VetScan® Prep Profile II

CE

For Veterinary use only
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1. Intended Use

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 alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (CRE), glucose (GLU), total protein (TP), and urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

2. Summary and Explanation of Tests

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

Alanine Aminotransferase	Liver diseases; including viral hepatitis and cirrhosis; heart diseases.
Alkaline Phosphatase	Liver, bone, parathyroid and intestinal diseases.
Creatinine	Renal disease.
Glucose	Diabetes**,** hyperglycemia, hypoglycemia, diabetes and liver disease.
Total Protein	Dehydration, kidney, liver disease, metabolic and nutritional disorders.
Blood Urea Nitrogen	Liver and kidney diseases.

As with any diagnostic test procedure, all other test procedures including the clinical status of the patient should be considered prior to final diagnosis.

3. Principles of Procedure

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

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

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

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

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

Alkaline Phosphatase (ALP)
The VetScan procedure is modified from the AACC and IFCC methods. [3] 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.[4, 5] 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.[6] The American Association for Clinical Chemistry (AACC) reference method uses *p*-NPP as a substrate and a metal-ion buffer.[7]

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

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

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.[8, 9] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[10, 11, 12] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.[13]

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

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

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

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

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) [14, 15, 16] 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.[17] The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH.

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

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

Total Protein (TP)
The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity. [18] It was originally developed by Riegler and modified by Weichselbaum, Doumas, et al. The biuret reaction is a candidate total protein reference method.[19, 20, 21]

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

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

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)
A coupled-enzymatic reaction is used by the Abaxis system. In this reaction, urease hydrolyzes urea into ammonia and carbon dioxide.[22] Upon combining ammonia with 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD^+.

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

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

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

4. Principle of Operation

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

5. Description of Reagents

Reagents
Each VetScan Prep 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 reagent rotor for use in calculating concentrations of alanine aminotransferase, alkaline phosphatase, glucose, and urea nitrogen. Dedicated sample blanks are included in the rotor to calculate the concentration of creatinine and total protein levels. Each reagent rotor also contains a diluent consisting of surfactants and preservatives.

Warnings and Precautions

- For Veterinary *In vitro* Diagnostic Use
- The diluent container in the reagent rotor is automatically opened when the analyzer drawer closes. A rotor with an opened diluent container can not be re-used. Ensure that the sample or control has been placed into the rotor before closing the drawer.
- Reagent beads may contain acids or caustic substances. The operator does not come into contact with the reagent beads when following the recommended procedures. In the event that the beads are handled (e.g., cleaning up after dropping and cracking a reagent rotor), avoid ingestion, skin contact, or inhalation of the reagent beads.
- Some Reagent beads contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Reagents will not come into contact with lead and copper plumbing when following recommended procedures. However, if the reagents do come into contact with such plumbing, flush with a large volume of water to prevent azide buildup.

Instructions for Reagent Handling
Reagent rotors may be used directly from the refrigerator without warming. Open the sealed foil pouch and remove the rotor being careful not to touch the bar code ring located on the top of the reagent rotor. Use according to the instructions provided in the VetScan Operator's Manual. A rotor not used within 20 minutes of opening the pouch should be discarded. Rotors in opened pouches can not be placed back in the refrigerator for use at a later time.

Storage
Store reagent rotors in their sealed pouches at 2-8˚C (36-46˚F). Do not expose opened or unopened rotors to direct sunlight or temperatures above 32˚C (90˚F). Do not allow the rotors sealed in their foil pouches to remain at room temperature longer than 48 hours prior to use. Open the pouch and remove the rotor just prior to use.

Indications of Reagent Rotor Instability or Deterioration
- All reagents contained in the reagent rotor, when stored as described above, are stable until the expiration date printed on the rotor pouch. Do **not** use a rotor after the expiration date. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on the VetScan Chemistry Analyzer display if the reagents have expired.
- A torn or otherwise damaged pouch may allow moisture to reach the unused rotor and adversely affect reagent performance. Do not use a rotor from a damaged pouch.

6. Instrument

See the VetScan Operator's Manual for complete information on using the analyzer.

