

# Laboratory Procedure Manual

*Analyte:* **Biochemistry Profile**

*Matrix:* **Serum**

*Method:* **Hitachi Model 917 Multichannel  
Analyzer**

*Method No.:*

*Revised:*

*as performed by:* *Coulston Foundation  
Alamogordo, New Mexico*

*Contact:* *Ms. Love Julian*

## Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999–2000 data.

Two laboratories performed this testing during 1999–2000. To maintain confidentiality of the participants, the quality control summary statistics and graphs were combined to mask the individual analysis dates from the two laboratories. Methods for both labs are included in this release.

A tabular list of the released analytes follows:

| Lab Number | Analyte  | SAS Label (and SI units)     |
|------------|----------|------------------------------|
| lab18      | LBXSAL   | Albumin (g/dL)               |
|            | LBDSALSI | Albumin (g/L)                |
|            | LBXSATSI | ALT (U/L)                    |
|            | LBXSASSI | AST (U/L)                    |
|            | LBDSAPSI | Alkaline phosphatase (U/L)   |
|            | LBXSBU   | Blood urea nitrogen (mg/dL)  |
|            | LBDSBUSI | Blood urea nitrogen (mmol/L) |
|            | LBXSCA   | Total calcium (mg/dL)        |
|            | LBDSCASI | Total calcium (mmol/L)       |
|            | LBXSCH   | Cholesterol (mg/dL)          |
|            | LBDSCHSI | Cholesterol (mmol/L)         |
|            | LBXSC3SI | Bicarbonate (mmol/L)         |
|            | LBXSGTSI | GGT (U/L)                    |
|            | LBXSGL   | Glucose, serum (mg/dL)       |
|            | LBDSGLSI | Glucose, serum (mmol/L)      |
|            | LBXSIR   | Iron (µg/dL)                 |
|            | LBDSIRSI | Iron (µmol/L)                |
|            | LBDSLDSI | LDH (U/L)                    |

|       |          |                           |
|-------|----------|---------------------------|
| lab18 | LBDSPH   | Phosphorus (mg/dL)        |
|       | LBDSPHSI | Phosphorus (mmol/L)       |
|       | LBDSTB   | Bilirubin, total (mg/dL)  |
|       | LBDSTBSI | Bilirubin, total (µmol/L) |
|       | LBXSTP   | Total protein (g/dL)      |
|       | LBDSTPSI | Total protein (g/L)       |
|       | LBXSTR   | Triglycerides (mg/dL)     |
|       | LBDSTRSI | Triglycerides (mmol/L)    |
|       | LBXSUA   | Uric acid (mg/dL)         |
|       | LBDSUASI | Uric acid (µmol/L)        |
|       | LBDSCR   | Creatinine (mg/dL)        |
|       | LBDSCRSI | Creatinine (µmol/L)       |
|       | LBXSNASI | Sodium (mmol/L)           |
|       | LBXSKSI  | Potassium (mmol/L)        |
|       | LBXSCLSI | Chloride (mmol/L)         |
|       | LBXSOSI  | Osmolality (mOsm/kg)      |
|       | LBXSGB   | Globulin (g/dL)           |
|       | LBDSGBSI | Globulin (g/L)            |

## 1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The 22 analytes described in this method constitute the routine biochemistry profile. The analyses are performed with a Hitachi Model 917 multichannel analyzer (Roche Diagnostics, Indianapolis, IN). Each analyte is described separately within each pertinent section of this document. NOTE: Glucose, cholesterol, and triglycerides were analyzed as part of this profile, but the results do not replace the formalized reference methods data from NHANES 1999–2000 samples analyzed at other institutions.

### a. Alanine Aminotransferase (ALT)

$\alpha$ -Ketoglutarate reacts with L-alanine in the presence of ALT to form L-glutamate plus pyruvate. The pyruvate is used in the indicator reaction for a kinetic determination of the reduced form of nicotinamide adenine dinucleotide (NADH) consumption. The International Federation of Clinical Chemistry (IFCC) has now recommended standardized procedures for ALT determination, including 1) optimization of substrate concentrations, 2) the use of Tris buffers, 3) preincubation of a combined buffer and serum solution to allow side reactions with NADH to occur, 4) substrate start ( $\alpha$ -ketoglutarate), and 5) optimal pyridoxal phosphate activation.

As a group, the transaminases catalyze the interconversion of amino acids and  $\alpha$ -keto acids by transferring the amino groups. The enzyme ALT been found to be in highest concentration in the liver, with decreasing concentrations found in kidney, heart, skeletal muscle, pancreas, spleen, and lung tissue. Alanine aminotransferase measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases. Elevated levels of the transaminases can indicate myocardial infarction, hepatic disease, muscular dystrophy, or organ damage. Serum elevations of ALT activity are rarely observed except in parenchymal liver disease, since ALT is a more liver-specific enzyme than aspartate aminotransferase (AST) (1).

### b. Albumin

At the reaction pH, the bromcresol purple (BCP) in the Roche Diagnostics (RD) albumin system reagent binds selectively with albumin. This reaction is based on a modification of a method described by Doumas (4). Although BCP is structurally similar to the conventional bromcresol green (BCG), its pH color change interval is higher (5.2–6.8) than the color change interval for BCG (3.8–5.4), thus reducing the number of weak electrostatic dye/protein interactions. The BCP system eliminates many of the nonspecific reactions with other serum proteins as a result of the increased pH. In addition, the use of a sample blank eliminates background spectral interferences not completely removed by bichromatic analyses.

Albumin constitutes about 60% of the total serum protein in normal, healthy individuals. Unlike most of the other serum proteins, albumin serves a number of functions which include transporting large insoluble organic anions (e.g., long-chain fatty acids and bilirubin), binding toxic heavy metal ions, transporting excess quantities of poorly soluble hormones (e.g., cortisol, aldosterone, and thyroxine), maintaining serum osmotic pressure, and providing a reserve store of protein. Albumin measurements are used in the diagnosis and treatment of numerous diseases primarily involving the liver or kidneys (2).

### c. Alkaline Phosphatase (ALP)

In the presence of magnesium ions, *p*-nitrophenylphosphate is hydrolyzed by phosphatases to phosphate and *p*-nitrophenol. The rate of *p*-nitrophenol liberation is proportional to the ALP activity and can be measured photometrically.

Increased ALP activity is associated with two groups of diseases: those affecting liver function and those involving osteoblastic activity in the bones. In hepatic disease, an increase in ALP activity is generally accepted as an indication of biliary obstruction. An increase in serum phosphatase activity is associated with primary hyperparathyroidism, secondary hyperparathyroidism owing to chronic renal disease, rickets, and osteitis deformans juvenilia due to vitamin D deficiency and malabsorption or

renal tubular dystrophies. Increased levels of ALP are also associated with Von Recklinghausen's disease with bone involvement and malignant infiltrations of bone. Low levels are associated with hyperthyroidism, and with the rare condition of idiopathic hypophosphatasia associated with rickets and the excretion of excess phosphatidyl ethanolamine in the urine (3).

d. Aspartate Aminotransferase (AST)

$\alpha$ -Ketoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate. The indicator reaction uses the oxaloacetate for a kinetic determination of NADH consumption. The International Federation of Clinical Chemistry (IFCC) has now recommended standardized procedures for ALT determination, including 1) optimization of substrate concentrations, 2) the use of Tris buffers, 3) preincubation of a combined buffer and serum solution to allow side reactions with NADH to occur, 4) substrate start ( $\alpha$ -ketoglutarate), and 5) optimal pyridoxal phosphate activation.

As a group, the transaminases catalyze the interconversion of amino acids and  $\alpha$ -keto acids by transferring the amino groups. The enzyme AST has been demonstrated in every animal and human tissue studied. Although the enzyme is most active in the heart muscle, significant activity has also been seen in the brain, liver, gastric mucosa, adipose tissue, skeletal muscle, and kidneys of humans. AST measurements are used in the diagnosis and treatment of certain types of liver and heart disease. AST is present in both the cytoplasm and mitochondria of cells. In cases involving mild tissue injury, the predominant form of serum AST is from the cytoplasm, with smaller amounts from the mitochondria. Severe tissue damage results in more of the mitochondrial enzyme being released. Elevated levels of the transaminases can signal myocardial infarction, hepatic disease, muscular dystrophy, or organ damage (4).

e. Bicarbonate ( $\text{HCO}_3^-$ )

Bicarbonate reacts with phosphoenolpyruvate (PEP) in the presence of PEPC to produce oxaloacetate and phosphate. This reaction occurs in conjunction with the transfer of a hydrogen ion from NADH to oxaloacetate using MDH. The resultant formation of NAD causes a decrease in absorbance in the UV range (320–400 nm). The change in absorbance is directly proportional to the concentration of bicarbonate in the sample being assayed.

Bicarbonate is the second largest fraction of the anions in plasma. Included in this fraction are the bicarbonate ( $\text{HCO}_3^-$ ) and carbonate ( $\text{CO}_3^{2-}$ ) ions and the carbamino compounds. At the pH of blood, the ratio of carbonate to bicarbonate is 1:1000. The carbamino compounds are also present, but are generally not mentioned specifically. The bicarbonate content of serum or plasma is a significant indicator of electrolyte dispersion and anion deficit. Together with pH determination, bicarbonate measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with acid-base imbalance in the respiratory and metabolic systems (5).

f. Blood Urea Nitrogen (BUN)

Urea is hydrolyzed by urease to form  $\text{CO}_2$  and ammonia. The ammonia formed then reacts with  $\alpha$ -ketoglutarate and NADH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and  $\text{NAD}^+$ . The decrease in absorbance due to consumption of NADH is measured kinetically.

Urea is synthesized in the liver from ammonia produced as a result of deamination of amino acids. This biosynthetic pathway is the human body's chief means of excreting surplus nitrogen. BUN measurements are used in the diagnosis of certain renal and metabolic diseases. The determination of serum urea nitrogen is the most widely used test for the evaluation of kidney function. The test is frequently requested in conjunction with the serum creatinine test for the differential diagnosis of prerenal, renal, and postrenal uremia. High BUN levels are associated with impaired renal function, increased protein catabolism, nephritis, intestinal obstruction, urinary obstruction, metallic poisoning, cardiac failure, peritonitis, dehydration, malignancy, pneumonia, surgical shock, Addison's disease, and uremia. Low BUN levels are associated with amyloidosis, acute liver disease, pregnancy, and nephrosis. Normal variations are observed according to a person's age and sex, the time of day, and

diet, particularly protein intake (6).

g. Calcium

Calcium reacts with o-cresolphthalein complexone in the presence of 8-hydroxyquinoline-5-sulfonic acid to form a purple complex. The intensity of the final reaction color is proportional to the amount of calcium in the specimen.

Elevated total serum calcium levels are associated with idiopathic hypercalcemia, vitamin D intoxication, hyperparathyroidism, sarcoidosis, pneumocystic carinii pneumonia and blue diaper syndrome. Low calcium levels are associated with hypoparathyroidism, pseudohypoparathyroidism, chronic renal failure, rickets, infantile tetany, and steroid therapy (7).

h. Cholesterol

All cholesterol esters present in serum or plasma are hydrolyzed quantitatively into free cholesterol and fatty acids by microbial cholesterol esterase. In the presence of oxygen, free cholesterol is oxidized by cholesterol oxidase to cholest-4-en-3-one. The  $H_2O_2$  reacts in the presence of peroxidase (POD) with phenol and 4-aminophenazone to form an o-quinone-imine dye. The intensity of the color is proportional to the cholesterol concentration and is measured photometrically.

An elevated cholesterol level is associated with diabetes, nephrosis, hypothyroidism, biliary obstruction, and those rare cases of idiopathic hypercholesterolemia and hyperlipemia; low levels are associated with hyperthyroidism, hepatitis, and sometimes severe anemia or infection (8).

i. Creatinine

This method, which uses the Jaffe reaction, is based on the work of Popper, Seeling, and Wuest. In an alkaline medium, creatinine forms a yellow-orange-colored complex with picric acid. The rate of color formation is proportional to the concentration of creatinine present and may be measured photometrically.

Creatinine measurement serves as a test for normal glomerular filtration. Elevated levels are associated with acute and chronic renal insufficiency and urinary tract obstruction. Levels below 0.6 mg/dL are of no significance (9).

j. Gamma Glutamyltransaminase ( $\gamma$ -GT)

In this rate method, L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide is used as a substrate and glycylglycine as a acceptor. The rate at which 5-amino-2-nitrobenzoate is liberated is proportional to  $\gamma$ -GT activity and is measured by an increase in absorbance.

$\gamma$ -GT measurement is principally used to diagnose and monitor hepatobiliary disease. It is currently the most sensitive enzymatic indicator of liver disease, with normal values rarely found in the presence of hepatic disease. It is also used as a sensitive screening test for occult alcoholism. Elevated levels are found in patients who chronically take drugs such as phenobarbital and phenytoin (10).

k. Glucose

Hexokinase catalyzes the phosphorylation of glucose by adenosine triphosphate (ATP). G-6-PD is oxidized to 6-phosphogluconate in the presence of NAD by the enzyme glucose-6-phosphate dehydrogenase. No other carbohydrate is oxidized.

The glucose hexokinase method, based on the work of Schmidt, Peterson, and Young, has long been recognized as the most specific method for the determination of glucose. Glucose measurements are used in the diagnosis and treatment of pancreatic islet cell carcinoma and of carbohydrate metabolism disorders, including diabetes mellitus, neonatal hypoglycemia, and idiopathic hypoglycemia (11).

l. Iron

Iron ( $\text{Fe}^{3+}$ ) is separated from transferrin by means of guanidinium chloride in the weakly acidic pH range and reduced to  $\text{Fe}^{2+}$  with ascorbic acid.  $\text{Fe}^{2+}$  then forms a colored complex with ferrozine.

Ingested iron is absorbed primarily from the intestinal tract and is temporarily stored in the mucosal cells as  $\text{Fe}_n^{3+}$ -ferritin, a complex of ferric hydroxide-ferric phosphate attached to the protein apoferritin. On demand, iron is released from the mucosal cells into the blood as  $\text{Fe}_2^{3+}$ -transferrin in equilibrium with a very small amount of free  $\text{Fe}^{3+}$ . Transferrin is the plasma iron transport protein that binds iron strongly at physiological pH levels.

Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, chronic renal disease, and hemochromatosis (a disease associated with widespread deposit in the tissues of two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin) (12).

m. Lactate Dehydrogenase (LDH)

This enzyme converts lactate and NADH to pyruvate and NAD<sup>+</sup> respectively. The rate at which NADH is formed is determined by the rate of absorbance and is directly proportional to enzyme activity.

LDH measurements are used in the diagnosis and treatment of liver diseases such as acute viral hepatitis, cirrhosis, and metastatic carcinoma of the liver; cardiac diseases such as myocardial infarction; and tumors of the lungs or kidneys (13).

n. Phosphorus

Inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form ammonium phosphomolybdate with a formula of  $(\text{NH}_4)_3[\text{PO}_4(\text{MoO}_3)_{12}]$ . The ammonium phosphomolybdate is quantified in the ultraviolet range (340 nm), through the use of a sample-blanked endpoint method.

More than 80% of the body's phosphorus is present in the bones as calcium phosphate. The remainder is involved in the intermediary metabolism of carbohydrates and is a component of such physiologically important substances as phospholipids, nucleic acids, and ATP. Phosphorus is present in blood as inorganic and organic phosphates, nearly all the latter residing in the erythrocytes. The small amount of extracellular organic phosphate exists almost exclusively in the form of phospholipid; the remainder of serum phosphorus is present as inorganic phosphate.

There is a reciprocal relationship between serum calcium and inorganic phosphorus. Any increase in the level of inorganic phosphorus causes a decrease in the calcium level by a mechanism not clearly understood. Hyperphosphatemia is associated with vitamin D hypervitaminosis, hypoparathyroidism, and renal failure. Hypophosphatemia is associated with rickets, hyperparathyroidism, and Fanconi syndrome.

Measurements of inorganic phosphorus are used in the diagnosis and treatment of various disorders, including parathyroid gland and kidney diseases and vitamin D imbalance (14).

o. Sodium, Potassium, and Chloride

An ion-selective electrode (ISE) makes use of the unique properties of certain membrane materials to develop an electrical potential (electromotive force, EMF) for the measurement of ions in solution. The electrode has a selective membrane in contact with both the test solution and an internal filling solution. The internal filling solution contains the test ion at a fixed concentration. Because of the particular nature of the membrane, the test ions will closely associate with the membrane on each side. The membrane EMF is determined by the difference between the ion concentration in the test solution and that in the internal filling solution. The EMF develops according to the Nernst equation for

a specific ion in solution:

$$[1] \quad E = E_0 + RT/nf \times \ln (f \times C_t/f \times C_i)$$

Where:

E = electrode EMF  
E<sub>0</sub> = standard EMF  
R = constant  
T = temperature  
n = charge of ion  
F = Faraday's constant  
ln = natural logarithm (base e)  
f = activity coefficient  
C<sub>t</sub> = ion concentration in test solution  
C<sub>i</sub> = ion concentration in internal filling solution

For sodium, potassium, and chloride, which all carry a single charge, R, T, n, and f are combined into a single value referred to as the slope (S). For determinations on the Hitachi 917 ISE module, where the sample is diluted 1:31, the ionic strength (and therefore the activity coefficient) is essentially constant. The concentration of the test ion in the internal filling solution is also constant. These constants may be combined into the E<sub>0</sub> term. The value of E<sub>0</sub> is also specific for the type of reference electrode used. Equation [1] can be rewritten to reflect these conditions:

$$[2] \quad E = E'_0 + S \times \ln(C_t)$$

The complete measurement system for a particular ion includes the ISE, a reference electrode, and electronic circuits to measure and process the EMF to give the test ion concentration. The direct-liquid-junction type reference electrode renews the reference electrode solution before and after sample measurement. The electromotive force is then measured to prevent drift.

