VETSCAN® Phenobarbital Profile

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

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1. Intended Use

The VetScan® 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:

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
Gamma Glutamyl Transferase (GGT)	Liver disease, primary and secondary liver tumors
Phenobarbital (PHB)	Anticonvulsant drug used to prevent seizures
Total Bilirubin (TBIL)	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). [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]

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

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]

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

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).

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

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

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^+.

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

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

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.

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.[19,20] The change to L-γ-glutamyl-*p*-nitroanilide as the substrate in the reaction eliminated the dye-formation step.[21] 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.[22] The International Federation of Clinical Chemistry (IFCC) recommended GGT method is based on the latter substrate, with glycylglycine as the other substrate.[23]

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.

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

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^+) to NADH.

$$\text{PHB + Enzyme Conjugate + } NAD^+ \xrightarrow{\text{Ab}} \text{Ab-PHB + Ab-Enzyme Conjugate + NADH + } H^+$$

The rate of change of the absorbance at 340 nm is due to the conversion of NAD^+ 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.[24,25] A newer, more specific method has been developed using the enzyme bilirubin oxidase.[26–28] 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.

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

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.[29] 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**.[30]

- 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**.[31]

- **Total bilirubin** results may be adversely affected by photodegradation.[32] 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.[33]

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 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.

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. 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

12. Bibliography

1. Wrobleski F, LaDue JS. Serum glutamic pyruvic transaminase in cardiac with hepatic disease. *Proc Soc Exp Biol Med*. 1956;91(4):569-571.
2. Bergmeyer HU; Hørder 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(8):521-534.
3. Webster D, Bignell AH, Attwood EC. An assessment of the suitability of bromocresol green for the determination of serum albumin. *Clin Chim Acta*. 1974;53(1):101-108.
4. Bowers GN; Bergmeyer HU; Hørder M; Moss DW; 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:(1-2)pp:163F-174F.
5. Ohmori Y. Uber die Phosphomonoesterase. *Enzymologia*. 1937;4(July 29):217-231.
6. Fujita H. Über die Mikrobestimmung der Blutphosphatase. *J Biochem*. 1939;30(1):69-87.
7. PetitClerc C, Delisle M, Martel M, Fecteau C, Brière N. 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 Phosphatases. *Can J Biochem*. 1975;53(10):1089-1100.
8. Tietz NW, Burtis CA, Duncan P, et al. A reference method for measurement of alkaline phosphatase activity in human serum. *Clin Chem*. 1983;29(5):751-761.
9. Bergmeyer HU, Bowers GN, Horder M, Moss DW. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. IFCC method for aspartate aminotransferase. *Clin Chim Acta*. 1977;23:887-899.
10. Bergmeyer HU, Bowers GN, Horder M, Moss DW. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. Revised IFCC method for aspartate aminotransferase. *Clin Chim Acta*. 1978;24:720-721.
11. Fales FW. Urea in serum, direct diacetyl monoxime method. *Sel Methods Clin Chem*. 1982;9:365-373.
12. Van Slyke, Donald D; Cullen GE. A permanent preparation of urease, and its use in the determination of urea. *J Biol Chem*. 1914;19(2):211-228.
13. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. *J Clin Pathol*. 1960;13(2):156-159.
14. Chaney AL, Marbach EP. Urea and ammonia determinations. *Clin Chem*. 1962;8:130-132.
15. Talke H, Schubert GE. Enzymatische Harnstoffbestimmung in Blut und Serum im optischen Test nachWarburg. *Klin Wochenschr*. 1965;43(3):174-175.

16. Hallett CJ, Cook JGH. Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. *Clin Chim Acta*. 1971;35(1):33-37.
17. Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal Chem*. 1977;49(3):464-469.
18. Sampson EJ, Baird MA, Burtis CA, Smith EM, Witte DL, Bayse DD. 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(7):816-826.
19. Ball EG, Cooper O, Revel JP. The quantitative measurement of gamma glutamyl transpeptidase activity. *J Biol Chem*. 1956;221(2):895-908.
20. Goldbarg JA, Friedman OM, Pineda EP, et al. The colorimetric determination of γ-glutamyl transpeptidase with a synthetic substrate. *Arch Biochem Biophys*. 1960;91(1):61-70.
21. Orlowski M, Meister A. γ-Glutamyl-p-nitroanilide: a new convenient substrate for determination and study of l-and d-γ-glutamyltranspeptidase activities. *Biochim Biophys Acta (BBA)-Specialized Sect Enzymol Subj*. 1963;73(4):679-681.
22. Persijn JP, van der Slik W. A New Method for the Determination of γ-Glutamyltransferase in Serum. *Clin Chem Lab Med*. 1976;14(1-12):421-428.
23. Shaw ML. 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.
24. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. *J Biol Chem*. 1937;119(2):481-490.
25. Meites S. Bilirubin, directing reacting and total, modified Mally-Evelyn method. *Sel Methods Clin Chem Faulkner WR, Meites S, eds Washington, DC Am Assoc Clin Chem*. 1982;9:119-124.
26. Murao S, Tanaka N. Agricultural and Biological Chemistry A New Enzyme "Bilirubin Oxidase" Produced by Myrothecium verrucaria MT-1. *Agric Biol Chem Agric BioI Chem*. 1981;45(10):2383-2384.
27. Osaki S, Anderson S. Enzymatic determination of bilirubin. *Clin Chem*. 1982;30:971 (Abstract).
28. Perry B, Doumas BT, Buffone G, Glick M, Ou CN, Ryder K. Measurement of total bilirubin by use of bilirubin oxidase. *Clin Chem*. 1986;32(2):329-332.
29. The Clinical & Laboratory Standard Institute. *Procedures for Handling and Processing of Blood Specimens; Tentative Standard. CLSI Document H18-A2*. 2nd ed. Wayne, PA; 1999.
30. Bergqvist Y, Eckerbom S, Funding L. Effect of use of gel-barrier sampling tubes on determination of some antiepileptic drugs in serum. *Clin Chem*. 1984;30(3):465-466.
31. Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. *Clin Chem*. 1988;34(10):2111-2114.
32. Sherwin JE, Obernolte R. Bilirubin. In: *Clinical Chemistry: Theory, Analysis, and Correlation*. 2nd ed. St. Louis: The C.V. Mosby Company; 1989:1009-1015.
33. Henry RJ, Cannon DC, Winkleman JW. *Clinical Chemistry: Principles and Technics*. 2nd ed. New York: Harper and Row; 1974.