7. Sample Collection and Preparation

The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma, serum or control. The reagent rotor sample chamber can contain up to 120 µL of sample.

- Specimens collected in a heparinized micropipette should be dispensed into the reagent rotor **immediately** following sample collection.
- Use only lithium heparin (green stopper) evacuated specimen collection tubes for whole blood or plasma samples. Use no additive (red stopper) evacuated specimen collection tubes or serum separator tubes (red or red/black stopper) for serum samples.
- Whole blood samples obtained by venipuncture must be homogenous before transferring a sample to the reagent rotor. Gently invert the collection tubes several times just prior to sample transfer. Do **not** shake the collection tube. Shaking may cause hemolysis.
- The test must be started within 10 minutes of transferring the sample into the reagent rotor.
- Whole blood venipuncture samples should be run within 60 minutes of collection; if this is not possible, separate the sample and transfer it into a clean test tube.[23] Run the separated plasma or serum sample within 5 hours of centrifugation. If this is not possible, refrigerate the sample in a stoppered test tube at 2-8°C (36-46°F) for no longer than 48 hours. A plasma or serum sample can be stored at -10°C (14°F) for up to 5 weeks in a freezer that does not have a self-defrost cycle.
- **Glucose** concentrations decrease approximately 5-12 mg/dL in 1 hour in uncentrifuged samples stored at room temperature.[24]
- Refrigerating whole blood samples can cause significant changes in concentrations of **glucose** and **creatinine**.[25]

Known Interfering Substances
- The only anticoagulant recommended for use with the VetScan Chemistry Analyzer is lithium heparin. 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 Prep Profile II reagent rotor.
- Physical interferents (hemolysis, icterus, and lipemia) may cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each result card to inform the operator about the levels of interferents present in each sample. The VetScan Chemistry Analyzer suppresses any results that are affected by >10% interference from hemolysis, lipemia, or icterus. "HEM", "LIP", "ICT" is printed on the result card in place of the result.
- **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.[26]
- Interference may be seen in the total protein test when analyzing samples with a 3 + lipemic index.[27] Samples with a triglyceride concentration >400 mg/dL may show an increased total protein level. The VetScan Chemistry Analyzer suppresses any results that are affected by >10% interference from lipemia. "LIP" is printed on the result card in place of the result.

8. Procedure

Materials Provided
- One VetScan Prep Profile II Reagent Rotor

Materials Required but not Provided
- VetScan Chemistry Analyzer

Test Parameters
The VetScan System operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each VetScan Prep Profile II Reagent Rotor is less than 14 minutes. The analyzer maintains the reagent rotor at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure
The complete sample collection and step-by-step operating procedures are detailed in the VetScan Operator's Manual.

Calibration
The VetScan Chemistry Analyzer is calibrated by the manufacturer before shipment. The barcode printed on the barcode ring provides the analyzer with rotor-specific calibration data. Please see the VetScan Operator's Manual**.**

Quality Control
Controls may be run periodically on the VetScan Chemistry Analyzer to verify the accuracy of the analyzer. Abaxis recommends that a serum-based commercially available control be run. Run controls on the reagent rotor in the same manner as for patient samples. See the VetScan Operator's Manual to run controls.

9. Results

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

10. Limitations of Procedure

General procedural limitations are discussed in the VetScan Systems Operator's Manual**.**

- Samples with hematocrits in excess of 60% packed red cell volume may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down and the plasma then re-run in a new reagent rotor.

Warning: Extensive testing of the VetScan Chemistry Analyzer has shown that in very rare instances, sample dispensed into the reagent rotor may not flow smoothly into the sample chamber. Due to the uneven flow, an inadequate quantity of sample may be analyzed and several results may fall outside your established reference ranges. The sample may be re-run using a new reagent rotor.

11. Expected Values

These normal intervals are provided only as a guideline. The most definitive reference intervals are those established for your patient population. Test results should be interpreted in conjunction with the patient's clinical signs. To customize specific normal ranges in your VetScan Chemistry Analyzer for the "Other" bank, refer to your VetScan Operator's Manual under the Menu Key functions.