The type of ISE used on the ISE Module is classified as the liquid/liquid junction type. The sodium and potassium electrodes are based on neutral carriers, and the chloride electrode is based on an ion exchanger.

Sodium is the major cation of extracellular fluid. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. Hyponatremia (low serum sodium level) is associated with a variety of conditions, including severe polyuria, metabolic acidosis, Addison's disease, diarrhea, and renal tubular disease. Hypernatremia (increased serum sodium level) is associated with Cushing's syndrome, severe dehydration due to primary water loss, certain types of brain injury, diabetic coma after therapy with insulin, and excess treatment with sodium salts.

Potassium is the major intracellular cation. Hypokalemia (low serum potassium level) is associated with body potassium deficiency, excessive potassium loss caused by prolonged diarrhea or prolonged periods of vomiting and increased secretion of mineralocorticosteroids. Hyperkalemia (increased serum potassium level) is associated with oliguria, anuria, and urinary obstruction.

Chloride is the major extracellular anion. Low serum chloride values are associated with salt-losing nephritis, Addisonian crisis, prolonged vomiting, and metabolic acidosis caused by excessive production or diminished excretion of acids. High serum chloride values are associated with dehydration and conditions causing decreased renal blood flow, such as congestive heart failure (15).

p. Total Bilirubin

Total bilirubin is coupled with diazonium salt DPD (2,5-dichlorophenyldiazonium tetrafluoroborate) in a strongly acidic medium (pH 1-2). The intensity of the color of the azobilirubin produced is proportional to the total bilirubin concentration and can be measured photometrically.



Bilirubin is an organic compound formed by the reticuloendothelial system during the normal and abnormal destruction of red blood cells. Elevated levels are associated with hemolytic jaundice, paroxysmal hemoglobinuria, pernicious anemia, polycythemia, icterus neonatorum, internal hemorrhage, acute hemolytic anemia, malaria, and septicemia. Low bilirubin levels are associated with aplastic anemia, and certain types of secondary anemia resulting from toxic therapy for carcinoma and chronic nephritis (16).

q. Total Protein

In alkaline solution, a colored chelate forms between cupric ions and compounds containing at least two  $-\text{CONH}_2$ ,  $-\text{CSNH}_2$ ,  $-\text{CH}_2\text{NH}_2$  or similar groups, joined directly or through a carbon or nitrogen atom. In proteins, the chelate is formed between one cupric ion and about six nearby peptide bonds. The intensity of the color is proportional to the total number of peptide bonds undergoing reaction and thus to the total amount of protein present. This is similar to the biuret reaction. Although compounds undergoing the biuret reaction give colors ranging from pink to purple, the violet colors given by serum albumins and globulins are essentially the same. Peptides of low molecular weight are present in serum, but their concentration is too low to cause interference.

Serum proteins perform a number of different functions in the body. In addition to being major structural components of cells, proteins are involved in transport, enzymatic catalysis, homeostatic control, hormonal regulation, blood coagulation, immunity, growth and repair, and heredity. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders (17).

r. Triglycerides

This method uses microbial lipase to promote rapid and complete hydrolysis of triglycerides to glycerol with subsequent oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The peroxide reacts with 4-aminophenazone and 4-chlorophenol in a Trinder reaction to a colorimetric endpoint.

Triglyceride measurements are used in the diagnosis of diabetes mellitus, nephrosis, liver obstruction, and other diseases involving lipid metabolism and various endocrine disorders and in the treatment of patients with these diseases (18).

s. Uric Acid

Uric acid is oxidized by the specific enzyme uricase to form allantoin and  $\text{H}_2\text{O}_2$ . The  $\text{H}_2\text{O}_2$  reacts with 2,4,6-tribromo-3-hydroxybenzoic acid (TBHB) and 4-aminophenazone in the presence of peroxidase to form quinone-imine dye and hydrogen bromide (HBr). The intensity of the red color is proportional to the uric acid concentration.

Uric acid measurements are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions and in the treatment of patients receiving cytotoxic drugs (19).

## 2. SPECIAL SAFETY PRECAUTIONS

Wear gloves, scrubs, laboratory coats, and face shields while handling all human blood products. Dispose of all biological samples and diluted specimens in a biohazard container at the end of the analysis. Place all disposable plastic, glass, and paper (pipet tips, Hitachi analyzer cups, tubes, gloves, etc.) that contact blood in the biohazard container located at the work sites. These containers will be used until they are 75% full, they then will be sealed, labeled, and transported to a biohazard storage facility until their removal by commercial contractor. Wipe down all work surfaces with 10% sodium hypochlorite solution when work is finished. Waste reagents from the Hitachi 917 and all control serum samples are considered a source of infectious material and must be treated with the same degree of caution as a high-risk specimen.

Material Safety Data Sheets (MSDS) for BCP chromogen; magnesium-L-aspartate; 2-amino-2-methyl-1-propanol buffer; magnesium; NADH/LDH; Tris/L-alanine buffer solution;  $\alpha$ -ketoglutarate solution; NADH; magnesium acetate; and phosphoenolpyruvate (PEP) buffer solution; bicarbonate diluent; PEPC/MDH, detergent/HCl solution; 2,5-dichlorophenyldiazonium tetrafluoroborate (DPD); GLDH/NADH/ $\alpha$ -ketoglutarate; urease substrate; o-cresolphthalein complexone/acetate buffer; solution contains 3,4-dichlorophenol; phenol; 4-aminophenazone; solution of cholesterol oxidase, cholesterol esterase, and horseradish; sodium hydroxide; picric acid; glycylglycine; Tris buffer; L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide; sodium chloride; sodium chloride/sulfuric acid; ammonium molybdate; solution containing sodium hydroxide, potassium sodium tartrate and potassium iodide; solution containing cupric sulfate, sodium hydroxide, potassium sodium tartrate and potassium iodide; boric acid; solution containing boric acid, sodium chloride, sodium bicarbonate, and potassium phosphate; potassium chloride; ATP/enzymes; buffer/4-chlorophenol; phosphate buffer/TBHB; solution of uricase/4-aminophenazone, phosphate buffer, and sodium hypochlorite are located adjacent to the RD HITACHI 917 in the WSRC clinical laboratory.

### 3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- a. The integrity of specimen and analytical data generated by this method is maintained by proofreading all transferred data from a printed copy of the output file and storing data in multiple computer systems. Data files containing the date, analytical run ID, specimen analytical results by specimen ID, and method code are stored in archive files in the Hitachi 917 main computer system in an ASCII format. Files are downloaded from the Hitachi 917 to the host computer (CompuAdd 386) via an RS232 port. The data are stored in two files: 1) the H\_917.DBF, which contains all data received from the Hitachi 917 and includes all participant data and analytical results, and 2) the H\_TABLE.DBF file, which contains the names of the tests, their respective Hitachi 917 test code numbers, and the date and time the samples were entered into the Hitachi 917 workstation. An output file, created by selecting fields from the NHANES files, is downloaded. An ASCII file of the data, created and copied to a 5¼" HD diskette, is sent to NCHS as an email attachment. The file is also copied onto another CompuAdd 386 in the laboratory administration area.
- b. Routine backup procedures include: 1) weekly backup of hard disks and 2) archival of data on a 3½" HD floppy diskette. Floppy diskettes containing sensitive data are stored in locked cabinets.
- c. Documentation for system maintenance is contained in hard copies of data recorded, as well as in files on the local tape drives used for archival of data.

### 4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

- a. Use a nonhemolyzed specimen from a fasting subject.
- b. Specimen type: serum or plasma with EDTA, heparin, citrate, or fluoride anticoagulants. Do not use oxalate.  
  
Separate serum or plasma from cells within 1 hour of collection.
- c. The optimal amount of specimen is 1.0 mL serum; the minimum is 0.5 mL serum or plasma.
- d. Acceptable containers for collection include 10- or 15-mL red-top or serum-separator Vacutainer tubes. Store serum in 2.0-mL Nalge tubes.
- e. Specimens should be refrigerated if not used immediately. Specimens stored longer than 24 hours should be frozen at  $\leq -20^{\circ}\text{C}$ . Specimen stability has been demonstrated for 1 year at  $\leq -20^{\circ}\text{C}$ .
- f. The criteria for unacceptable specimens are low volume ( $<0.25$  mL), hemolysis, improper labeling, and prolonged contact of serum or plasma with cells.
- g. Specimen handling conditions are outlined in the White Sands Clinical Laboratory's Collection

Procedures and Specimen Requirements Manual located in all sections of the laboratory and available to clients upon request. Collection, transport, and special requirements are discussed. In general, serum specimens from NCHS collection sites are transported on dry ice and stored at  $\leq -70^{\circ}\text{C}$  until analysis. Residual samples should be refrozen at  $\leq -70^{\circ}\text{C}$ .

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Instrumentation

- (1) Hitachi 917 automated analyzer (Roche Diagnostics, Indianapolis, IN). The analyzer includes a tungsten-halogen lamp, DMS 16-bit computer with 1MB RAM and 20 MB hard disk, remote computer workstation, and Okidata Microline 320 9-pin printer.
- (2) Sealpette variable-volume micropipets: 2-20, 20-200, and 200-1000  $\mu\text{L}$  volumes (Cole Scientific, Moorpark, CA).
- (3) Tek Pro Tek-tator V variable rotator (Baxter Healthcare, Valencia, NC).
- (4) Pipet-aid (Drummond Scientific Co., Broomall, PA).
- (5) Fisher hematology mixer (Fisher Scientific, Pittsburgh, PA).

b. Other Materials

- (1) Reagents (RD).
- (2) RD Precial calibrators (RD).
- (3) RD Precitrol normal and abnormal human assayed control serum (RD).
- (4) Physiological saline, 0.9% (Ricca Chemical, Arlington, TX). Must contain no additives or preservatives.
- (5) 3.0-mL, class A volumetric pipets (any vendor).
- (6) Conical-bottom 2.0-mL polystyrene autosampler cups (RD).
- (7) Ultrapure water, Barnstead E-pure water purification system with a resistivity of  $>16$  megohm-cm Culligan Water Systems, Alamogordo, NM).
- (8) 3.5-mL Beral polypropylene transfer pipettes (Sarstedt, Newton, NC).
- (9) Kim-Wipe tissues (Kimberly-Clark Corp., Roswell, GA).
- (10) Clean-room vinyl gloves (Baxter Healthcare).
- (11) Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Atlanta, GA).
- (12) Bleach (10% sodium hypochlorite solution) (any vendor).
- (13) 9½" x 11" 20-lb white computer paper (Viking Office Products, Irving, TX).

c. Reagent Preparation

(1) ALT

- (a) Reagent 1 (R1) working solution: (Bottles 1 and 1a) Tris buffer: 125 mmol/l, pH 7.3; L-alanine: 625 mmol/l; NADH: 0.23 mmol/l (yeast); LDH D 1.5 U/ml (microorganisms); preservative

Connect Bottle 1 to Bottle 1a and dissolve the granule into the buffer.

- (b) Reagent 2 (R2) working solution: Ketoglutarate: 94 mmol/l; preservative

Use  $\alpha$ -ketoglutarate solution, supplied "ready to use". Store capped at 2–8°C until the expiration date on the package.

(2) Albumin

- (a) Reagent 1 (R1) working solution: Citrate buffer: 95 mmol/l, pH 4.1; preservative

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: Citrate buffer: 95 mmol/l, pH 4.1; bromcresol green: 0.66 mmol/l;

Use supplied ready to use. Transfer the contents of BCP chromogen to an analyzer bottle. Store at 2–8°C until the expiration date on the package.

(3) ALP

- (a) Reagent 1 (R1) working solution: Buffer/magnesium (bottles 1 and 1a); 2-Amino-2-methyl-1-propanol D 0.93 mol/l, pH 10.5; magnesium-L-aspartate: 1.24 mmol/l; hydrochloric acid; zinc sulfate hepta-hydrate

Using a funnel, transfer 6 tablets of magnesium-L-aspartate (Bottle 1a) into contents of one Bottle 1 (Buffer). Swirl gently to dissolve. Aliquot into clean analyzer bottles and store capped at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: 2-Amino-2-methyl-1-propanol D 0.93 mol/l, pH 10.5; p-nitrophenyl phosphate: 101 mmol/l; hydrochloric acid; zinc sulfate heptahydrate

Dissolve 6 tablets of magnesium from one Bottle 2 (Substrate) by adding R1 Working Solution (Buffer/Magnesium) up to the base of the bottle neck (23 mL). Swirl gently to dissolve. Aliquot into clean analyzer bottles and store capped at 2–8°C until expiration date on package.

(4) AST

- (a) Reagent 1 (R1) working solution: Tris buffer: 100 mmol/l, pH 7,8; L-aspartate: 300 mmol/l; NADH: 0.23 mmol/l (yeast); MDH D 0,53 U/ml (porcine heart); LDH D 0,75 U/ml (microorganisms); preservative

Tap the bottom of the granulate bottle (Bottle 1a) before opening. Connect one Bottle 1a (Enzyme/Coenzyme) to Bottle 1 (Buffer) using one of the enclosed adapters. Pour granulate into the buffer and completely dissolve by inverting gently. Aliquot into clean analyzer bottles and store capped at 2–8°C.

- (b) Reagent 2 (R2) working solution: ketoglutarate: 75 mmol/l; preservative

Use  $\alpha$ -ketoglutarate solution, supplied "ready to use." Store capped at 2–8°C until the expiration date on the package.

- (5) Bicarbonate ( $\text{HCO}_3$ )

- (a) Reagent 1 (R1) working solution:  $\text{MgSO}_4$ : 780  $\mu\text{mol/l}$ ; preservative; surfactant; inhibitor; sodium oxamate

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: PEPC (plant):  $\geq 1.24$  KU/l; MDH (porcine heart):  $\geq 33.2$  KU/l; NADH:  $\geq 6.45$  mmol/l; PEP:  $\geq 21.1$  mmol/l;  $\text{MgSO}_4$ : 370  $\mu\text{mol/l}$ ; buffer; stabilizer; preservative; surfactant

Connect one bottle of bicarbonate diluent to one bottle of PEPC/MDH using one of the adapters. Mix by gentle inversion. R2 working solution is ready for use after 15 minutes. Invert several times prior to use. Store at 2–8°C until the expiration date on the package.

- (6) BUN

- (a) Reagent 1 (R1) working solution: CAPSO buffer: 5 mmol/l, pH 9.65; NADH  $\geq 0.23$  mmol/l (east); preservative

Use supplied "ready to use." Stable at 2–8°C until the expiration date on the package when protected from light and from contamination by microorganisms. Discard any solution with visible microbial growth, or when controls demonstrate shifts or trends.

- (b) Reagent 2 (R2) working solution: BICIN buffer: 1000 mmol/l, pH 7.6; urease  $\geq 7.2$  U/ml (jack bean); dextran-linked GLDH  $\geq 0.90$  U/ml (bovine liver);  $\alpha$ -ketoglutarate  $\geq 8.3$  mmol/l; preservative

Use supplied "ready to use." Stable at 2–8°C until the expiration date on the package when protected from contamination by microorganisms. Discard any solution with visible microbial growth, or when controls demonstrate shifts or trends.

- (7) Calcium

- (a) Reagent 1 (R1) working solution: Ethanolamine buffer: 1 mol/l, pH 10.6

Use contents of the blank reagent, supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: *o*-cresolphthalein complexone: 0.3 mmol/l; 8-hydroxyquinoline: 13.8 mmol/l; hydrochloric acid: 122 mmol/l

Use contents of the *o*-cresolphthalein complexone/acetate buffer reagent, supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (8) Cholesterol

Reagent 1 (R1) working solution: PIPES buffer (Piperazine-1,4-bis(2-ethane sulfonic acid)): 75 mmol/l, pH 6.8;  $\text{Mg}^{2+}$ : 10 mmol/l; sodium cholate: 0.2 mmol/l; 4-aminophenazone D 0.15 mmol/l; phenol D 4.2 mmol/l; fatty alcohol polyglycol ether: 1%; cholesterol esterase (*Pseudomonas spec.*) D 0.5 U/ml; cholesterol oxidase (*E. coli*) D 0.15 U/ml; peroxidase (horseradish) D 0.25 U/ml; stabilizers; preservative.

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

(9) Creatinine

- (a) Reagent 1 (R1) working solution: Sodium hydroxide: 0.20 mol/l

Use one bottle of NaOH, supplied "ready to use." Stable until the expiration date on the package if stored capped at 2–8°C.

- (b) Reagent 2 (R2) working solution: Picric acid

Use one bottle of picric acid, supplied ready to use. Stable until the expiration date on the package if stored capped at 2–8°C.

