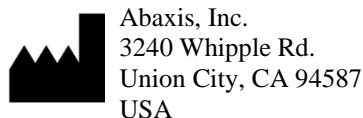


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## 1. Intended Use

The VetScan<sup>®</sup> Phenobarbital Profile 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), gamma glutamyl transferase (GGT), phenobarbital (PHB), and total bilirubin (TBIL) in heparinized whole blood, heparinized plasma, or serum.

## 2. Summary and Explanation of Tests

The VetScan Phenobarbital Profile reagent rotor and the VetScan VS2 Chemistry Analyzer comprise an *in vitro* diagnostic system that aids veterinarians in monitoring phenobarbital levels while determining liver health:

<b>Alanine Aminotransferase (ALT)</b>	Liver diseases, including viral hepatitis and cirrhosis; heart diseases
<b>Albumin (ALB)</b>	Liver and kidney diseases
<b>Alkaline Phosphatase (ALP)</b>	Liver, bone, parathyroid, and intestinal diseases
<b>Aspartate Aminotransferase (AST)</b>	Liver disease including hepatitis and viral jaundice; shock
<b>Blood Urea Nitrogen (BUN)</b>	Liver and kidney diseases
<b>Gamma Glutamyl Transferase (GGT)</b>	Liver disease, primary and secondary liver tumors
<b>Phenobarbital (PHB)</b>	Anticonvulsant drug used to prevent seizures
<b>Total Bilirubin (TBIL)</b>	Hepatic disorders

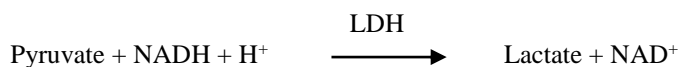
**As with any diagnostic test procedure, all other test procedures including the clinical status of the patient should be considered prior to final diagnosis.**

## 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.

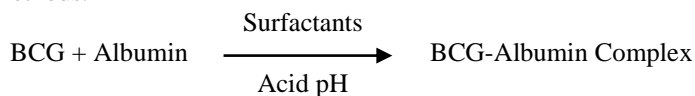




The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD<sup>+</sup> and is directly proportional to the amount of ALT present in the sample.

### Albumin (ALB)

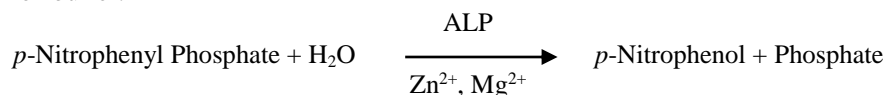
Dye binding techniques are the most frequently used methods for measuring albumin. Bromocresol green (BCG) is the most commonly used of the dye binding methods.<sup>3</sup>



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)

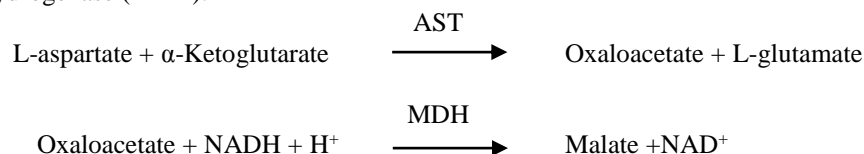
The VetScan procedure is modified from the AACC and IFCC methods.<sup>4</sup> Alkaline phosphatase hydrolyzes p-NPP in a metal-ion buffer and forms p-nitrophenol and phosphate. The use of p-nitrophenyl phosphate (p-NPP) increases the speed of the reaction.<sup>5,6</sup> The reliability of this technique 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>



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 α-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).



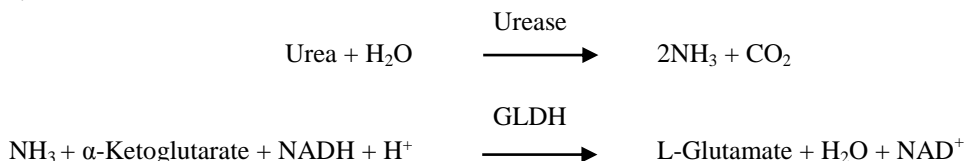
The rate of absorbance change caused by the conversion of NADH to NAD<sup>+</sup> is determined bichromatically at 340 nm and 405 nm. This rate is directly proportional to the amount of AST present in the sample.

### 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>.

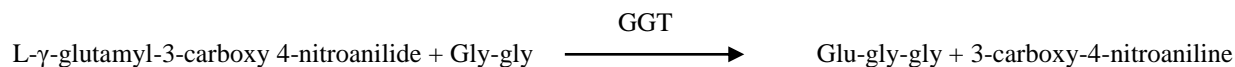


The rate of change of the absorbance difference between 340 nm and 405 nm is caused by the conversion of NADH to NAD<sup>+</sup> and is directly proportional to the amount of urea present in the sample.