Table 1: Reference Intervals

	Canine	Feline	Equine
ALT	10 – 118 U/L (10 – 118 U/L)	20 – 100 U/L (20 – 100 U/L)	5 – 20 U/L (5 – 20 U/L)
ALP	20 – 150 U/L (20 – 150 U/L)	10 – 90 U/L (10 – 90 U/L)	50 – 170 U/L (50 – 170 U/L)
CRE	0.3 – 1.4 mg/dL (27 – 124 µmol/L)	0.3 – 2.1 mg/dL (27 – 186 µmol/L)	0.6 – 2.2mg/dL (53 – 194 µmol/L)
GLU	60 – 110 mg/dL (3.3 – 6.1 mmol/L)	70 – 150 mg/dL (3.9 – 8.3 mmol/L)	65 – 110 mg/dL (3.6 – 6.1 mmol/L)
TP	5.4 – 8.2 g/dL (54 – 82 g/L)	5.4 – 8.2 g/dL (54 – 82 g/L)	5.7 – 8.0 g/dL (57 – 80 g/L)
BUN	7 – 25 mg/dL (2.5 – 8.9 mmol/L)	10 – 30 mg/dL (3.6 – 10.7 mmol/L)	7 – 25 mg/dL (2.5 – 8.9 mmol/L)

12. Performance Characteristics (Linearity)

The chemistry for each analyte is linear over the dynamic range listed below when the VetScan System is operated according to the recommended procedure (see the VetScan Operator's Manual). The Dynamic Range table referenced below represents the spectrum that the VetScan System can detect. **The intervals below do not represent normal ranges.**

Table 2: VetScan Dynamic Ranges

Analyte	Dynamic Ranges Common Units	SI Units
ALT	5-2000 U/L	5-2000 U/L
ALP	5-2400 U/L	5-2400 U/L
CRE	0.2-20 mg/dL	18-1768μmol/L
GLU	10-700 mg/dL	0.6-39mmol/L
TP	2-14 g/dL	20-140 g/L
BUN	2-180 mg/dL	0.7-64.3 mmol urea/L

Precision

Precision studies were conducted using the NCCLS EP5-A[27] guidelines with modifications based on NCCLS EP18-P[28] for unit-use devices. Results for within-run and total precision were determined by testing bi-level controls.

Table 3: Precision

Analyte		Sample Size	Within-Run	Total
ALT (U/L)		n=80		
Control 1				
	Mean		21	21
	SD		2.76	2.79
	%CV		13.1	13.3
Control 2				
	Mean		52	52
	SD		2.70	3.25
	%CV		5.2	6.3
ALP (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
CRE (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
Glu (mg/dL)		n=80		
Control 1				
	Mean		66	66
	SD		0.76	1.03
	%CV		1.2	1.6

Table 3: Precision Continued

Analyte		Sample Size	Within-Run	Total
Control 2				
	Mean		278	278
	SD		2.47	3.84
	%CV		0.9	1.4
TP (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
BUN (mg/dL)		n=120		
Control 1				
	Mean		19	19
	SD		0.35	0.40
	%CV		1.8	2.1
Control 2				
	Mean		65	65
	SD		1.06	1.18
	%CV		1.6	1.8

Correlation

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

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

		Correlation Coefficient	Slope	Intercept	N	Sample Range
ALT (U/L)	Canine	1.00	0.95	0	22-180	10 – 1549
	Feline	0.98	0.92	0	21-55	27 – 99
	Equine	0.97	0.94	6	7-101	11 – 30
ALP (U/L)	Canine	1.00	0.89	-5	22-180	15 - 1722
	Feline	0.97	0.81	1	21-55	6 – 54
	Equine	1.00	0.90	-4	7-101	119 - 1476
Cre (mg/dL)	Canine	0.99	1.00	0.0	22-180	0.6 – 10.6
	Feline	1.00	1.01	-0.1	21-55	0.3– 13.6
	Equine	0.95	1.00	-0.4	7-101	0.3 – 6.2
Glu (mg/dL)	Canine	0.96	1.01	-6	22-180	28 – 348
	Feline	1.00	0.97	3	21-55	52 – 607
	Equine	0.97	0.94	16	7-101	36 – 353
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
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

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