(10)  $\gamma$ -GT

- (a) Reagent 1 (R1) working solution: Reactive ingredients (approximate concentration after reconstitution): 143 mmol/L Tris (hydroxymethyl) aminomethane, pH 8.25; 14.3 mmol/L thiourea, 140 mmol/L glycylglycine; nonreactive ingredient: preservative.

Connect one bottle 1 to one bottle 1a using the enclosed adapter. Dissolve the granulate completely in the buffer. Aliquot into clean analyzer bottles and store capped at 2–8°C until the expiration date.

- (b) Reagent 2 (R2) working solution: Reactive ingredients (approximate concentration after reconstitution): 10.4 mmol/L L-g-Glutamyl-3-carboxy-4-nitroanilide; nonreactive ingredient preservative.

Connect one bottle 2 to one bottle 2a using the enclosed adapter and dissolve the granulate completely in the diluent. Aliquot into clean analyzer bottles and store capped at 2–8°C until the expiration date.

(11) Glucose

- (a) Reagent 1 (R1) working solution: Tris buffer\*: 100 mmol/L, pH 7.8; Mg<sup>2+</sup>: 4 mmol/L; ATP @ 1.7 mmol/L; NADP @ 1.0 mmol/L; preservative;\*\* Tris = Tris(hydroxymethyl)-aminomethane

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: R2 HEPES buffer\*: 30 mmol/L, pH 7.0; Mg<sup>2+</sup>: 4 mmol/L; HK @ 8.3U/mL (yeast); G-6-PDH @ 15 U/mL (E. coli); preservative; \* HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethane sulfonic acid.

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

(12) Iron

- (a) Reagent 1 (R1) working solution: Buffer/detergent, citric acid: 200 mmol/l; thiourea: 115 mmol/l; detergent

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: ascorbate/ferrozine; sodium ascorbate: 150 mmol/l; ferrozine: 6 mmol/l; preservative

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

(13) LDH

- (a) Reagent 1 (R1) working solution: *N*-methylglucamine: 400 mmol/l, pH 9.4 (37°C); lithium lactate: 61 mmol/l

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: NAD: 61 mmol/l; stabilizers and preservatives

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

(14) Phosphorus

- (a) Reagent 1 (R1) working solution: Sulfuric acid: 0.36 mol/l; detergent

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: Ammonium molybdate: 3.5 mmol/l; sulfuric acid: 0.36 mol/l; sodium chloride: 150 mmol/l

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

(15) Sodium, Potassium, and Chloride

- (a) ISE diluent: reactive ingredient (approximate concentration after reconstitution): 650 mmol/l boric acid; nonreactive ingredient: preservative

For working solution, add appropriate amount of distilled or deionized water to one bottle of ISE Diluent. Mix thoroughly by inversion. Store until the expiration date on the package.

- (b) ISE internal reference solution: Reactive ingredients (approximate concentration after reconstitution): 650 mmol/L boric acid, 32.3 mmol/L sodium chloride, 12.9 mmol/L sodium bicarbonate, 1.6 mmol/L potassium phosphate; nonreactive ingredient: preservative

Store until the expiration date on the package.

- (c) Reference electrode internal solution: Reactive ingredient: 1 mol/l potassium chloride

Use reagent as provided. Stable until the expiration date on the package.

(16) Total Bilirubin

- (a) Reagent 1 (R1) working solution: C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub> (sodium acetate buffer): 85 mmol/l; H<sub>3</sub>NO<sub>3</sub>S (sulfamic acid): 110 mmol/l; surfactant; solubilizer R2

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: HCl: 100 mmol/l; diazonium ion: 3 mmol/l

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

(17) Total Protein

- (a) Reagent 1 (R1) working solution: Sodium hydroxide: 400 mmol/l; potassium sodium tartrate:

89 mmol/l

Use contents of blank, supplied "ready to use." Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: Sodium hydroxide: 400 mmol/l; potassium sodium tartrate: 89 mmol/l; potassium iodide: 61 mmol/l; copper sulfate: 24.3 mmol/l.

Use supplied "ready to use." Store at 2–8°C until the expiration date on the package.

(18) Triglycerides

- (a) Reagent 1 (R1) working solution: Tris buffer: 0.15 mol/l, pH 7.6; magnesium sulfate: 17.5 mmol/l; EDTA, disodium salt: 10 mmol/l; 4-chlorophenol: 3.5 mmol/l; Potassium hexacyanoferrate (II): 6 µmol/l; Sodium cholate: 0.15%; hydroxypolyethoxy-*n*-alkanes: 0.12%; ATP: ≥1 mmol/l; glycerol kinase (*Candida mycoderma*): 0.4 ≥U/ml; glycerol phosphate oxidase (microbial): 5 ≥U/ml; peroxidase (horseradish): ≥0.3 U/ml; preservative

Connect one Bottle 1a (enzymes) to one Bottle 1 (buffer) using one of the enclosed adapters. Mix by gentle inversion. Store at 2–8°C until the expiration date on the package.

- (b) Reagent 2 (R2) working solution: Tris buffer: 0.15 mol/l, pH 7.6; magnesium sulfate: 17.5 mmol/l; EDTA, disodium salt: 10 mmol/l; 4-chlorophenol: 3.5 mmol/l; potassium hexacyanoferrate (II): 6 µmol/l; sodium cholate: 0.15%; hydroxypolyethoxy-*n*-alkanes: 0.12%; lipase (*Pseudomonas* species): ≥6 U/ml; 4-aminophenazone: 0.7 mmol/l; preservative

Connect one Bottle 2a (lipase/4-aminophenazone) to one Bottle 2 (buffer) using one of the enclosed adapters. Mix by gentle inversion.

(19) Uric acid

- (a) Reagent 1 (R1) working solution: Buffer/enzyme/TOOS  
Phosphate buffer: 0.05 mol/l, pH 7.8; TOOS: 7 mmol/l; fatty alcohol polyglycol ether: 4.8%; ascorbate oxidase (EC 1.10.3.3; zucchini).

Use supplied "ready to use." Store capped at 2–8°C until the expiration date.

- (b) Reagent 2 (R2) working solution: Buffer/enzymes/4-aminophenazone  
Phosphate buffer: 0.1 mol/l, pH 7.8; potassium hexacyanoferrate (II): 0.30 mmol/l; 4-aminophenazone D 3 mmol/l; uricase (EC 1.7.3.3; *Arthrobacter protophormiae*; 25°C) D 0.5 U/ml; peroxidase (POD) (EC 1.11.1.7; horseradish; 25°C) D 1 U/ml.

Use supplied "ready to use." Store capped at 2–8°C until the expiration date.

d. Standards Preparation

- (1) Precical diluent

Store unopened diluent until the expiration date on the vial.

- (2) RD Precical calibrator serum

A human serum with added chemicals, human and animal tissue extracts, and preservatives. Constituent concentrations are specific for each lot used. Store unopened Precical calibrator serum at 2–8°C until the expiration date on the vial.

- (a) Bring Precical diluent to 20–25°C before use.



- (b) Remove Precical calibrator serum from 2–8°C storage. Tap the calibrator serum bottle lightly to dislodge the lyophilized material.
  - (c) Invert the bottle containing the calibrator serum several times to cover all inside surfaces of the bottle. Immediately place the calibrator serum on a mechanical rotator.
  - (e) Remove the calibrator serum from the mechanical rotator and store prior to first use. Visually inspect the calibrator serum for total dissolution before use.
- (3) Calibration standards
- (a) Use the standards according to manufacturer's specifications
  - (c) Dispense Precitrol normal, and Precitrol abnormal serum into separate Hitachi sample cups.
  - (d) Place all barcoded tubes on the Hitachi 917 sample wheel starting at correct position with the barcodes facing towards the center. Place the calibration standards on the disk before control samples. This will ensure that the instrument is calibrated prior to the control sample analysis. In the case of photometric, linear chemistries, place the saline solution before the calibration samples.
  - (e) At the computer terminal, request "CALIBRATION SELECTION".
  - (f) Press the function key to order controls for the run. The Control Select Menu will appear on the screen. Press ENTER. Deselect any tests not matching selected controls. Ensure that all controls are selected for each parameter. Type the number of control groups.
  - (g) The cursor will move to the "HOME" position. Monitor the run.
- e. Preparation of Quality Control Materials

The quality control materials are commercial preparations of human serum with added human and animal tissue extracts and preservatives. The constituent concentrations are specific for each lot.

- (1) Reconstitute Precitrol normal and abnormal control serum as follows:
- (a) Bring all vials of control serum and diluent to 20–25°C before reconstitution.
  - (b) Tap the control serum bottle lightly to dislodge the lyophilized material.
  - (c) Using a volumetric pipette, transfer the appropriate diluent into a bottle of the control serum. Do not mix lot numbers of diluent and controls. Do not pour diluent directly into the control serum vial.
  - (d) Invert bottles several times and place them on a mechanical rotator.
  - (e) Remove bottles of control serum from the rotator and store the bottles prior to use.
  - (f) Store reconstituted control serum at 2–8°C between each use. Invert the bottles gently before each use to ensure total homogeneity.

## 7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

### a. Calibration Curve

Endpoint/endpoint with sample blank, kinetic, and ISE are the three calibration curves generated by this instrument. Calibration is automatically performed by the analyzer. A blank calibration occurs daily and with a bottle or reagent lot change.

#### (1) Endpoint/endpoint with sample blank

This instrument generates an endpoint/endpoint with sample blank calibration curve for albumin, bicarbonate ( $\text{HCO}_3$ ), total bilirubin, calcium, cholesterol, glucose, iron, phosphorus, total protein, triglycerides, and uric acid parameters.

- (a) A calibration sequence must be performed to ensure accurate chemistry results on the Hitachi 917. This calibration establishes the calibration factors. The factors are then used to convert the electronic response of the instrument into concentration or activity for the constituent being measured.
- (b) To determine the reagent blank absorbance and thus establish a baseline for each test, analyze the blank sample in duplicate for each requested test. When reagents are added to the blank sample in the reaction cell, the final absorbance readings reflect the absorbance of the reagents. The absorbance readings for the two blank samples are averaged and the mean blank absorbance thus determined is stored in memory.
- (c) The calibrator is analyzed in duplicate. The absorbance readings are averaged and the mean calibration value thus determined is stored in memory. A calibration factor is then calculated by the computer.
- (d) The computer retains two sets of calibration data for each test (current and previous). The computer updates the current calibration if the data are acceptable.
- (e) A calibration report is then printed by the computer. It contains information on the calibration ID, the set point, the ABS or MV reading, the factor calibrated from the curve, and the sensitivity. It also prints the previous calibration and calculates a ratio. The "ratio" column is calculated by dividing the previous factor by the current results. This number gives the operator a quick indication of the stability of the calibration analysis for each channel.

#### (2) Kinetic

This instrument generates a kinetic calibration curve for alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, gamma glutamyl transaminase, and lactate dehydrogenase.

- (a) A calibration sequence must be performed to ensure accurate chemistry results on the Hitachi 917. This calibration establishes the calibration factors. These factors in turn are used to convert the electronic response of the instrument into concentration or activity for the constituent being measured.
- (b) To determine the reagent blank absorbance and thus establish a baseline for each test, analyze the blank sample in duplicate for each requested test. When reagents are added to the blank sample in the reaction cell, the final absorbance readings reflect the absorbance of the reagents. The absorbance readings for the two blank samples are printed and used in the factor calculation.
- (c) The calibration factor K for this parameter assay is established according to the following formula when the instrument is installed by a Roche Diagnostics representative.

$$K = \frac{\text{total reaction volume} \times 100}{\text{extinction coefficient} \times \text{lightpath (cm)} \times \text{specimen vol.}}$$

- (d) The factor is then typed into the "factor (fixed)" column in system 9, 12. Because this factor remains constant for this instrument, no recalibration is required. This factor is monitored with the QC program and a daily zero point calibration against saline.
- (e) A calibration report must then be requested from the computer, which contains information on the calibration ID, set point, ABS or MV reading, factor setting, and the sensitivity. It also prints the previous calibration and calculates a ratio. The "ratio" column is calculated by dividing the previous factor by the current results. This number gives the operator a quick indication of the stability of the calibration analysis for each channel.

(3) ISE

This instrument generates an ISE calibration curve for sodium, potassium, and chloride.

- (a) The ISE module may be calibrated with the chemistry channels. The calibration requires the use of high and low standard solutions to determine the slope factor, and a serum-based calibrator to adjust the ISE calibration for differences between the response of aqueous standards and the response of serum.
- (b) An internal reference solution is measured during calibration and between each sample to correct the calibration for drift between calibrations.
- (c) The electromotive force of the internal reference solution must fall within the following ranges:

$$\begin{aligned} \text{Na}^+ &: -90 \text{ to } -10 \text{ mV} \\ \text{K}^+ &: -90 \text{ to } -10 \text{ mV} \\ \text{Cl}^- &: 80 \text{ to } 160 \text{ mVs} \end{aligned}$$

- (d) The values must also fall between the EMF values for the low standard and the high standard.
- (e) The slope values must fall within the following ranges:

$$\begin{aligned} \text{Na}^+ &: 32.0 \text{ to } 68.0 \text{ mV/decade} \\ \text{K}^+ &: 32.0 \text{ to } 68.0 \text{ mV/decade} \\ \text{Cl}^- &: -35 \text{ to } -68 \text{ mV/decade} \end{aligned}$$

- (f) A calibration report is then printed by the computer; it contains information on the calibration ID, the set point, the ABS or MV reading, the factor calibrated from the curve, and the sensitivity. It also prints the previous calibration and calculates a ratio. The "ratio" column is calculated by dividing the previous factor by the current results. This number gives the operator a quick indication of the stability of the calibration analysis for each channel.

b. Verification

None required. WSRC Clinical Laboratory utilizes PreciLin Linearity Solutions to verify calibration and reportable recovery.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

- (1) For information regarding the range of linearity and how to handle results outside this range, refer to the calculations section of this document (Section 8.g).
  - (2) Allow frozen blood specimens, quality control serum, and calibration serum to reach 20–25°C and mix by inversion for 10 sec.
  - (3) Prepare a sufficient number of barcoded tubes for the samples being tested.
- b. Sample Preparation
- (1) Store specimens at 4–8°C until analysis.
  - (2) Dispense each specimen into an analyzer cup in the appropriately barcoded analyzer tube.
  - (3) Place all barcoded tubes on the Hitachi 917 sample wheel starting at position #1 with the barcodes facing center. Ensure that the instrument is calibrated and verified before starting the unknown sample analysis.
- c. Instrument Setup for the Hitachi 917 Chemistry Analyzer
- (1) Set the parameters for the Hitachi 917.
  - (2) Turn on the water by opening the valve on the Barnstead unit.
  - (3) Power up the Hitachi 917 analyzer by turning the main circuit breaker ON. The computer will boot the operating program. Allow 30 min for the waterbath on the instrument and ISE chamber to reach 37°C and the mechanical devices to perform a synchronization.
  - (4) The screen will display reagent volumes and the number of tests remaining for each chemical profile. Determine if sufficient reagent is available for calibration and the scheduled run. Prepare any needed reagents, place them in the in appropriate channel in the reagent compartment, and update new reagent volumes by inputting the new volume.
  - (5) Depress the HOME key,
  - (6) The operator must now request a start-up report. The system will initiate a system function check. Quickly review the report and verify the current photometer and temperature conditions as well as the programmed system parameters.
  - (7) If a problem is detected at this point, the supervisor must be notified for technical assistance.
  - (8) Initiate priming of reagents,
  - (9) Request "CALIBRATION SELECTION".
  - (10) Request "CONTROL SELECTION".
  - (11) Enter information from the NHANES transmittal form submitted with specimens.
  - (12) Obtain a work pending list.
- d. Operation of Assay Procedure
- (1) Request "MONITOR RUN".
  - (2) After all calibration and control material has been analyzed, request a calibration report. If the

channel has been calibrated, proceed to control verification. If the channel did not calibrate, the channel should be recalibrated.

- (3) Verify the calibration by printing a control report. Check that the quality control materials are within the specified limits and that no shifts or trends are present.
- (4) If the values observed for the control materials are "in control," proceed with the analysis of the NHANES specimens.
- (5) Load all barcoded specimens on the Hitachi 917 assay tray with the barcoded label facing the center of the tray.
- (6) At the computer workstation, request "MONITOR RUN".

e. Recording of Data

(1) Quality Control Data

The quality control data are automatically stored on the hard disk daily. At the end of each day, request all control data accumulated during the instrument operation period. The report will be printed on two-part carbonless computer paper. This report must be given to a computer analyst for entry into the QC data table. At the end of each month, print out Levey-Jennings charts, means, 2 SD ranges and %CVs. This report must be posted on the QC board in the laboratory for one month and then placed in a bound QC book for archival.

The quality control data are automatically stored on the computer in the QC program. Results are printed daily, and Levey-Jennings charts are printed monthly. These charts are included in the quarterly report to NCHS.

(2) Analytical Results

Results which are collated by the Hitachi 917 computer system include 1) participant demographic information, 2) names of tests performed, 3) units for each parameter, 4) normal ranges, 5) result obtained, 6) any flags pertaining to those results (high or low), and 7) the results depicted graphically. To obtain a report of the participant results, the supervisor must first review the data. If the supervisor decides that any of the results are unacceptable, the operator must perform a rerun of the necessary parameters. Request a printout of the participant report by pressing the function key F10.