### Gamma Glutamyl Transferase (GGT)

The first quantitative methods developed to measure gamma glutamyl transferase (GGT) involved a second reaction to form an azo dye that combined with a chromophore.<sup>19,20</sup> The change to L- $\gamma$ -glutamyl-*p*-nitroanilide as the substrate in the reaction eliminated the dye-formation step.<sup>21</sup> Due to the poor solubility and stability of L- $\gamma$ -glutamyl-*p*-nitroanilide, this procedure was modified to use the substrate L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide.<sup>22</sup> The International Federation of Clinical Chemistry (IFCC) recommended GGT method is based on the latter substrate, with glycylglycine as the other substrate.<sup>23</sup>

Abaxis has modified the IFCC method to react at 37°C. The addition of sample containing gamma glutamyl transferase to the substrates L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide and glycylglycine (gly-gly) causes the formation of L- $\gamma$ -glutamyl glycylglycine (glu-gly-gly) and 3-carboxy-4-nitroaniline.



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

### Phenobarbital (PHB)

Abaxis has adapted a commercially available homogeneous method for phenobarbital (PHB) for use in the VetScan VS2 Chemistry Analyzer. In the reaction, PHB competes with PHB labeled glucose-6-phosphate dehydrogenase enzyme (enzyme conjugate) for antibody (Ab) binding sites. Antibody bound enzyme conjugate has lower activity than unbound conjugate. High levels of PHB binding to antibody result in an increase in enzyme conjugate activity. The active enzyme reduces nicotinamide adenine dinucleotide (NAD<sup>+</sup>) to NADH.



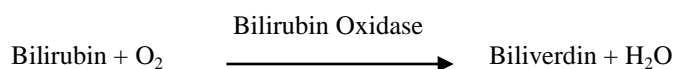
The rate of change of the absorbance at 340 nm is due to the conversion of NAD<sup>+</sup> to NADH and is directly proportional to the amount of phenobarbital in the sample.

### Total Bilirubin (TBIL)

Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.<sup>24,25</sup> A newer, more specific method has been developed using the enzyme bilirubin oxidase.<sup>26-28</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.



#### 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 Phenobarbital Profile reagent rotor contains dry test specific reagent beads. A dry sample blank reagent (comprised of buffer, surfactants, excipients and preservatives) is included in each reagent rotor for use in calculating concentrations of alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), gamma glutamyl transferase (GGT) and phenobarbital (PHB). Dedicated sample blanks are included in the rotor to calculate the concentration of 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, and serum or control material. The reagent rotor sample chamber can hold 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.<sup>29</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.
- Blood collection tubes with gel **cannot** be used for plasma and serum separation since it can cause changes in the concentration of **Phenobarbital**.<sup>30</sup>
- Samples are recommended to be filled to the manufacturer fill-line indicated on the label of the tube regardless of the tube size. They are designed to maintain the blood-to-additive ratio throughout the shelf life of the tube. At minimum, the tube should be at least half-filled.
- Refrigerating whole blood samples can cause significant changes in concentrations of **aspartate aminotransferase**.<sup>31</sup>
- **Total bilirubin** results may be adversely affected by photodegradation.<sup>32</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>33</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 Phenobarbital Profile reagent rotor.
- Physical interferents (hemolysis, icterus, and lipemia) may cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each 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.

## Exogenous Interference

Listed below are substances that do not interfere with the phenobarbital assay at the tested concentration.

Substances	Concentration Tested (µg/mL)
Potassium Bromide	3000
Diazepam	0.6
Levetiracetam	45
Zonisamide	40
Valproic Acid	100
Pregabalin	11
Phenytoin Sodium	15
5-(4-Hydroxyphenyl)-5-phenylhydantoin	20
Chlorpromazine hydrochloride	100
Amitriptyline hydrochloride	100

## 8. Procedure

### Materials Provided

- One VetScan Phenobarbital Profile Reagent Rotor PN: 500-1049 (a box of 12 rotors PN: 500-0049-12)

### 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 Phenobarbital Profile 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. 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, refer to your VetScan VS2 Operator's Manual.

