VetScan® Mammalian Liver Profile

For Veterinary Use Only
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1. Intended Use

The VetScan® Mammalian Liver Profile reagent rotor used with the VetScan Chemistry Analyzer utilizes dry and liquid reagents to provide veterinary *in vitro* quantitative determinations of alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), bile acids (BA), total bilirubin (TBIL), total cholesterol (CHOL), gamma glutamyl transferase (GGT), and (blood) urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

2. Summary and Explanation of Tests

The VetScan Mammalian Liver Profile reagent rotor and the VetScan 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 disease.
Alkaline Phosphatase (ALP)	Liver, bone, parathyroid and intestinal diseases.
Bile Acids (BA)	Hepatobiliary disease; portosystemic vascular anomaly (PSVA); extrahepatic shunting.
Total Bilirubin (TBIL)	Hepatic disorders.
Total Cholesterol (CHOL)	Detection of hyperlipidemia; screening test for hypothyroidism and hyperadrenocorticism.
Gamma Glutamyl Transferase (GGT)	Liver disease, primary and secondary liver tumors
Blood Urea Nitrogen (BUN)	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
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.

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

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

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

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

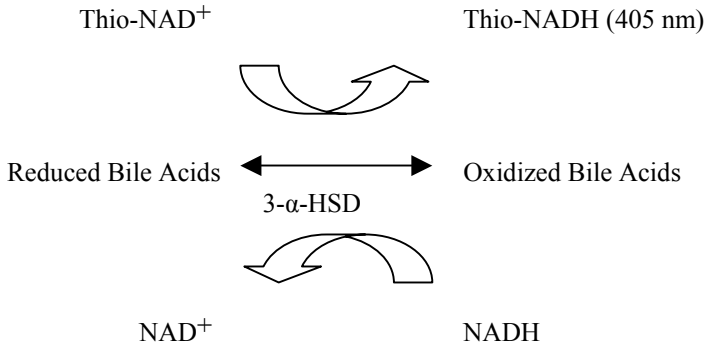
The VetScan procedure is modified from the AACC and IFCC methods.[4] Alkaline phosphatase hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate. The use of *p*-nitrophenyl phosphate (*p*-NPP) increases the speed of the reaction.[5, 6] The reliability of this technique is greatly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

$$\textit{p}\text{-Nitrophenyl Phosphate} + \text{H}_2\text{O} \xrightarrow[\text{Zn}^{2+},\ \text{Mg}^{2+}]{\text{ALP}} \textit{p}\text{-Nitrophenol} + \text{Phosphate}$$

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

Bile Acids

In the presence of the thio-derivative of nicotinamide adenine dinucleotide (Thio-NAD+) the enzyme 3-α-Hydroxysteroid Dehydrogenase (3-α-HSD) reversibly oxidizes bile acids to oxidized bile acids (3-α-keto forms) with the concomitant conversion of Thio-NAD+ to its reduced form (Thio-NADH). In a cycling reaction, the oxidized bile acids are returned to their reduced state when excess NADH is present. The NADH is converted to NAD+. In the Abaxis system, Thio-NAD+, NADH, and 3-α-HSD are supplied as dry reagent beads. The cycling reaction amplifies the levels of bile acids from the sample. The rate of increase in absorbance at 405 nm (Thio-NADH) is measured and is proportional to the concentration of bile acids in the sample. The rate is measured bichromatically at 405 and 500 nm.



Total Bilirubin

Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.[9,10] A newer, more specific method has been developed using the enzyme bilirubin oxidase.[11-13] 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 Cholesterol

The Abaxis Piccolo CHOL assay is an enzymatic end-point method that uses cholesterol esterase (CE) and cholesterol dehydrogenase (CHDH).[14]

$$\text{Cholesterol Esters} + H_2O \xrightarrow{\text{CE}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + NAD^+ \xrightarrow{\text{CHDH}} \text{Cholest-4-en-3-one} + NADH + H^+$$

CE hydrolyzes cholesterol esters to form cholesterol and fatty acids. The CHDH reaction converts cholesterol to cholest-4-en-3-one. The NADH is measured bichromatically at 340 nm and 405 nm. NADH production is directly proportional to the amount of cholesterol present. An assay-specific blank is also monitored to ensure no extraneous reactions interfere with the calculations of CHOL levels.

Gamma Glutamyl Transferase

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.[15,16] The change to L-γ-glutamyl-*p*-nitroanilide as the substrate in the reaction eliminated the dye-formation step.[17] Due to the poor solubility and stability of L-γ-glutamyl-*p*-nitroanilide, this procedure was modified to use the substrate L-γ-glutamyl-3-carboxy-4-nitroanilide.[18] The International Federation of Clinical Chemistry (IFCC) recommended GGT method is based on the latter substrate, with glycylglycine as the other substrate.[19]

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

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

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.