Give the form to the computer analyst so that results can be verified against the ASCII file, which is printed from the host computer system. After verifying the results, the computer analyst will transfer the ASCII file to a 5¼" HD diskette and send the results as an email attachment to the NHANES coordinator. A printout of the ASCII file of the results will be filed in the study notebook.

f. Replacement and Periodic Maintenance of Key Components

- (1) Clean the dispenser nozzles daily and the reagent lines monthly with a 10% sodium hypochlorite solution. If a QC or calibration problem occurs, clean the lines and nozzles as part of the problem-solving procedure.
- (2) Clean the reaction cells daily. Maintain a complete set of spare cells so that replacements can be made when a cell breaks.
- (3) Take photometer lamp readings daily and record the results in the Maintenance Log. Maintain spare lamps so that a replacement lamp can be installed if readings significantly change.
- (4) Maintain spare ISE cartridges and reference and ground electrodes so that these can be replaced when problems occur with the ISE channel.

g. Calculations

This instrument performs separate calculations for each assay type. The four assay types calculated by the Hitachi are endpoint with sample blank, endpoint, kinetic, and ISE. Specimen analysis must be repeated when results are outside the  $\pm 2$  SD range. Duplicates must agree within 10%.

(1) Assay type: endpoint with sample blank

The analyzer computer uses absorbance measurements to calculate albumin, bicarbonate ( $\text{HCO}_3$ ), calcium, glucose, iron, phosphorus, and total protein concentrations as follows:

The analyzer computer uses absorbance measurements to calculate concentrations as follows:

$$C_x = K(A_x \text{ to } A_b) + C_b$$

Where:

$C_x$  = Concentration of Sample.

$K$  = Concentration Factor (determined during calibration).

$A_x$  = Mean of absorbances of Sample + R1 read during designated cycles.

$A_b$  = Mean of absorbances of STD 1 (Blank/CALIB 1)+ R1 read during designated cycles.

$C_b$  = Concentration of STD 1 (Blank/CALIB 1).

(a) Albumin

The albumin method is linear up to 10.0 g/dL.

When reanalyzing any specimen with a concentration greater than 10 g/dL, prepare a twofold (1+1) dilution of the specimen with distilled deionized water. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 0.2 g/dL. Results below the detection limit are reported as <0.2 g/dL, and the specimen is reassayed with a microprotein assay.

(b) Bicarbonate ( $\text{HCO}_3$ )

The method is linear up to 40.0 mmol/L.

When reanalyzing any specimen with a concentration greater than 40 mmol/L, prepare a twofold (1+1) dilution of the specimen with deionized carbon dioxide-free water. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 5 mmol/L. Results below the detection limit are reported as <5 mmol/L.

(c) Calcium

The method is linear up to 20.0 mg/dL.

When reanalyzing any specimen with a concentration greater than 20 mg/dL, prepare a twofold (1+1) dilution of the specimen with physiological saline. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 0.2 mg/dL. Results below the detection limit are reported as < 0.2 mg/dL.

(d) Glucose

The method is linear up to 750 mg/dL.

When reanalyzing any specimen with a concentration greater than 750 mg/dL, prepare a twofold (1+1) dilution of the specimen with distilled deionized water. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 2.0 mg/dL. Results below the detection limit are reported as <2.0 mg/dL.

(e) Iron

The method is linear up to 1000 µg/dL.

When reanalyzing any specimen with a concentration greater than 1000 µg/dL, prepare a twofold (1+1) dilution of the specimen with distilled deionized water. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 5.0 µg/dL. Results below the detection limit are reported as <5.0 µg/dL.

(f) Phosphorus

The method is linear up to 20.0 mg/dL.

When reanalyzing any specimen with a concentration greater than 20 mg/dL, prepare a twofold (1+1) dilution of the specimen with distilled deionized water. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 0.3 mg/dL. Results below the detection limit are reported as <0.3 mg/dL.

(g) Total protein

The method is linear up to 15.0 g/dL.

When reanalyzing any specimen with a concentration greater than 15 g/dL, prepare a twofold (1+1) dilution of the specimen with distilled deionized water. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 0.2 g/dL. Results below the detection limit are reported as <0.2 g/dL, and the specimen is reassayed with a microprotein assay.

(2) Assay type: Endpoint

The analyzer computer uses absorbance measurements to calculate concentrations as follows:

$$C_x = K(A_x - A_b) + C_b$$

Where:

|       |   |   |
|-------|---|---|
| $C_x$ | = | Concentration of sample.                              |
| $K$   | = | Concentration factor (determined during calibration). |
| $A_x$ | = | Mean of absorbances of sample + R1 read during        |

$A_b$  = designated cycles.  
Mean of absorbances of STD 1 (Blank/CALIB 1) + R1 read during designated read cycles.  
 $C_b$  = Concentration of STD 1 (Blank/CALIB 1).

(a) Total bilirubin

The method is linear up to 35.0 mg/dL.

When reanalyzing any specimen with a concentration greater than 35.0 mg/dL, prepare a twofold (1+1) dilution of the specimen with saline. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 0.1 mg/dL. Results below the detection limit are reported as <0.1 mg/dL.

(b) Cholesterol

The method is linear up to 800.0 g/dL.

When reanalyzing any specimen with a concentration greater than 800 mg/dL, prepare a twofold (1+1) dilution of the specimen with distilled deionized water. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 3.0 mg/dL.

(c) Triglycerides

The method is linear up to 1000 mg/dL.

When reanalyzing any specimen with a concentration greater than 1000 mg/dL, prepare a twofold (1+1) dilution of the specimen with distilled deionized water. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 4 mg/dL. Results below the detection limit are reported as <4 mg/dL.

(d) Uric acid

The method is linear up to 25.0 mg/dL.

When reanalyzing any specimen with a concentration greater than 25 mg/dL, prepare a twofold (1+1) dilution of the specimen with physiological saline. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 0.2 mg/dL. Results below the detection limit are reported as <0.2 mg/dL.

(3) Assay type: Kinetic

The analyzer computer uses absorbance measurements to calculate alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), BUN, creatinine, gamma glutamyl transaminase ( $\gamma$ -GT), and lactate dehydrogenase (LDH) concentrations as follows:



The analyzer computer uses absorbance measurements to calculate activity as follows:

$$C_x = K(_A_x - _A_b) + C_b$$

Where:

$C_x$  = Activity of Sample.

$K$  = Factor for determining enzyme activity, established for each kinetic assay during installation.

$_A_x$  = Change in absorbance per minute of Sample + R1 + R2 during designated cycles.

$_A_b$  = Change in absorbance per minute of STD 1  
(Blank/CALIB 1) + R1 + R2 during designated cycles.

$C_b$  = Concentration of STD 1 (Blank/CALIB 1)

(a) Alkaline phosphatase (ALP)

The method is linear up to 1000 U/L.

When reanalyzing any specimen with a concentration greater than 1000 U/L, prepare a 10-fold (1+9) dilution of the specimen with saline.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 3 U/L. Results below the detection limit are reported as <3 U/L.

(b) Alanine aminotransferase (ALT)

The method is linear up to 400 U/L.

When reanalyzing any specimen with a concentration greater than 400 U/L, prepare a dilution of the specimen with saline.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 4 U/L. Results below the detection limit are reported as <4 U/L.

(c) Aspartate aminotransferase (AST)

The method is linear up to 800 U/L.

When reanalyzing any specimen with a concentration greater than 800 U/L, prepare a dilution of the specimen with saline. The results must then be multiplied by the dilution factor to account for the dilution.

The minimum detection limit, based on linear a regression curve of certified material analyzed 20 times, is 4 U/L. Results below the detection limit are reported as <4 U/L./

(d) Blood urea nitrogen (BUN)

The method is linear up to 400 mg/dL.

When reanalyzing any specimen with a concentration greater than 400 mg/dL, prepare a twofold (1+1) dilution of the specimen with physiological saline. The results must then be multiplied by 2 to account for this dilution.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 5.0 mg/dL. Results below the detection limit are reported as <5.0 mg/dL.

(e) Creatinine

The method is linear up to 25.0 mg/dL.

When reanalyzing any specimen with a concentration greater than 25.0 mg/dL, prepare a twofold (1+1) dilution of the specimen with saline.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 0.1 mg/dL. Results below 0.6 mg/dL are of no significance and can be reported as <0.1 mg/dL.

(f) Gamma glutamyl transaminase ( $\gamma$ -GT)

The method is linear up to 1200 U/L.

When reanalyzing any specimen with a concentration greater than 1200 U/L, prepare a 10-fold (1+9) dilution of the specimen with saline.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 3 U/L. Results below the detection limit are reported as <3 U/L.

(g) Lactate dehydrogenase (LDH)

The method is linear up to 1000 U/L.

When reanalyzing any specimen with a concentration greater than 1000 U/L, prepare a 10-fold (1+9) dilution of the specimen with saline.

The minimum detection limit, based on a linear regression curve of certified material analyzed 20 times, is 5 U/L. Results below the detection limit are reported as <5 U/L.

(4) Assay type: ISE

The analyzer computer uses changes in EMF to determine ion concentrations. The slope is determined by using ISE standard solutions, and a compensation coefficient is determined by using Precical calibrator serum. The concentrations of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> in the test samples are determined by measuring the EMFs and calculating the results using the following equation:  
 $C_x = (CISS \cdot 10^S) + K$

Where:

C<sub>x</sub> = concentration of test ion

K = compensation factor

CISS = concentration of Internal Standard Solution as determined during calibration

$\Delta E_x$  = EMF of test sample - EMF of Internal Standard Solution (determined just prior to the sample)

S = slope, S, is calculated from the equation  $S = mV/\text{decade}$

Where:

EL = EMF of low standard for each electrolyte

EH = EMF of high standard for each electrolyte

aH = input value of the concentration of the high standard

aL = input value of the concentration of the low standard

The concentration of the Internal Standard Solution is established using the following equation:

$$CISS = aL \times 10$$

Where:

EISS = EMF of internal standard solution

S = slope

aL and EL are the same as described above

The compensation factor, K, is calculated from the following equation:

$K = \frac{\text{assigned value (Std 3)} - \text{calculated value (Std 3)}}{\text{(EISS - EL)/S}}$

$\frac{\Delta E_x/S}{EH - EL}$

$\log(aH / aL)$

Sodium, potassium, and chloride

The method is linear up to 180 mmol/L for Na<sup>+</sup>, 10.0 mmol/L for K<sup>+</sup>, and 140 mmol/L for Cl<sup>-</sup>.

The minimum detection limits, based on linear regression curve of certified material analyzed 20 times, is 80 mmol/L Na<sup>+</sup>, 1.5 mmol/L K<sup>+</sup>, and 10 mmol/L Cl<sup>-</sup>. Results below the detection limit are reported as less than the minimum detection limit (as outlined above).

## 9. REPORTABLE RANGE OF RESULTS

- a. ALT. Serum ALT values are reportable in the range 4-400 U/L without dilution. If the ALT value is >400 U/L, the specimen should be diluted and then the results must then be multiplied by dilution factor to account for this dilution.
- b. Albumin. Serum albumin values are reportable in the range 0.2-10.0 g/dL without dilution. If the albumin value is >10.0 g/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- c. ALP. Serum ALP values are reportable in the range 3-1000 U/L without dilution. If the value is >1000 U/L, the specimen should be diluted 10-fold (1+9) and reanalyzed. The results must then be multiplied by 10 to account for this dilution.
- d. AST. Serum AST values are reportable in the range 4-800 U/L without dilution. If the AST value is >800 U/L, the specimen should be diluted and reanalyzed. The results must then be multiplied to account for this dilution.
- e. Bicarbonate (HCO<sub>3</sub>). Serum bicarbonate values are reportable in the range 5-40 mmol/L without dilution. If the bicarbonate value is >40 mmol/L, the specimen should be diluted twofold (1+1) and reanalyzed.
- f. BUN. Serum BUN values are reportable in the range 5.0-400 mg/dL without dilution. If the BUN value is >400 mg/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- g. Calcium. Serum calcium values are reportable in the range .2-20.0 mg/dL without dilution. If the value is >20.0 mg/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- h. Cholesterol. Serum cholesterol values are reportable in the range 3-800 mg/dL without dilution. If the cholesterol value is >800 mg/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- i. Creatinine. Serum creatinine values are reportable in the range 0.1-25.0 mg/dL without dilution. If the value is >25.0 mg/dL, the specimen should be diluted twofold (1+1) and reanalyzed. The results must then be multiplied by 2 to account for this dilution.
- j. γ-GT. Serum GGT values are reportable in the range 3–1200 U/L without dilution. If the value is >1200 U/L, the specimen should be diluted 10-fold (1+9) and reanalyzed. The results must then be multiplied by 10 to account for this dilution.

- k. Glucose. Serum glucose values are reportable in the range 2.0–1000 mg/dL without dilution. If the value is >1000 mg/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- l. Iron. Serum iron values are reportable in the range 5.0–1000 µg/dL without dilution. If the iron value is >1000 µg/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- m. LDH. Serum LDH values are reportable in the range 5–1000 U/L without dilution. If the value is >1000 U/L, the specimen should be diluted 10-fold (1+9) and reanalyzed. The results must then be multiplied by 10 to account for this dilution.
- n. Phosphorus. Serum phosphorus values are reportable in the range 0.3–20.0 mg/dL without dilution. If the value is >20.0 mg/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- o. Sodium, Potassium, and Chloride. Serum sodium values are reportable in the range 133–145 mmol/L, potassium values in the range 3.3–5.1 mmol/L and chloride values in the range of 96–108 mmol/L without dilution.
- p. Total Bilirubin. Serum total bilirubin values are reportable in the range 0.1–35.0 mg/dL without dilution. If the total bilirubin value is >35.0 mg/dL, the specimen should be diluted (1+1) and re-analyzed.
- p. Total Protein. Serum total protein values are reportable in the range 0.2–15.0 g/dL without dilution. If the value is <0.2 g/dL an alternate method capable of detecting micrograms of protein must be used. If the value is >15.0 g/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- r. Triglycerides. Serum triglyceride values are reportable in the range 4–1000 mg/dL without dilution. If the value is >1000 mg/dL, the specimen should be diluted twofold (1+1) and reanalyzed.
- s. Uric acid. Serum uric acid values are reportable in the range 0.20–25.0 mg/dL without dilution. If the uric acid value is >25.0 mg/dL, the specimen should be diluted twofold (1+1) and reanalyzed.

## 10. QUALITY CONTROL (QC) PROCEDURES

The method described in this protocol is in compliance with Clinical Laboratory Improvement Act of 1988 (CLIA) and the College of American Pathologists (CAP) guidelines. The Hitachi analyzer has proven to be accurate, precise, and reliable. Precision studies were generated from the use of a long-term lot number of quality control material.

Three types of quality control material are used to establish the White Sands Research Center quality control program: (1) a normal and abnormal level of quality control material assayed by the RD, (2) a normal and abnormal level of in-house quality control material, which will have a moving 2 SD range as determined by assay within the laboratory on its instrument and compared nationwide with the 2SD range of peers, and (3) the CAP Proficiency Survey. All levels of this parameter are assessed by taking these samples through the complete analytical process. The data from these materials are used to estimate methodological imprecision, shifts, and trends.

Two levels of unassayed control materials are used. A particular lot of each level is purchased from Dade Scientific in a sufficient quantity to last a minimum of 2 years. The daily data points from the results generated on the Hitachi 917 are stored on the hard disk in the QC program, and are printed at the end of each month. This program generates a monthly mean, SD, %CV, and number of data points that were evaluated. At the end of each month, the laboratory submits this summary to the Dade computer center and the center calculates a monthly mean, SD, and %CV for the peer group which consists of laboratories using the same method and lot number of control material for each analyte. The Standard Deviation Index (SDI) is used to compare results obtained by the members of the peer group. The SDI indicates where the laboratory stands with respect to the other members of the group and the group mean. This material is useful in assessing within-run and run-to-run shifts and trends in the data. One set of normal and one set of abnormal unassayed controls are analyzed according to the method protocol at the beginning and at two 4-hour intervals during the analyzer run period.

The assayed quality control materials are used to form a normal and an abnormal level of each analyte for the Hitachi 917, manufactured by RD. These materials are lyophilized preparations of pooled human serum with added chemicals, human and animal tissue extract and preservatives. The controls are assayed by the RD which establishes concentrations for each lot and determines the mean and  $\pm 2$  SD ranges for each parameter. One set of assayed controls is analyzed at the beginning of the analyzer run period to verify calibration and as an accuracy check. If the inventory of these materials becomes low, another lot should be ordered in time to analyze it concurrently with the lot currently in use so that a bridge may be formed between the materials.