**Table 1: Reference Intervals**

Analyte	Units	Canine	Feline
Alanine Aminotransferase (ALT)	U/L	10 – 118	20 – 100
Albumin (ALB)	g/dL g/L	2.5 – 4.4 25 – 44	2.2 – 4.4 22 – 44
Alkaline Phosphatase (ALP)	U/L	20 – 150	10 – 90
Aspartate Aminotransferase (AST)	U/L	14 – 45	12 – 43
Blood Urea Nitrogen (BUN)	mg/dL mmol urea/L	7 – 25 2.5 – 8.9	10 – 30 3.6 – 10.7
Gamma Glutamyl Transferase (GGT)	U/L	0 – 7	0 – 2
Phenobarbital (PHB)	µg/mL µmol/L	10 – 45 43.1 – 194.0	10 – 45 43.1 – 194.0
Total Bilirubin (TBIL)	mg/dL µmol/L	0.1 – 0.6 2 – 10	0.1 – 0.6 2 – 10

## 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.**

**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
Gamma Glutamyl Transferase (GGT)	5 – 3000 U/L	5 – 3000 U/L
Phenobarbital (PHB)	5.0 – 60.0 µg/mL	21.6 – 258.6 µmol/L
Total Bilirubin (TBIL)	0.1 – 30 mg/dL	1.7 – 513 µmol/L



**Precision**

Precision studies were conducted using the Clinical and Laboratory Standards Institute (CLSI) EP5-A<sup>34</sup>. Guidelines with modifications based on CLSI EP18-P<sup>35</sup> for unit-use devices. Results for within-run and total precision were determined by testing bi-level controls with the exception of Phenobarbital which used tri-level controls.

**Table 3: Precision**

Analyte	Sample Size	Within-Run	Total
<b>Alanine Aminotransferase (U/L)</b>			
<u>Control 1</u>	N=80		
Mean		21	21
SD		2.76	2.79
%CV		13.1	13.3
<u>Control 2</u>			
Mean		52	52
SD		2.70	3.25
%CV		5.2	6.3
<b>Albumin (g/dL)</b>			
<u>Control 1</u>	N=80		
Mean		3.9	3.9
SD		0.13	0.14
%CV		3.3	3.6
<u>Control 2</u>			
Mean		2.3	2.3
SD		0.09	0.10
%CV		3.9	4.3
<b>Alkaline Phosphatase (U/L)</b>			
<u>Control 1</u>	N=80		
Mean		39	39
SD		1.81	2.29
%CV		4.6	5.9
<u>Control 2</u>			
Mean		281	281
SD		4.08	8.75
%CV		1.5	3.1
<b>Aspartate Aminotransferase (U/L) N=80</b>			
<u>Control 1</u>			
Mean		47	47
SD		0.98	1.84
%CV		2.1	3.9
<u>Control 2</u>			
Mean		145	145
SD		1.83	4.62
%CV		1.3	3.2
<b>Blood Urea Nitrogen (mg/dL)</b>			
<u>Control 1</u>	N=120		
Mean		19	19
SD		0.35	0.40
%CV		1.8	2.1
<u>Control 2</u>			
Mean		65	65
SD		1.06	1.18
%CV		1.6	1.8

**Table 3: Precision (continued)**

Analyte	Sample Size	Within-Run	Total
<b>Gamma Glutamyl Transferase (U/L)</b>	N=80		
<u>Control 1</u>			
Mean		25	25
SD		0.59	0.74
%CV		2.3	2.9
<u>Control 2</u>			
Mean		106	106
SD		1.52	2.29
%CV		1.4	2.2
<b>Phenobarbital (µg/mL)</b>	N=40		
<u>Control 1</u>			
Mean		8.3	8.3
SD		0.73	0.92
%CV		8.8	11.1
<u>Control 2</u>			
Mean		22.4	22.4
SD		1.43	1.80
%CV		6.4	8.0
<u>Control 3</u>			
Mean		44.9	44.9
SD		2.99	3.74
%CV		6.7	8.3
<b>Total Bilirubin (mg/dL)</b>	N=80		
<u>Control 1</u>			
Mean		0.8	0.8
SD		0.06	0.07
%CV		7.5	8.8
<u>Control 2</u>			
Mean		5.2	5.2
SD		0.09	0.15
%CV		1.7	2.9

### Correlation

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

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

Analyte	Units	Species	Correlation Coefficient	Slope	Intercept	N	Sample 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
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
Alkaline Phosphatase (ALP)	UL	Canine	1.00	0.89	-5	22 – 180	15 – 1722
		Feline	0.97	0.81	1	21 – 55	6 – 54
Aspartate Aminotransferase (AST)	UL	Canine	1.00	1.02	1	22 – 180	18 – 176
		Feline	1.00	1.03	1	21 – 55	18 – 125
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
Gamma Glutamyl Transferase (GGT)	U/L	Canine	1.0	0.96	2	22 – 180	5 – 65
		Feline	NA	NA	NA	NA	NA
Phenobarbital (PHB)	µg/mL	Canine	0.98	1.09	-0.9	52	0 – 45
		Feline	NA	NA	NA	NA	NA
Total Bilirubin (TBIL)	mg/dL	Canine	0.87	0.84	0.1	22 – 180	0.1 – -3.2
		Feline	1.00	0.92	-0.3	21 – 55	0.4 – 15.0

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