Urea Nitrogen

A coupled-enzymatic reaction is used by the Abaxis system. In this reaction, urease hydrolyzes urea into ammonia and carbon dioxide.[20] Upon combining ammonia with 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD^+.

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

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

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 Mammalian Liver 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, albumin, alkaline phosphatase, bile acids, total bilirubin, total cholesterol, gamma glutamyl transferase, and (blood) urea nitrogen. Dedicated sample blanks are included in the rotor to calculate the concentration of total bilirubin and total cholesterol 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 cannot 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 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 Chemistry Analyzer display if the reagents have expired.

Indications of Reagent Rotor Instability or Deterioration Continued

- 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.[21] 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.
- **Total bilirubin** results may be adversely affected by photodegradation.[22] Whole blood samples not run immediately should be stored in the dark for no longer than 60 minutes. If the sample can not be analyzed within that period, it should be separated into plasma or serum and stored in a capped sample tube in the dark at low temperatures.[23]

Known Interfering Substances
- The only anticoagulant recommended for use with the VetScan 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 Mammalian Liver 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 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.

8. Procedure

Materials Provided
- One Mammalian Liver Profile Reagent Rotor PN: 500-1040 (a box of 10 rotors PN: 500-0040)

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 Mammalian Liver Profile 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**.**

- **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 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)
ALB	2.5 – 4.4 g/dL (25 - 44 g/L)	2.2 – 4.4 g/dL (22 – 44 g/L)	2.2 – 3.7 g/dL (22 – 37 g/L)
ALP	20 – 150 U/L (20 – 150 U/L)	10 – 90 U/L (10 – 90 U/L)	50 – 170 U/L (SI = 50 – 170 U/L)
BA[24]	Fasting: 1 – 4 µmol/L (1 – 4 µmol/L) 2 Hrs Postprandial: 2 – 15 µmol/L (2 – 15 µmol/L) Cutoff: 25 µmol/L** (25 µmol/L)	Fasting: 1 – 3 µmol/L (1 – 3 µmol/L) 2 Hrs Postprandial: 7 – 9 µmol/L (7 – 9 µmol/L) Cutoff: 25 µmol/L** (25 µmol/L)	Fasting: NA Postprandial: NA Cutoff: 25 µmol/L** (25 µmol/L)
TBIL	0.1 – 0.6 mg/dL (2 – 10 µmol/L)	0. 1– 0.6 mg/dL (2 – 10 µmol/L)	0.5 – 2.3 mg/dL (9 – 39 µmol/L)
CHOL	125 – 270 mg/dL (3.2 – 7.0 mmol/L)	90 – 205 mg/dL (2.3 – 5.3 mmol/L)	50 – 140 mg/dL (1.3 – 3.6 mmol/L)
GGT	0 – 7 U/L (0 – 7 U/L)	0 – 2 U/L (0 – 2 U/L)	5 – 24 U/L (5 – 24 U/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)

* (SI Units)
** Cutoffs for Bile Acids (BA) set for 100% specificity with a sensitivity of 74% for dogs and cats. See Ettinger & Feldman, reference 24, pages 1288-1290.

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
ALB	1 – 6.5 g/dL	10 – 65 g/L
ALP	5 – 2400 U/L	5 – 2400 U/L
BA	1 – 140 µmol/L	1 – 140 µmol/L
TBIL	0.1 – 30 mg/dL	1.7 – 513 µmol/L
CHOL	20 – 520 mg/dL	0.52 – 13.5 mmol/L
GGT	5 – 3000 U/L	5 – 3000 U/L
BUN	2 – 180 mg/dL	0.7 – 64.3 mmol urea/L

Precision

Precision studies were conducted using the NCCLS EP5-A.[25] Guidelines with modifications based on NCCLS EP18-A[26] 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
Alanine Aminotransferase (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
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
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
Bile Acids (µmol/L)		n=40		
Control 1	Mean		24	24
	SD		0.33	0.33
	%CV		1.4	1.4

Table 3: Precision (Continued)

Analyte	Sample Size	Within-Run	Total
Control 2			
Mean		75	75
SD		1.03	1.33
%CV		1.4	1.8
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 Cholesterol (mg/dL)	n=80		
Control 1			
Mean		204	204
SD		6.64	6.84
%CV		3.3	3.4
Control 2			
Mean		275	275
SD		6.46	8.00
%CV		2.3	2.9
Gamma Glutamyl Transferase (U/L)	n=80		
Control 1			
Mean		25	25
SD		0.59	0.74
%CV		2.3	2.9
Control 2			
Mean		106	106
SD		1.52	2.29
%CV		1.4	2.2
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