After the standards and the assayed and unassayed quality control materials are analyzed, the 30-day Levey-Jennings control chart (which is stored in the Hitachi 917 computer system) is consulted to determine if the system is "in control." The system is out of control if any of the following events occur for any one of the parameters:

- a. Test the control data using the 2 SD rule. Accept the run when all controls are within 2SD of the mean. Report participants' results. When at least one control observation is more than 2SD from the mean, hold the participants' results and inspect the control data further, using additional Westgard control rules.
- b. Inspect control data within the run.
  - Reject the run when one observation is more than 3SD from the mean. Do not report participants' results.
  - Reject the run when two control observations are more than 2 SD on the same side of the mean. Do not report participants' results.
  - Reject the run when the range of one control observation is more than 2SD above the mean and that of another is more than 2SD below the mean. Do not report participants' results.
- c. Inspect control data across runs.
  - Reject the run when two consecutive control results are more than 2SD on the same side of the mean. Do not report participants' results.
  - Reject the run when four consecutive control observations fall more than 1SD on the same side of the mean. Do not report participants' results.
  - Reject the run when three consecutive control observations fall more than 1SD on the same side of the mean. Do not report participants' results.
  - Reject the run when 10 consecutive observations fall on the same side of the mean. Do not report participants' results.
  - Reject the run when 9 consecutive observations of the same control material fall on the same side of the mean. Do not report participants' results.
- d. Accept the run when none of the rules indicate a lack of statistical control. Report participants' results.

#### 11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If one or more quality control samples fall outside the  $\pm 2$  SD range or a within-run control sample shifts 2 SD or more from its previous value, then take the following steps:

- a. Determine the type of errors occurring (random, systematic, or both) on the basis of the control rules being violated.
- b. Refer to the RD troubleshooting guide to determine the elements of the method or the components of the instrument that can cause the type of error observed.
- c. Correct the problem, then reanalyze the participants' samples and control samples, testing for

statistical control by the same procedure.

- d. Consult the chemistry technical supervisor for any decision to report data when there is a lack of statistical control. The chemistry technical supervisor may make a decision to report data when there is a lack of statistical control in the following situations:
  - (1) The control problem can be due to the control materials themselves.
  - (2) The control problem has resulted from an isolated event that would not have affected the rest of the run.
  - (3) The control problem occurs in a concentration range that is different from the concentration range of the participants' samples. The method is in-control in the range of the participants' samples.
  - (4) The size of the analytical error is judged to be small relative to the medical usefulness requirements.

## 12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS (54-55)

- a. ALT. Bilirubin does not affect test results. Hemolysis causes elevated results, and lipemia may cause absorbance flagging as a result of an absorbance increase. The effects of various drugs on ALT activity should be taken into consideration in the case of participants receiving large doses of drugs.
- b. Albumin. The absorptivity of the dye-albumin complex differs for albumin obtained from different species. Therefore materials for the standardization control of test results must be human in origin. Bilirubin, hemolysis, and lipemia do not affect test results.
- c. ALP. Bilirubin and lipemia do not affect test results. Hemolysis causes significant negative interference at 200 mg/dL. Phosphatases catalyze the hydrolysis of a great number of phosphoric esters of which p-nitrophenylphosphate has proven to be the most convenient. The interaction with other esterases present in the specimen cannot be excluded. It is postulated that under the conditions of the assay, with a pH of 10.5, the measured activity is mainly related to ALP.
- d. AST. Bilirubin does not affect test results. Hemolysis causes elevated results, and lipemia may cause absorbance flagging as a result of an absorbance increase. The effects of various drugs on AST activity should be taken into consideration in the case of participants receiving large doses of drugs.
- e. Bicarbonate. Bilirubin, lipemia, and hemolysis have no effect on this method.
- f. BUN. Bilirubin and hemolysis do not affect test results. Highly lipemic samples may cause absorbance flags; if that occurs, the sample must be diluted.
- g. Calcium. Bilirubin, hemolysis, and lipemia cause no significant interference; however, extremely turbid samples may exhibit a negative interference. Oxalate, citrate, and EDTA anticoagulants interfere by binding calcium.
- h. Cholesterol. Bilirubin causes a significant negative interference at 15 mg/dL. Hemolysis, lipemia, uric acid, creatinine, and glutathione cause no significant interference. A two-fold toxic dose of  $\alpha$ -methyldopa lowered recovery by 50%. Noramidopyrine lowered recovery by 20%, and a 10-fold therapeutic concentration of ascorbic acid lowered cholesterol recovery by 5%.
- i. Creatinine. Bilirubin causes a significant interference at 15 mg/dL. Hemolysis and lipemia do not affect test results.
- j.  $\gamma$ -GT. Bilirubin and lipemia do not affect test results. Hemolysis causes significant negative interference at

100 mg/dL.

- k. Glucose. Bilirubin, hemolysis, and lipemia do not affect test results.
- m. Iron. Bilirubin, lipemia, and atypical gamma globulins do not affect test results. Hemoglobin levels of 31–199 mg/dL produce significant negative interference. Hemolyzed serum with abnormally low iron values may produce negative results. Hemoglobin levels >400 mg/dL cause significant positive interference. Deferoxamine-bound serum iron does not react in the test, resulting in falsely lowered values.
- m. LDH. Bilirubin, hemolysis, and lipemia do not affect test results.
- n. Phosphorus. Bilirubin and lipemia do not affect test results. RBC contamination will elevate results. The presence of monoclonal immunoglobulins in the serum may give erroneous results. Use of an alternative method is recommended.
- o. Sodium, Potassium, and Chloride. Bilirubin, hemolysis, and lipemia do not affect test results.
- p. Total Bilirubin.
  - (1) Urea and creatinine do not interfere; however, bilirubin may react with other metabolites whose levels are elevated in uremic serum.
  - (2) At indicant levels twice those found in dialysis patients, no significant bias (<+0.2 mg/dL bilirubin) is observed when 570 nm is used as the primary measuring wavelength.
  - (3) Elevated VLDL levels (triglyceride concentrations of at least 1000 mg/dL) produce a positive bias of approximately 0.3 mg/dL bilirubin. Intralipid at a concentration of approximately 60 mg/dL will produce a positive bias of 0.5 mg/dL at normal bilirubin levels.
  - (4) Typical hemolysis (<200 mg/dL hemoglobin) will produce less than +0.2 mg/dL bias at normal bilirubin levels and less than -0.7 mg/dL bias at elevated bilirubin levels (e.g., neonatal).
- q. Total Protein. Bilirubin and lipemia do not affect test results. Hemolysis will elevate results.
- r. Triglycerides. Bilirubin and lipemia do not affect test results. Occasionally, extremely elevated triglyceride levels (>3000 mg/dL) have been found to give a "normal" result. To ensure accurate results, dilute grossly lipemic serum with 1 part serum to 4 parts saline and multiply the result by 5. Hemolysis causes a significant positive interference at 400 mg/dL.
- s. Uric acid. Bilirubin levels >7.5 mg/dL cause significant negative interference. Hemoglobin levels >200 mg/dL cause significant positive interference. Lipid levels >750 mg/dL cause significant positive interference. Ascorbic acid causes no interference up to 5 mg/dL. Therapeutic levels of dipyrone give falsely low results. Uricase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.

### 13. REFERENCE RANGES (NORMAL VALUES)

- a. ALT. The normal range for serum ALT by this method, as determined by RD for the Hitachi 917, is  $\leq 41$  U/L (37°C) among males and  $\leq 31$  U/L (37°C) among females.
- b. Albumin. The normal range for serum albumin by this method, as determined by RD for the Hitachi 917, is 3.4–5.0 gm/dL (conventional units) or 34–50 g/L (SI Units).

Physiological factors are capable of modifying the levels of albumin in healthy individuals. Levels are subject to seasonal variation, tending to be lower in summer than in winter. Transient increases are found 11–12 hours after strenuous exercise. Dietary factors play a role in these normal value ranges.

Premature infants may show higher levels.

- c. ALP. The normal range for serum ALP by this method, as determined by RD for the Hitachi 917, is 117-390 U/L (37°C) for children (3-15 years) and 39-117 U/L (37°C) for adults.
- d. AST. The normal range for serum AST by this method, as determined by RD for the Hitachi 917, is <37 U/L (37°C) for males and <31 U/L (37°C) for females.
- e. Bicarbonate (HCO<sub>3</sub>). The normal range for serum bicarbonate by this method, as determined by RD for the Hitachi 917, is 22.0-29.0 mmol/L.
- f. BUN. The normal range for serum BUN by this method, as determined by RD for the Hitachi 917, is 10-50 mg/dL (conventional units) or 1.7-8.3 mmol/L urea (SI Units).
- g. Calcium. The normal range for serum calcium by this method, as determined by RD for the Hitachi 917, is 8.6-10.2 mg/dL (conventional units) or 2.15-2.6 mmol/L (SI Units).
- h. Cholesterol. According to the National Cholesterol Education Program, the desirable blood cholesterol level is <200 mg/dL, the borderline-high blood cholesterol level is 200-239 mg/dL, and the high blood cholesterol level is ≥240 mg/dL.
- i. Creatinine. The normal range for serum creatinine by this method, as determined by RD for the Hitachi 917, is 0.7-1.2 mg/dL for males and 0.5-0.9 mg/dL for females.
- j. γ-GT. The normal range for serum GGT by this method, as determined by RD for the Hitachi 917, is 11-49 U/L (37°C) for males and 7-32 U/L (37°C) for females.
- k. Glucose. The normal range for serum glucose by this method, as determined by RD for the Hitachi 917, is 70-115 mg/dL (conventional units) or 3.8-6.38 mmol/L (SI Units).
- l. Iron. The normal range for serum iron by this method, as determined by RD for the Hitachi 917, is 30-160 µg/dL (conventional units) or 5.4-28.6 µmol/L (SI Units) for females and 45-160 µg/dL (conventional units) or 8.1-28.6 µmol/L (SI Units) for males.
- m. LDH. The normal range for serum LDH by this method, as determined by RD for the Hitachi 917, is 135-225 U/L (37°C) for males and 135-214 U/L (37°C) for females.
- n. Phosphorus. The normal range for serum phosphorus by this method, as determined by RD for the Hitachi 917, is 2.7-4.5 mg/dL (conventional units) or 0.87-1.45 mmol/L (SI Units).
- o. Sodium, Potassium, and Chloride. The normal range by this method, as determined by RD for the Hitachi 917, is 133-145 mmol/L for sodium, 3.3-5.1 mmol/L for potassium, and 96-108 mmol/L for chloride.
- p. Total Bilirubin. The normal range for total bilirubin by this method, as determined by RD for the Hitachi 917, is up to 1.0 mg/dL for adults and children. Ranges for neonates are as follows:
  - 24 hrs: 1.0-6.0 mg/dL for premature neonates and 2.0-6.0 mg/dL for full term neonates.
  - 48 hrs: 6.0-8.0 mg/dL for premature neonates and 6.0-7.0 mg/dL for full term neonates.
  - 3-5 days: 10-15 mg/dL for premature neonates and 4.0-12.0 mg/dL for full term neonates.
- q. Total Protein. The normal range for serum total protein by this method, as determined by RD for the Hitachi 917, is 6.6-8.7 gm/dL (conventional units) or 66-87 g/L (SI Units).

Physiological factors are capable of modifying the levels of protein in healthy individuals. Levels are subject to seasonal variation, tending to be lower in summer than in winter. Transient increases are found 11-12 hours after strenuous exercise. Dietary factors play a role in these normal value ranges.



Premature infants may show higher levels.

- r. Triglycerides. The normal range for serum triglycerides by this method, as determined by RD for the Hitachi 917, is  $\leq 200$  mg/dL (conventional units) or  $\leq 2.26$  mmol/L (SI Units).
- s. Uric acid. The normal range for serum uric acid by this method, as determined by RD for the Hitachi 917, is 3.4-7.0 mg/dL (conventional units) or 0.2-0.41 mmol/L (SI Units) for males and 2.4-5.7 mg/dL (conventional units) or 0.14-0.34 mmol/L (SI Units) for females.

#### 14. CRITICAL CALL RESULTS ("PANIC VALUES")

Results requiring notification of the NHANES Coordinator or medical intervention are shown in Table 1.

#### 15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may reach and maintain 20–25°C during analysis, but they should be returned to 2–8°C storage as soon as possible. After a run is accepted, samples should be returned to  $\leq -70^\circ\text{C}$  for long-term storage.

#### 16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

The analysis for these parameters can be performed on the Hitachi 734 in the event of a malfunction of the Hitachi 917. The Hitachi 734 can be used as a backup instrument to the Hitachi 917. The same reagents, controls and calibrators are used for all three instruments.

#### 17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

White Sands will report the results to the NCHS Coordinator by telephone or fax. The NCHS physician will notify the participant.

**Table 1. Critical Call Results**

| Analyte              | Units  | Result Ranges to be Reported to NHANES Coordinator | Result Ranges Requiring Medical Intervention         |
|----------------------|--------|--|--|
| ALT (1)              | U/L    | --   | ≥60  |
| Albumin (2)          | gm/dL  | --   | <2.0   |
| ALP (3)              | U/L    | --   | --   |
| AST (4)              | U/L    | --   | ≥50  |
| Bicarbonate (5)      | mmol/L | --   | <16  |
| BUN (6)              | mg/dL  | --   | >50  |
| Calcium (7)          | mg/dL  | --   | ≤7.5 or ≥ 11.5                                       |
| Cholesterol (8)      | mg/dL  | --   | >400   |
| Creatinine (9)       | mg/dL  | --   | >2.5   |
| γ-GT (10)            | U/L    | --   | >51 M<br>>33 F                                       |
| Glucose (11)         | mg/dL  | --   | ≥ 126 (fasting ≥ 8 hrs)                              |
|                      | μg/dL  | --   | ≥ 300 (fasting < 8 hrs)                              |
| Iron (12)            | ug/dL  | --<br>--<br>--                                     | <20 (1-17 yrs)<br><32 M (≥18 yrs)<br><22 F (≥18 yrs) |
| LDH (13)             | U/L    | --   | >250   |
| Phosphorus (14)      | Mg/dL  | --   | <2.0 or >6.5   |
| Sodium (15)          | mmol/L | --   | ≤122 or ≥154   |
| Potassium (15)       | mmol/L | --   | ≤2.9 or ≥5.4   |
| Chloride (15)        | mmol/L | --   | ≤75 or ≥126  |
| Total bilirubin (16) | Mg/dL  | --   | ≤6.0   |
| Total protein (17)   | g/dL   | --   | <5.9 or >8.4   |
| Triglycerides (18)   | Mg/dL  | --   | >1000  |
| Uric acid (19)       | mg/dL  | --   | >13  |
| ALT (1)              | U/L    | --   | ≥60  |
| Albumin (2)          | gm/dL  | --   | <2.0   |

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN  
ACCOUNTABILITY AND TRACKING

Standard record-keeping means (electronic and log accession books) are used to track specimens. All records, including related QC data, are maintained for a minimum of 7 years, in electronic and hardcopy format. Only numerical identifiers are used to identify participants, with all related personal identifiers being maintained by the NCHS coordinator to safeguard confidentiality.

