Urea Candidate reference method. Clin Chem 1980; 26: 816-826.
19. Cali JP, Bowers GN, Young DS, et al. A reference method for the determination of total calcium in serum. In: GR Cooper, ed., Selected methods of Clinical Chemistry. Washington, DC: American Association for Clinical Chemistry 1977; 8: 3-8.
20. Kessler G, M Wolfman. An Automated procedure for the simultaneous determination of calcium and phosphorus. Clin Chem 1964; 10: 686-703.
21. Michaylova V, P Ilkova. Photometric determination of micro amounts of calcium with arsenazo III. Anal Chim Acta 1971; 53: 194-8.
22. Scarpa A, et al. Metallochromic indicators of ionized calcium. Ann NY Acad Sci 1978; 307: 86-112.
23. Ono T, et al. A new enzymatic assay of chloride in serum. Clin Chem 1988; 34: 552-553.
24. Knoll VE, et al. Spezifische kreatininbetimmung Im Serum. Z Klin Chemi Clin Biochem 1970; 8: 582-587.
25. Haeckel R, et al. Simplified determinations of the "true" creatinine concentration in serum and urine. J Clin Chem Clin Biochem 1980; 18: 385-394.
26. Moss GA, et al. Kinetic enzymatic method for determining serum creatinine. Clin Chem 1975; 21: 1422-1426.
27. Jaynes PK, et al. An enzymatic, reaction-rate assay for serum creatinine with a centrifugal analyzer. Clin Chem 1982; 28: 114-117.
28. Fossati P, et al. Enzymatic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin Chem 1983; 29: 1494-1496.
29. Whelton A, et al. Nitrogen metabolites and renal function. In: CA Burtis and ER Ashwood, Eds., Tietz Textbook of Clinical Chemistry, 3rd Ed. Philadelphia: W.B. Saunders Company. 1999; 1513-1575.
30. Folin O, and Wu H. A system of blood analysis. J Biol Chem 1919; 38: 81-110.
31. Somogyi M. A reagent for the copper-idiometric determination of very small amounts of sugar. J Biol Chem 1937; 117: 771-776.
32. Nelson N. A photometric adaption of the Somogyi method for the determination of glucose. J Biol 1944; 153: 375-380.
33. Kaplan LA. Glucose. In: LA Kaplan and AJ Pesce, eds., Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed St. Louis: The C.V. Mosby Company. 1989; 850-856.
34. Berry MN, et al. Enzymatic determination of potassium in serum. Clin Chem 1989; 35: 817-20.
35. Van Pelt J. Enzymatic determination of sodium, potassium and chloride in serum compared with determination by flame photometry, coulometry and ion selective electrodes. Clin Chem 1994; 40: 846-7.
36. Hubl W, et al. Enzymatic determination of sodium, potassium and chloride in abnormal (hemolyzed, icteric, lipemic, paraproteinemic, or uremic) serum samples compared with indirect determination with ion selective electrodes. Clin Chem 1994; 40: 1528-31.
37. Helgerson RC, et al. Host-guest Complexation. 50. Potassium and sodium ion-selective chromogenic ionophores. J Amer Chem Soc 1989; 111: 6339-50.
38. Kumar A, et al. Chromogenic ionophere-based methods for spectrophotometric assay of sodium and potassium in serum and plasma. Clin Chem 1988; 34: 1709-12.
39. Berry MN, et al. Enzymatic determination of sodium in serum. Clin Chem 1988; 34: 2295-8.
40. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937; 119:481-90.
41. Meites S. Bilirubin, directing reacting and total, modified Mally-Evelyn method. In: WR Faulkner and S Meites, eds., Selected Methods of Clinical Chemistry, Washington, DC: American Association for Clinical Chemistry. 1982; 9: 119-24.
42. Murao S, Tanaka N. A new enzyme "bilirubin oxidase" produced by Myrothecium verrucaria MT-1. Agric Biol Chem 1981; 45: 2383-4.
43. Osaki S, Anderson S. Enzymatic determination of bilirubin. Clin Chem 1982; 30: 971. (Abstract)

44. Perry B, et al. Measurement of total bilirubin by use of bilirubin oxidase. Clin Chem 1986; 32: 329-32.
45. Skeggs LT Jr. An automatic method for the determination of carbon dioxide in blood plasma. Am J. Clin Pathol 1960; 33: 181-185.
46. Korzun WJ, Miller WG. Carbon Dioxide. In: Clinical chemistry theory, analysis and correlation, 2nd ed. Kaplan LA, Pesce AJ, eds. St. Louis: The CV Mosby Company. 1989: 869-872.
47. Koller A and Kaplan LA. Total serum protein. In: LA Kaplan and AJ Pesce, eds., Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. St Louis: The C.V. Mosby Company. 1989: 1057-60.
48. Reigler E. Eine kolorimetrische Bestimmungsmethods des Eiweisses. Z Anal Chem 1914; 53: 242-5.
49. Weicheselbaum TE. An accurate and rapid method for determination of proteins in small amounts of blood serum and plasma. Am J Clin Path 1946; 16: 40-9.
50. Doumas BT, et al. A candidate reference method for determination of total protein in serum. I. Development and validation. Clin Chem 1981; 27: 1642-50.
51. CLSI. Procedures for Handling and Processing of Blood Specimens; tentative standard. CLSI document H18-A2. Wayne, PA: CLSI, 1999.
52. Overfield CV, Savory J, and Heintges MG. Glycosis: a re-evaluation of the effect on blood glucose. Clin Chim Acta 1972; 39: 35-40.
53. Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem 1988; 34: 2111-14.
54. Sherwin JE. Obernolte R. Bilirubin. In: LA Kaplan and AJ Pesce, eds. Clinical Chemistry: Theory, Analysis and Correlation, 2nd ed. St. Louis: The C.V. Mosby Company. 1989; 1009-1015.
55. Henry RJ, Canon DC and Winkelman. Clinical Chemistry Principles and Technics, 2nd ed. New York: Harper and Row. 1974; 417-421: 127-8.
56. Scott MG. Electrolytes and Blood Gases. In: Tietz Textbook of Clinical Chemistry. 3rd ed. Burtis CA, Ashwood ER, eds. Philadelphia: WB Saunders Company. 1999: 1065-1066.
57. Scott MG, Electrolytes and Blood Gases. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders Company. 1999: 617-721.
58. Witte DL, Brown LF, and RL Williams. Effects of bilirubin on detection of hydrogen peroxide by use of peroxidase. Clin Chem 1978; 24: 1778-82.
59. Melnik J, Potter JL. Variance in capillary and venous glucose levels during glucose tolerance test. Am J Med Tech 1982; 48: 543-5.
60. Henry RJ, Canon DC and Winkelman. Clinical chemistry principles and technics, 2nd ed. New York: Harper and Row. 1974; 417-21: 127-8.