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	Sample Range
Alanine Aminotransferase (U/L)	Canine	1.00	0.95	0	10 – 1549
	Feline	0.98	0.92	0	27 – 99
	Equine	0.97	0.94	6	11 – 30
Albumin (g/dL)	Canine	0.96	0.99	0.1	1.3 – 4.6
	Feline	0.75	1.02	0	2.1 – 4.8
	Equine	0.89	0.99	-0.6	1.2 – 3.2
Alkaline Phosphatase (U/L)	Canine	1.00	0.89	-5	15 – 1722
	Feline	0.97	0.81	1	6 – 54
	Equine	1.00	0.90	-4	119 – 1476
Bile Acids (µmol/L)	Canine	1.00	0.96	1	0 – 125
	Feline	1.00	1.09	-1	0 – 137
	Equine	*	*	*	*
Total Bilirubin (mg/dL)	Canine	0.87	0.84	0.1	0.1 – 3.2
	Feline	1.00	0.92	-0.3	0.4 – 15.0
	Equine	1.00	0.90	0.1	0.6 – 26.1
Total Cholesterol (mg/dL)	Canine	0.99	0.99	6	103 – 450
	Feline	0.99	1.06	-3	63 – 257
	Equine	*	*	*	*
Gamma Glutamyl Transferase (U/L)	Canine	1.00	0.96	2	5 – 65
	Feline	*	*	*	*
	Equine	0.99	1.11	0	5 – 317
Urea Nitrogen (mg/dL)	Canine	1.00	0.98	-2	4 – 117
	Feline	1.00	1.07	-5	14 – 165
	Equine	1.00	0.95	-1	3 – 64

* Not available

Note: Correlation studies for dogs included n = 22 – 180 samples; for cats included n = 21 – 55 samples; and for horses n = 7 – 101.

13. Bibliography

1. Wróbleski F, LaDue. 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 Phosphomomesterase. 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 Mg^{2+} and Zn^{2+} on rat placental alkaline phosphatase. I. Studies on the soluble Zn^{2+} and Mg^{2+} 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. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937; 119: 481-90.
10. Meites S. Bilirubin, directing reacting and total, modified Mally-Evelyn method. *In*: Selected Methods of Clinical Chemistry. Faulkner WR, Meites S, eds. Washington, DC: American Association for Clinical Chemistry. 1982: 119-24.
11. Murao S, Tanaka N. A new enzyme "bilirubin oxidase" produced by *Myrothecium* verrucaria MT-1. Agric Biol Chem 1981; 45: 2383-4.

12. Osaki S, Anderson S. Enzymatic determination of bilirubin. Clin Chem 1982; 30: 971 (Abstract).
13. Perry B, et al. Measurement of total bilirubin by use of bilirubin oxidase. Clin Chem 1986; 32: 329-32.
14. Kayamori, Y, et al. Endpoint colorimetric method for assaying total cholesterol in serum with cholesterol dehydrogenase. Clin Chem 1999; 45: 2158-2163.
15. Ball EG, Revel JP, Cooper O. The quantitative measurement of γ-glutamyl transpeptidase activity. J Biol Chem 1956; 221: 895-908.
16. Goldbarg JA, et al. The colorimetric determination of γ-glutamyl transpeptidase with a synthetic substrate. Arch Biochem Biophys 1960; 91: 61-70.
17. Orlowski M, Meister A. γ-glutamyl-*p*-nitroanilide: A new convenient substrate for determination and study of k-and d-γ-glutamyltranspeptidase activities. Biochem Biophys Acta 1963; 73: 679-681.
18. Persijn JP, can der Slik W. A new method for the determination of γ-glutamyl- transferase in serum. J Clin Chem Clin Biochem 1976; 14: 421-427.
19. Shaw LM, et al. IFCC methods for the measurement of catalytic concentration of enzymes. Part 4 IFCC method for γ-glutamyl-transferase. J Clin Chem Clin Biochem 1983; 21: 633-646.
20. Sampson, et al. A coupled-enzyme equilibrium method for measuring urea in serum: optimization and evaluation of the AACC study group on urea candidate reference method. Clin Chem 1980; 26: 816-826.
21. National Committee for Clinical Laboratory Standards. Procedures for handling and processing of blood specimens; approved guidline-2nd ed. NCCLS Document H18-A2. Wayne, PA: NCCLS, 1999.
22. Sherwin JE and Obernolte R. Bilirubin. *In:* Clinical Chemistry: Theory, Analysis and Correlation, 2nd ed. Kaplan LA, Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989: 1009-1015.
23. Henry RJ, Canon DC, Winkelman. Clinical Chemistry Principles and Technics, 2nd ed. New York: Harper and Row; 1974; 417-21 & 127-8.
24. Leveille-Webster CR. Chapter 141. Laboratory diagnosis of hepatobiliary disease. *In*: Textbook of Veterinary Internal Medicine, Vol 2, 5th ed. Ettinger SJ, Feldman EC, eds. St. Louis: W.B. Saunders Company. 2000: 1288-1290.
25. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS Document EP5-A. Wayne, PA: NCCLS, 1999.
26. National Committee for Clinical Laboratory Standards. Quality management for unit-use testing; approved guideline. NCCLS Document EP18-A. Wayne, PA: NCCLS, 2002.