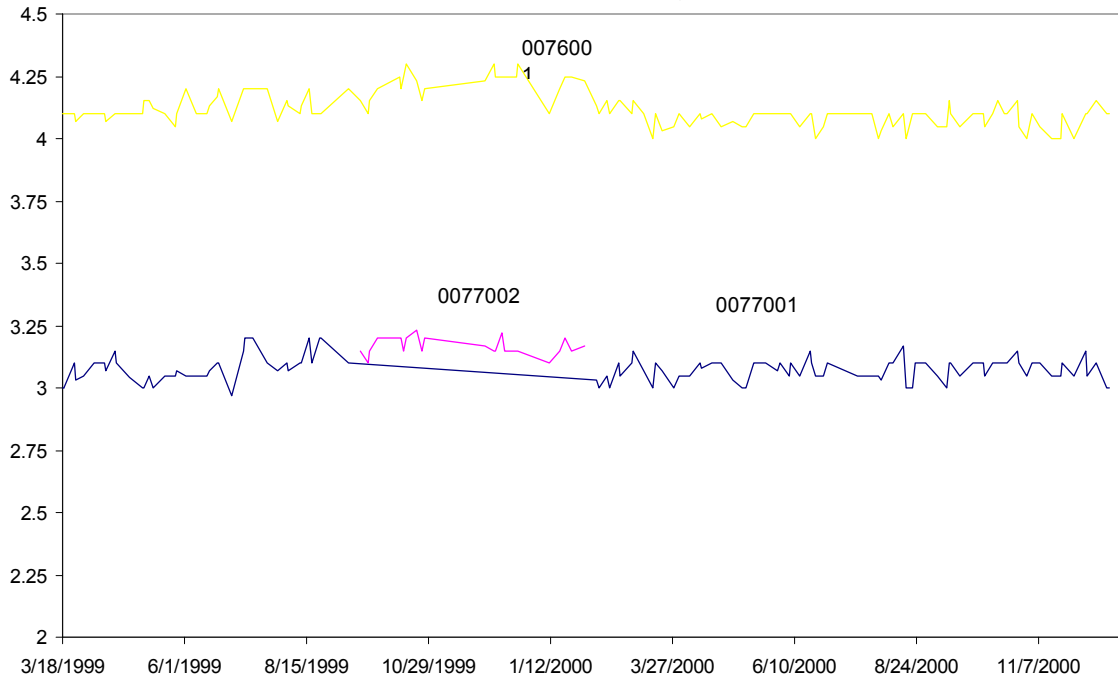
19. SUMMARY STATISTICS AND QC GRAPHS

a. Albumin

Summary Statistics for Albumin by Lot

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 3.08 | 0.05               | 1.65                     |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 4.12 | 0.07               | 1.63                     |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 3.17 | 0.03               | 1.07                     |

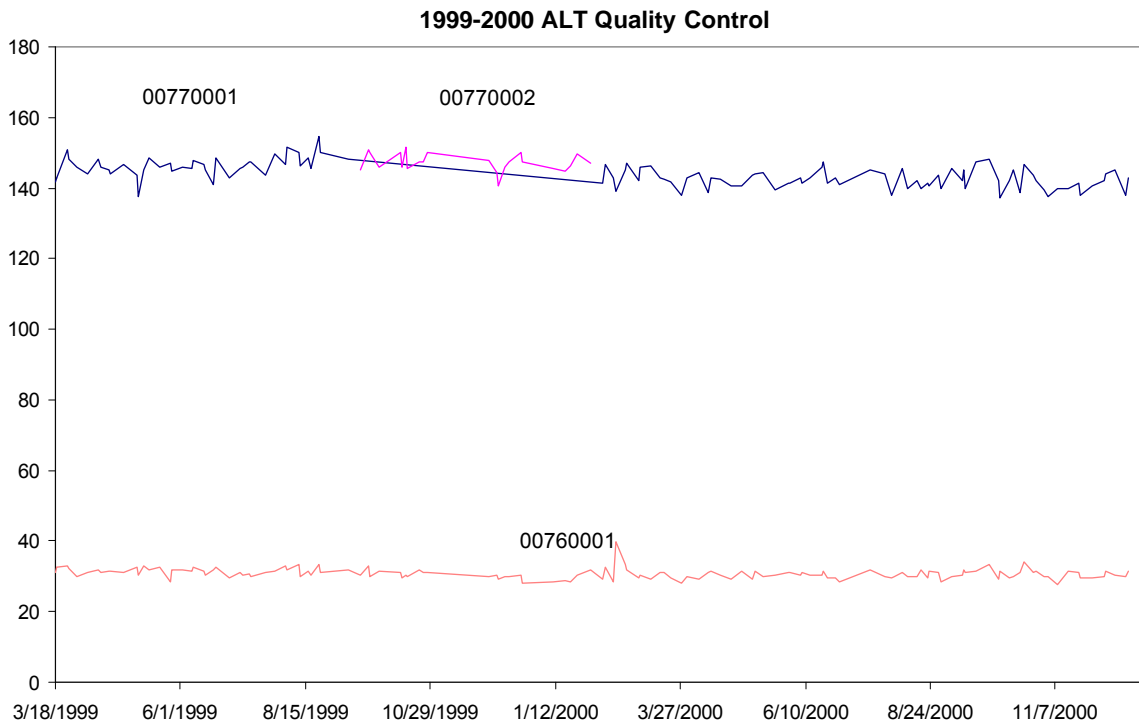
1999-2000 Albumin Quality Control



b. Alanine Aminotransferase

Summary Statistics for ALT by Lot

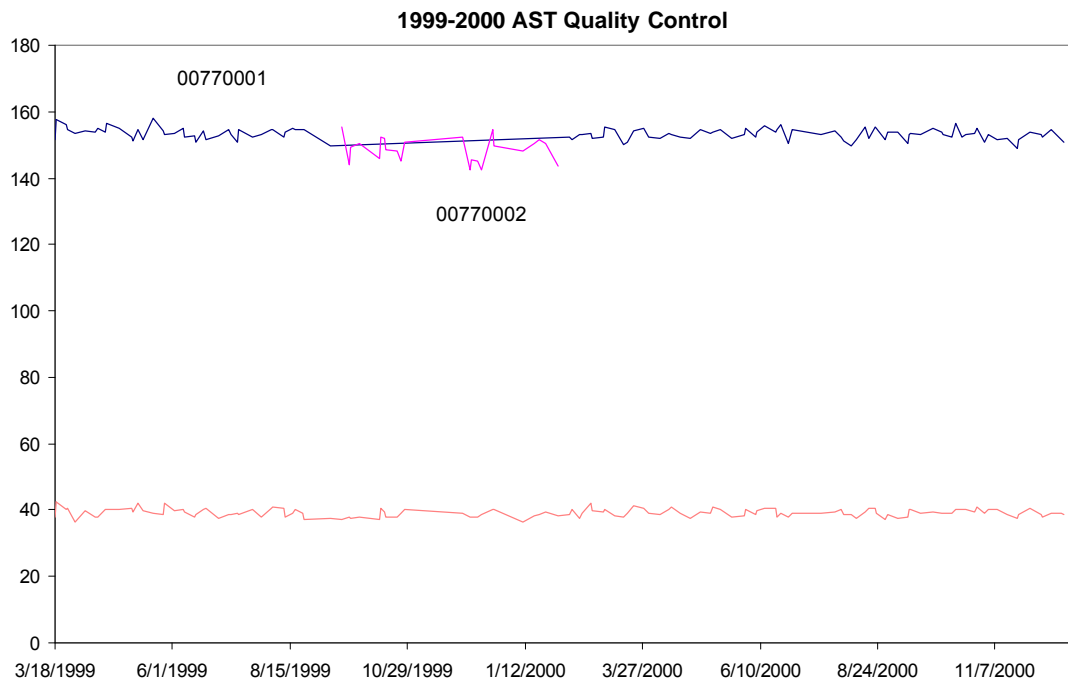
| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 143.8 | 3.4                | 2.4                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 30.8  | 1.5                | 4.9                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 147.2 | 2.6                | 1.7                      |



c. Aspartate Aminotransferase

Summary Statistics for AST by Lot

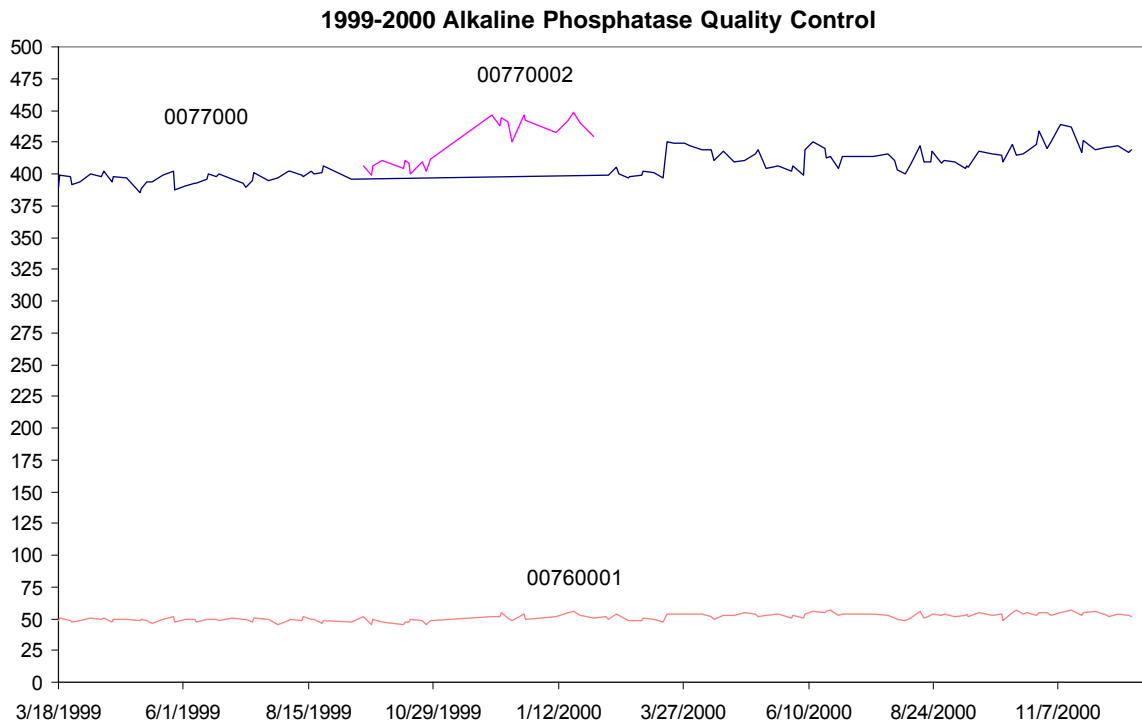
| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 153.3 | 1.7                | 1.1                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 39.2  | 1.2                | 3.0                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 148.6 | 3.8                | 2.5                      |



d. Alkaline Phosphatase

Summary Statistics for Alkaline Phosphatase by Lot

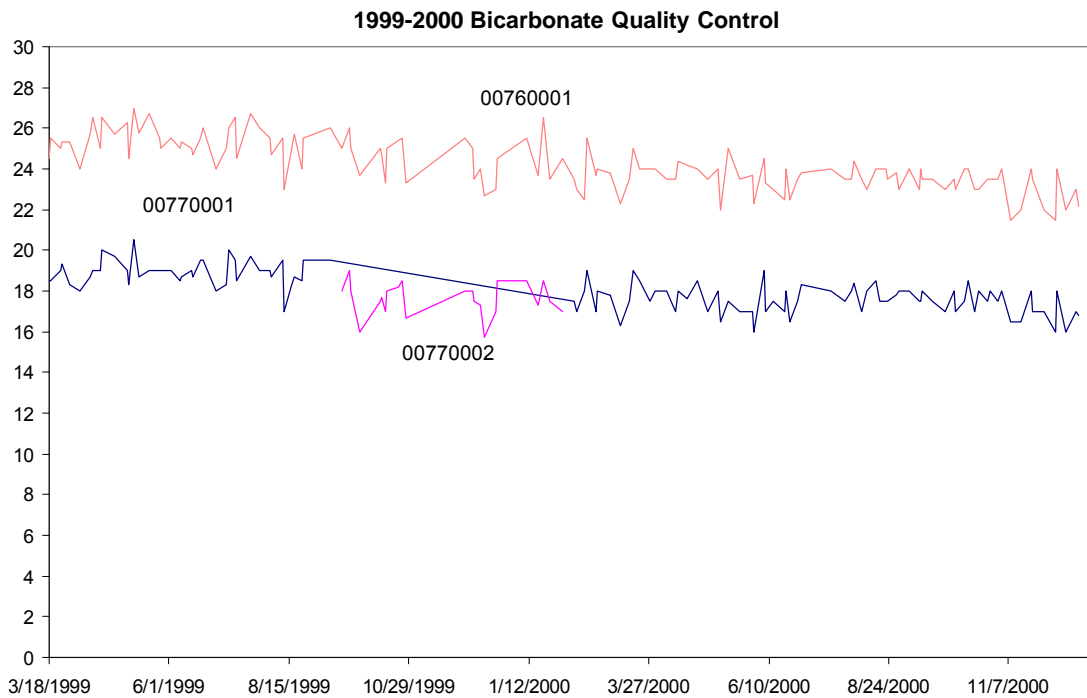
| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 407.2 | 11.6               | 2.8                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 51.1  | 2.8                | 5.4                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 423.7 | 18.1               | 4.3                      |



e. Bicarbonate

Summary Statistics for Bicarbonate by Lot

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 18.0 | 0.9                | 5.2                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 24.2 | 1.2                | 4.9                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 17.6 | 0.8                | 4.6                      |

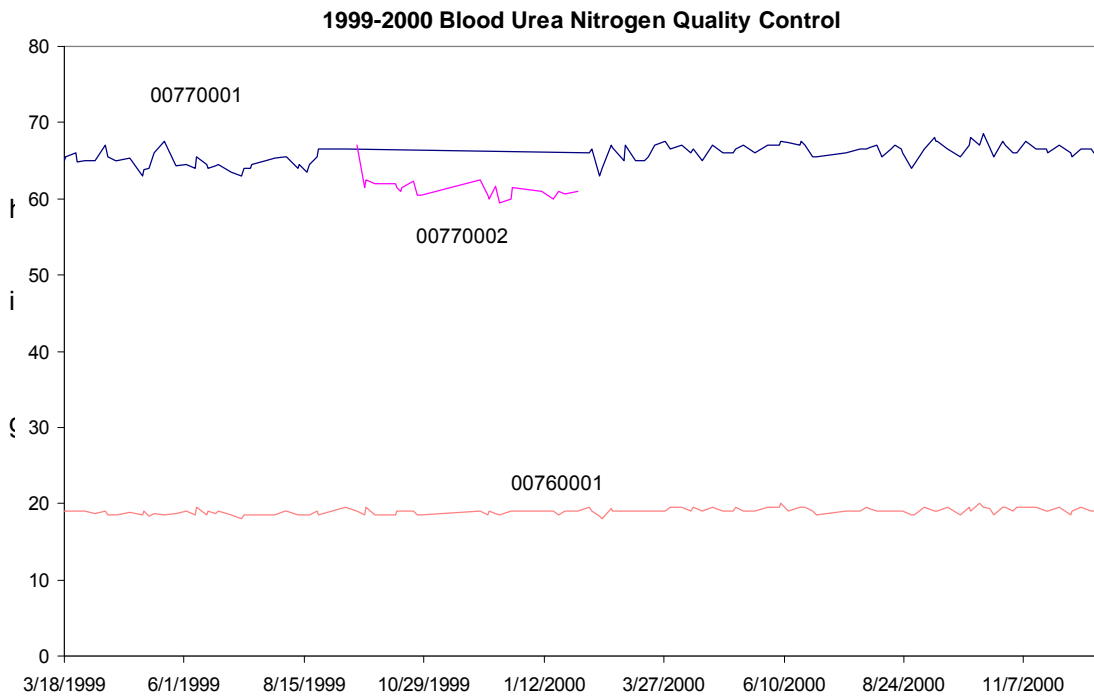




f. BUN

Summary Statistics for Blood Urea Nitrogen by Lot

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 65.8 | 1.2                | 1.9                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 19.0 | 0.4                | 2.0                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 61.4 | 1.5                | 2.4                      |



g. Cholesterol

Summary Statistics for Cholesterol by Lot

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 117.2 | 2.3                | 1.9                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 192.8 | 3.8                | 2.0                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 126.6 | 2.3                | 1.8                      |

1999-2000 Total Cholesterol Quality Control

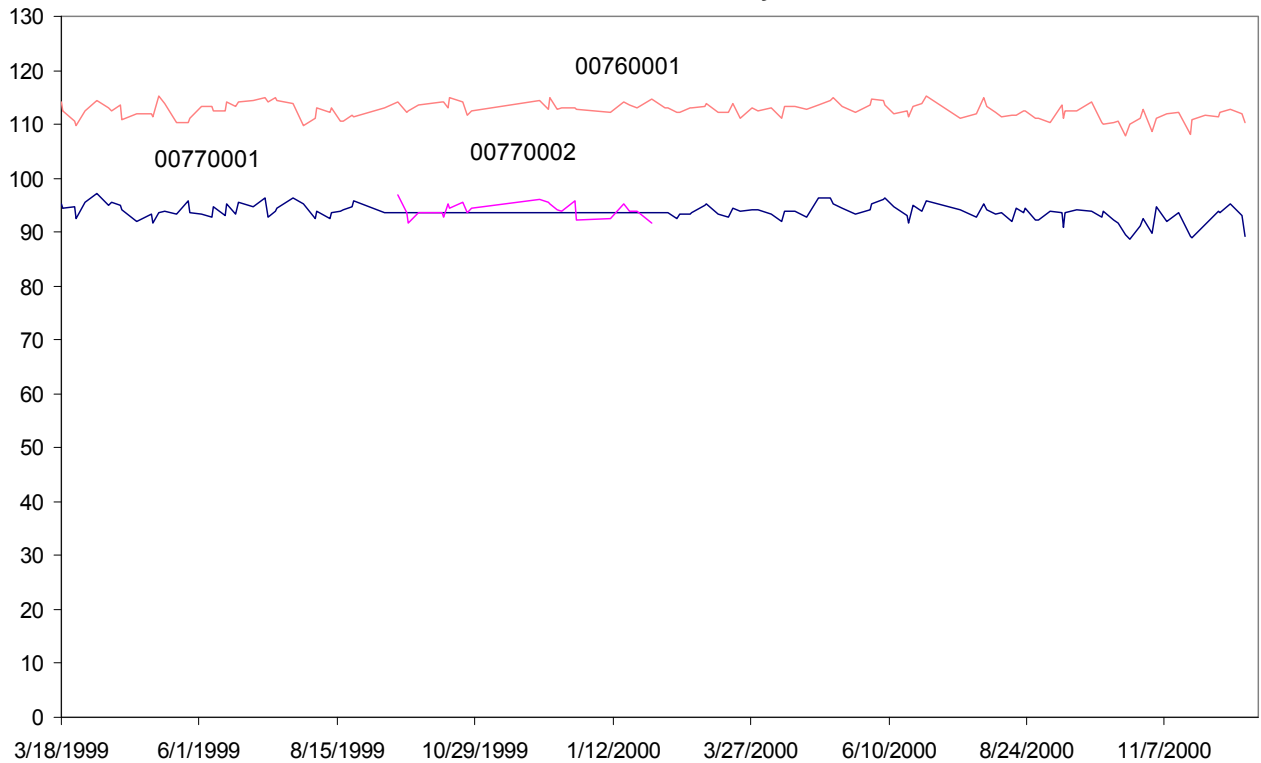


h. chloride

**Summary Statistics for Chloride by Lot**

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 93.6  | 1.6                | 1.7                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 112.5 | 1.5                | 1.3                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 94.2  | 1.4                | 1.5                      |

**1999-2000 Chloride Quality Control**

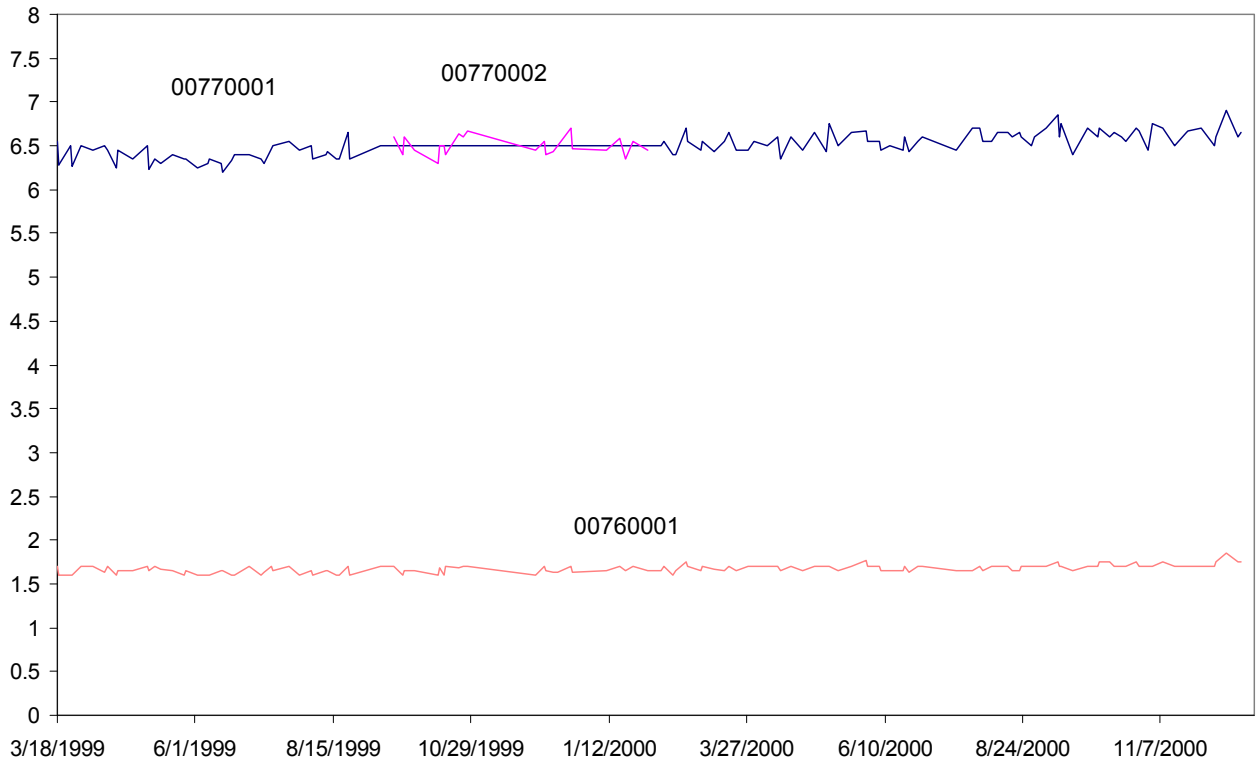


i. creatinine

**Summary Statistics for Creatinine by Lot**

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 6.51 | 0.14               | 2.17                     |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 1.67 | 0.05               | 2.72                     |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 6.50 | 0.10               | 1.59                     |

**1999-2000 Creatinine Quality Control**

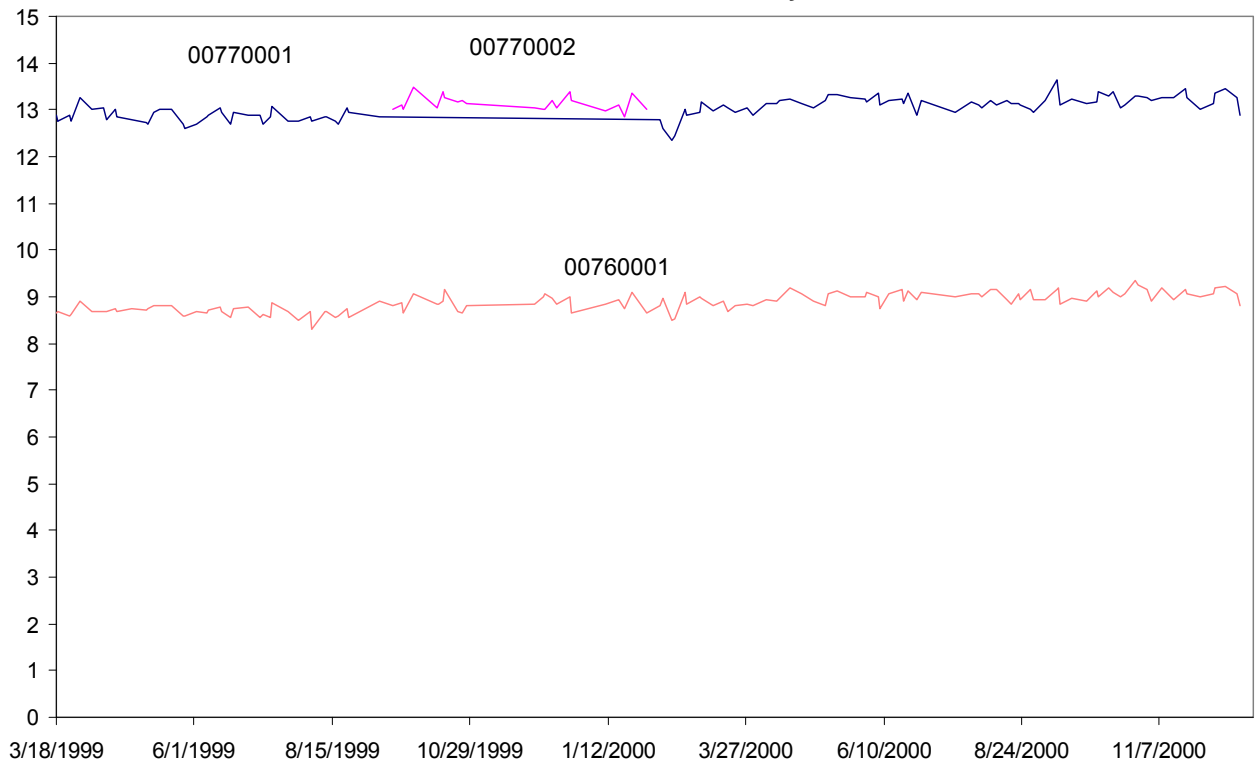


j. calcium

**Summary Statistics for Calcium by Lot**

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 13.04 | 0.23               | 1.75                     |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 8.88  | 0.20               | 2.24                     |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 13.14 | 0.16               | 1.23                     |

**1999-2000 Total Calcium Quality Control**

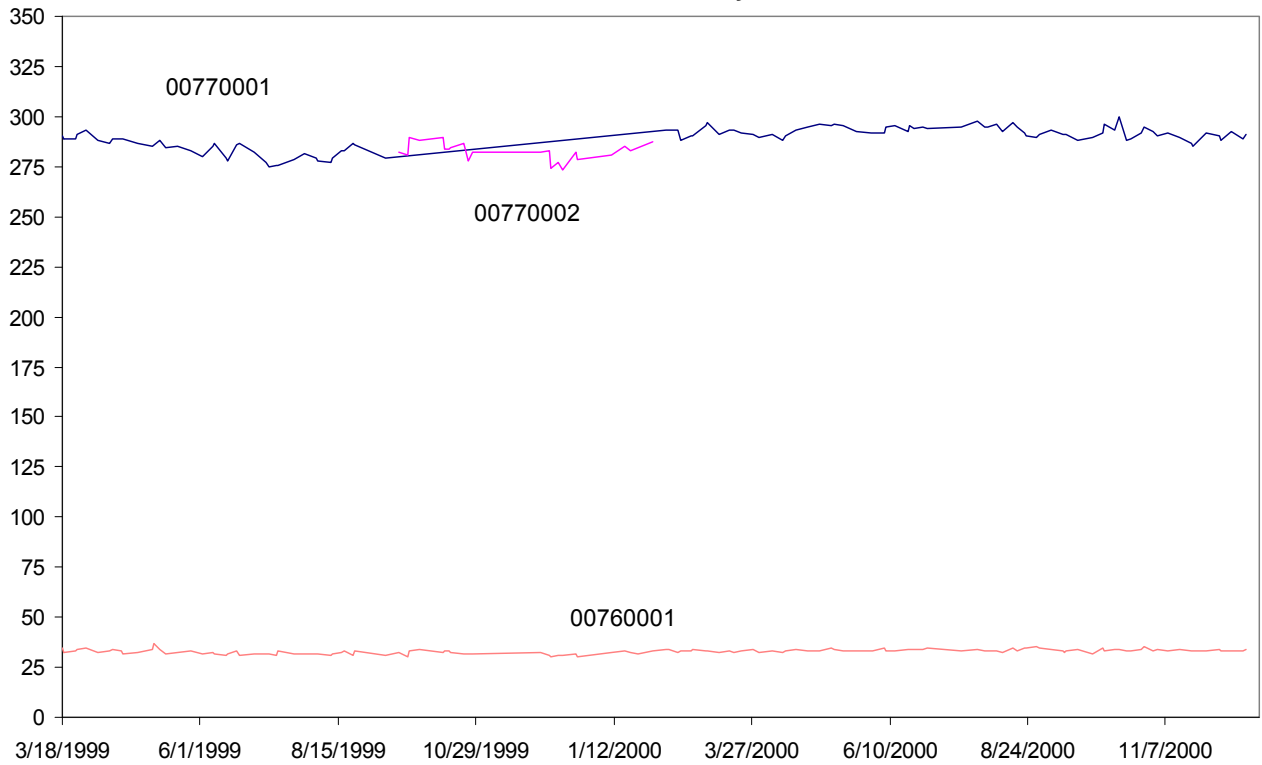


k. Gamma Glutamyl Transferase

Summary Statistics for GGT by Lot

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 111 | 3/18/1999  | 12/21/2000 | 289.3 | 5.6                | 1.9                      |
| 00760001 | 135 | 3/18/1999  | 12/21/2000 | 33.0  | 1.1                | 3.4                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 282.6 | 4.4                | 1.5                      |

1999-2000 GGT Quality Control



I. Glucose

Summary Statistics for Glucose by Lot

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 283.7 | 4.8                | 1.7                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 73.9  | 1.2                | 1.6                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 288.9 | 3.7                | 1.3                      |

1999-2000 Glucose Quality Control

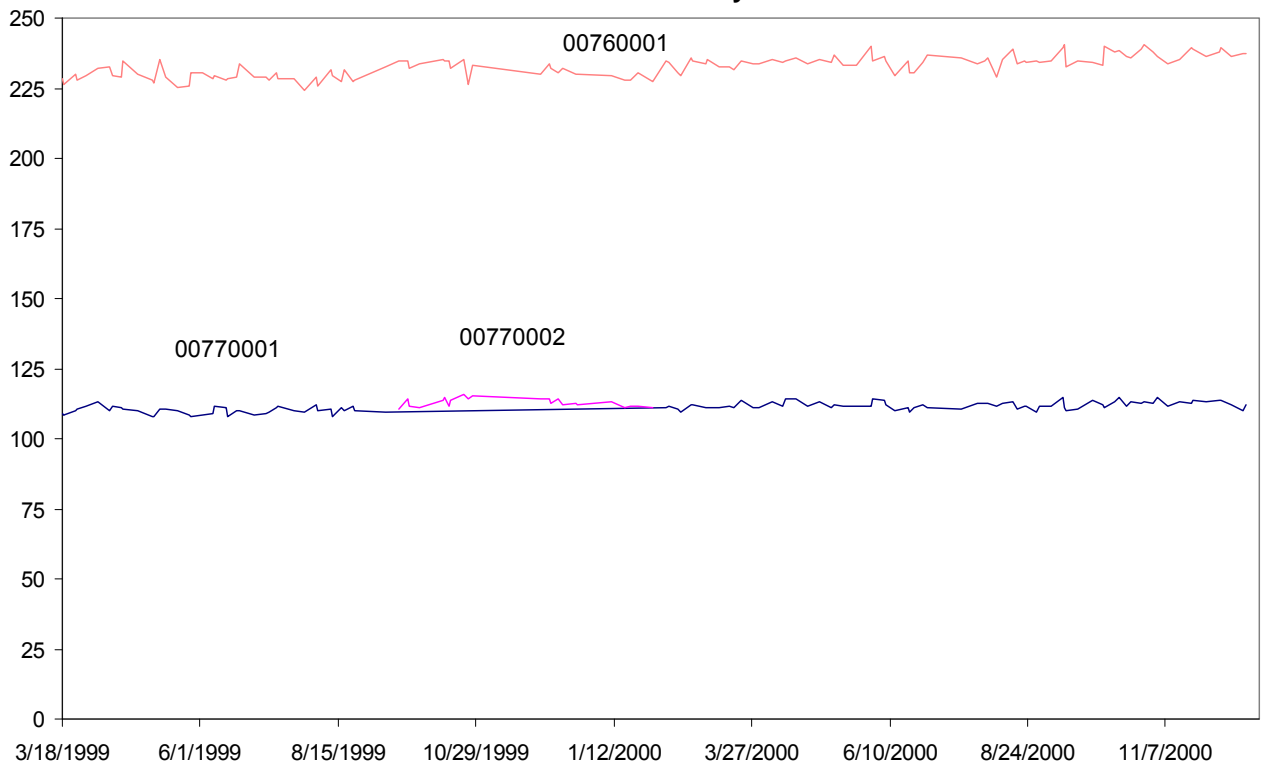


m. Iron

Summary Statistics for Iron by Lot

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 111.2 | 1.6                | 1.4                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 232.8 | 3.7                | 1.6                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 112.8 | 1.5                | 1.3                      |

1999-2000 Iron Quality Control



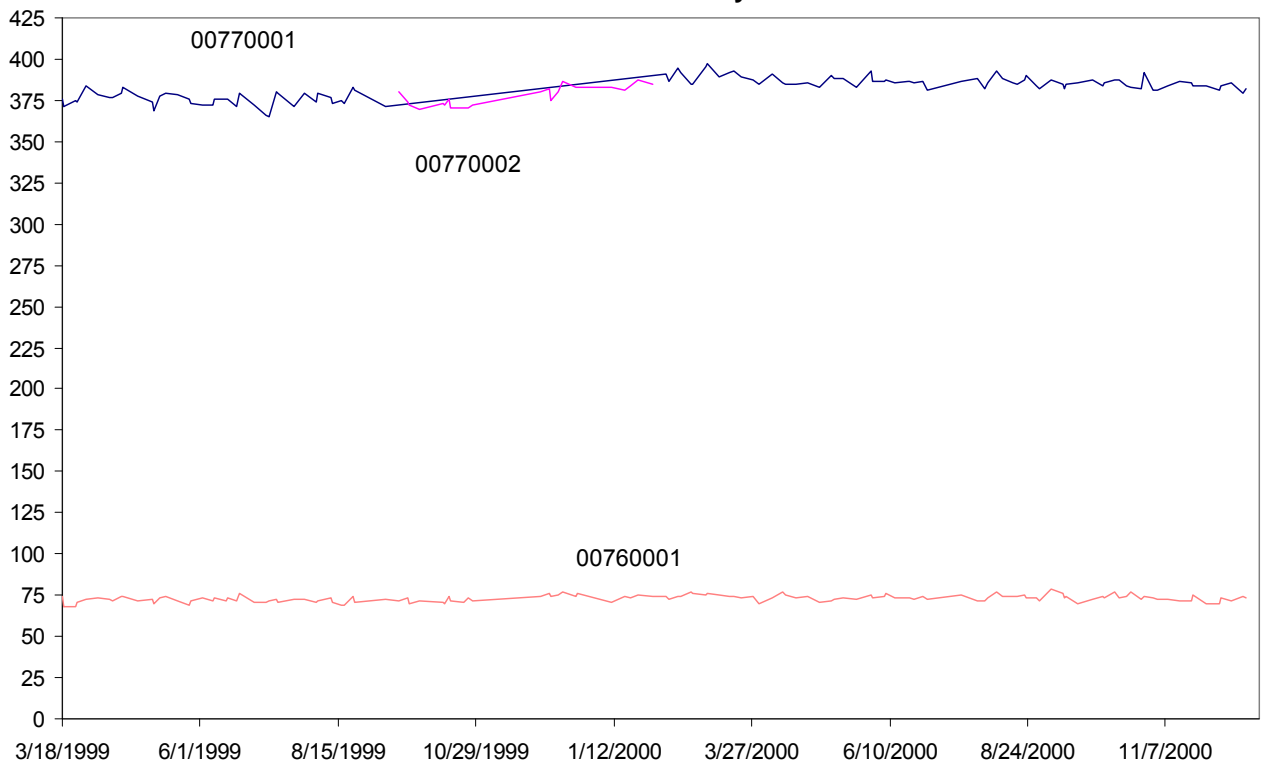


n. Lactate Dehydrogenase

Summary Statistics for LDH by Lot

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 382.5 | 6.5                | 1.7                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 73.1  | 2.0                | 2.8                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 377.8 | 5.9                | 1.6                      |

1999-2000 LDH Quality Control

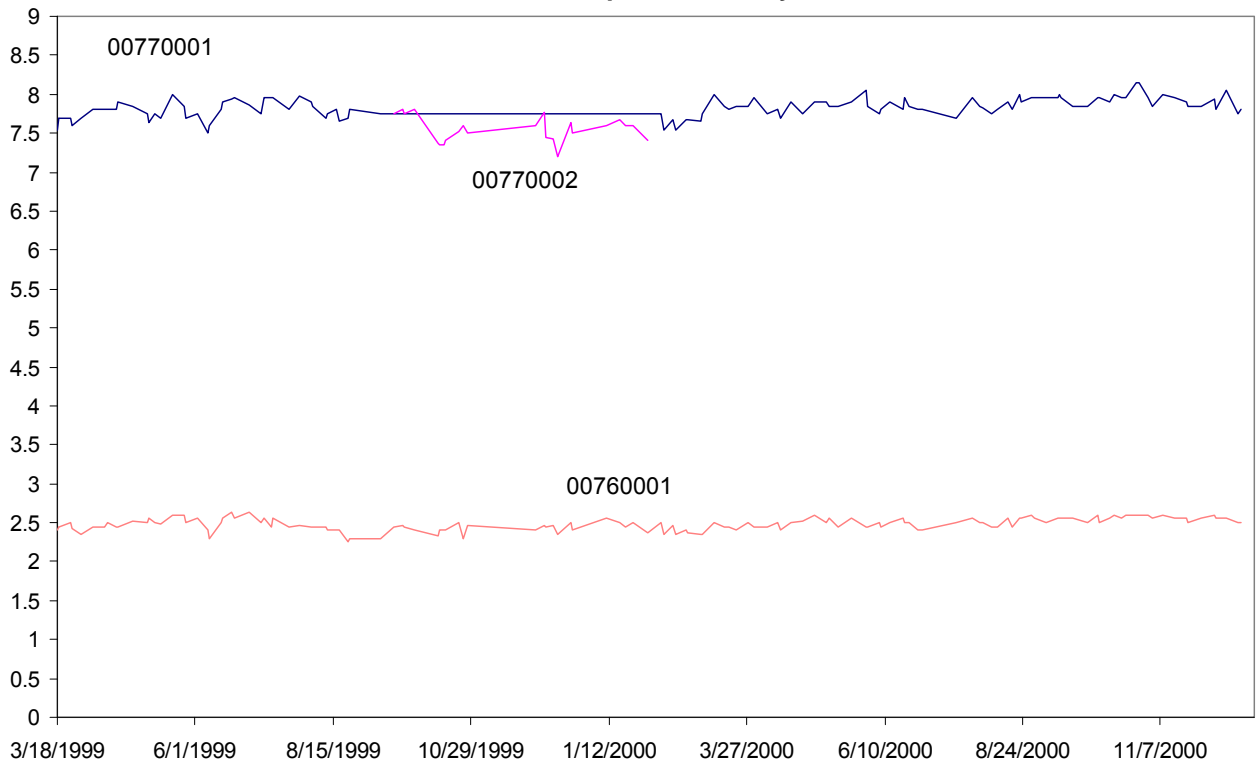


o. Phosphorus

Summary Statistics for Phosphorus by Lot

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 7.83 | 0.12               | 1.58                     |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 2.48 | 0.08               | 3.15                     |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 7.55 | 0.16               | 2.18                     |

1999-2000 Phosphorus Quality Control

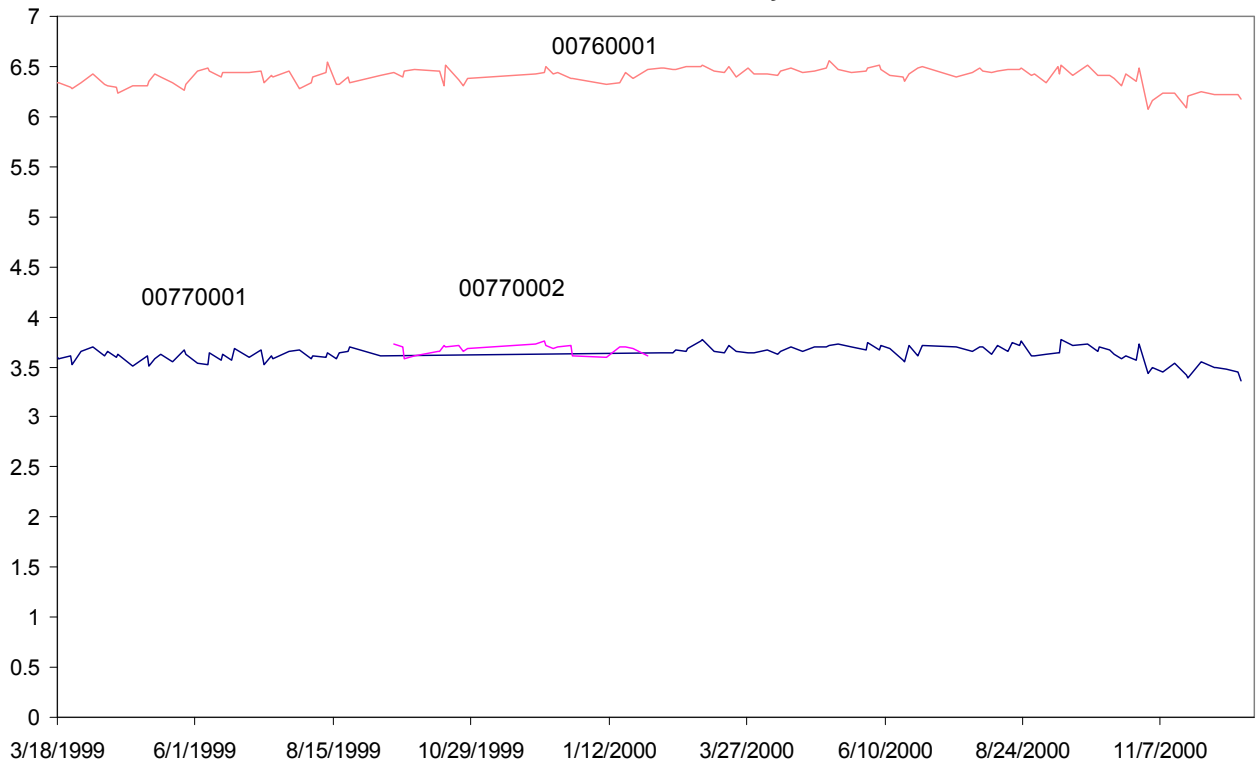


p. Potassium

**Summary Statistics for Potassium by Lot**

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 3.63 | 0.08               | 2.30                     |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 6.40 | 0.09               | 1.44                     |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 3.68 | 0.05               | 1.28                     |

**1999-2000 Potassium Quality Control**

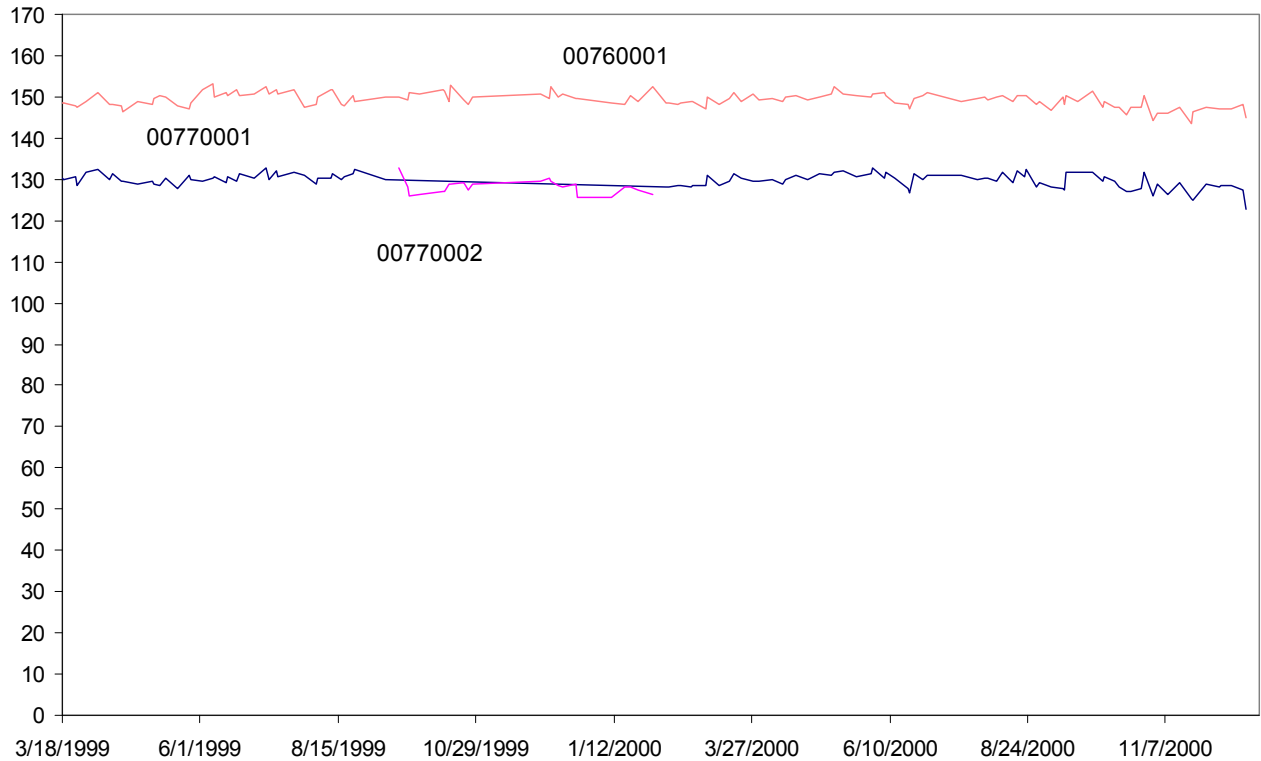


q. Sodium

Summary Statistics for Sodium by Lot

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 129.7 | 1.7                | 1.3                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 149.3 | 1.7                | 1.2                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 128.1 | 1.7                | 1.3                      |

1999-2000 Sodium Quality Control

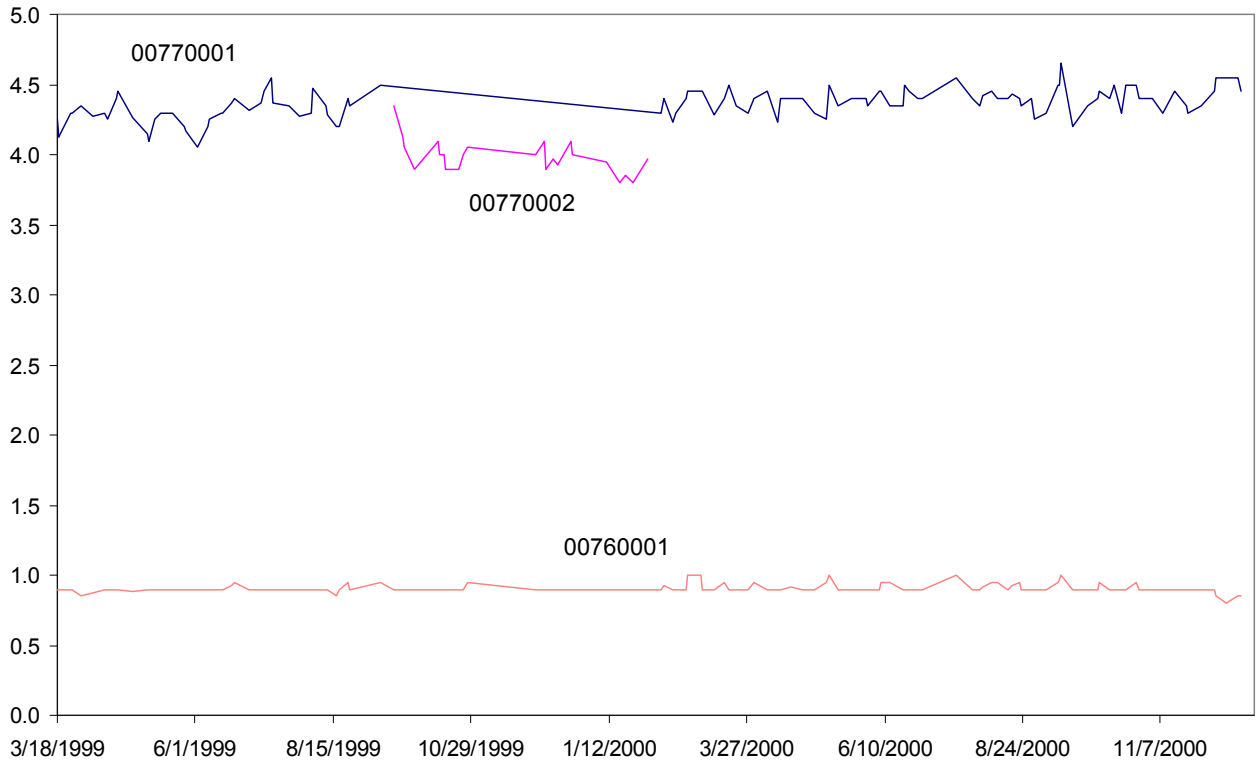


r. Total Bilirubin

Summary Statistics for Total Bilirubin by Lot

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 4.36 | 0.11               | 2.41                     |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 0.91 | 0.03               | 3.17                     |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 3.99 | 0.12               | 3.04                     |

1999-2000 Total Bilirubin Quality Control

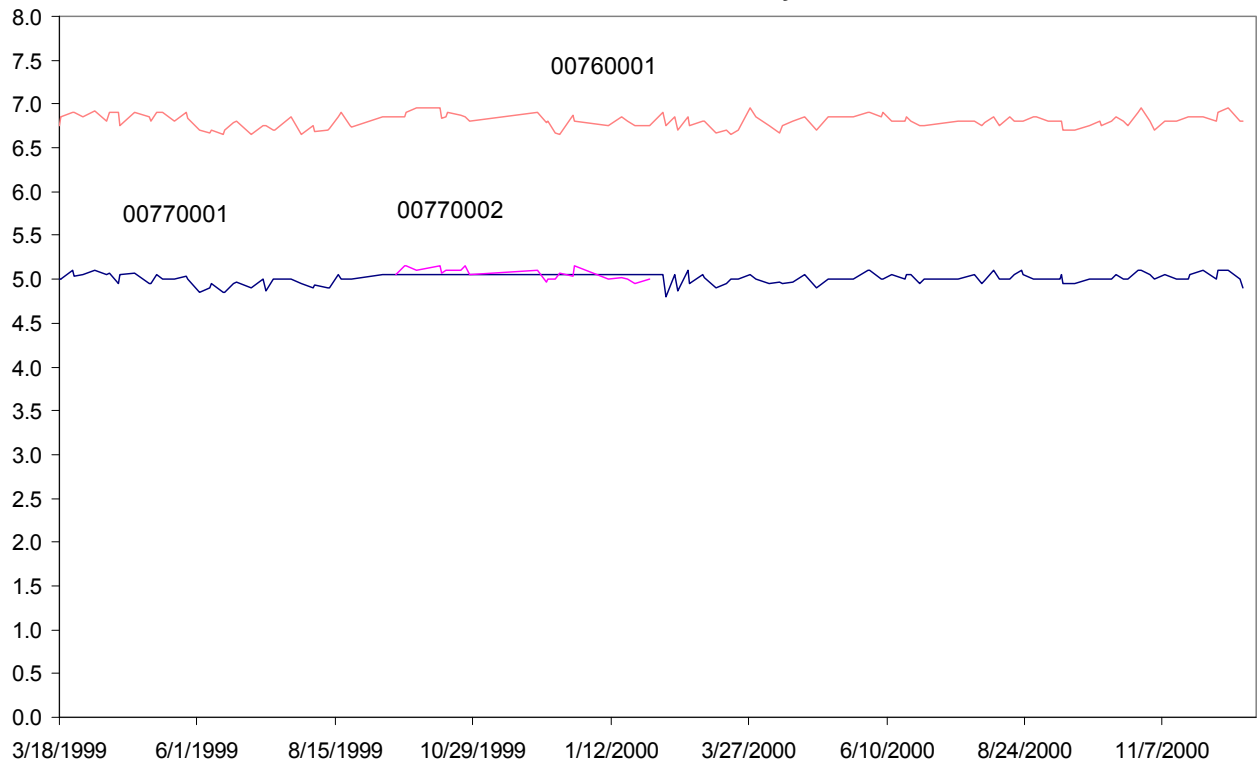


s. Total Protein

Summary Statistics for Total Protein by Lot

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 5.00 | 0.06               | 1.27                     |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 6.80 | 0.08               | 1.12                     |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 5.06 | 0.06               | 1.26                     |

1999-2000 Total Protein Quality Control



t. Triglycerides

Summary Statistics for Triglycerides by Lot

| Lot      | N   | Start Date | End Date   | Mean  | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|-------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 204.1 | 8.4                | 4.1                      |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 92.8  | 4.4                | 4.7                      |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 197.4 | 2.8                | 1.4                      |

1999-2000 Triglycerides Quality Control

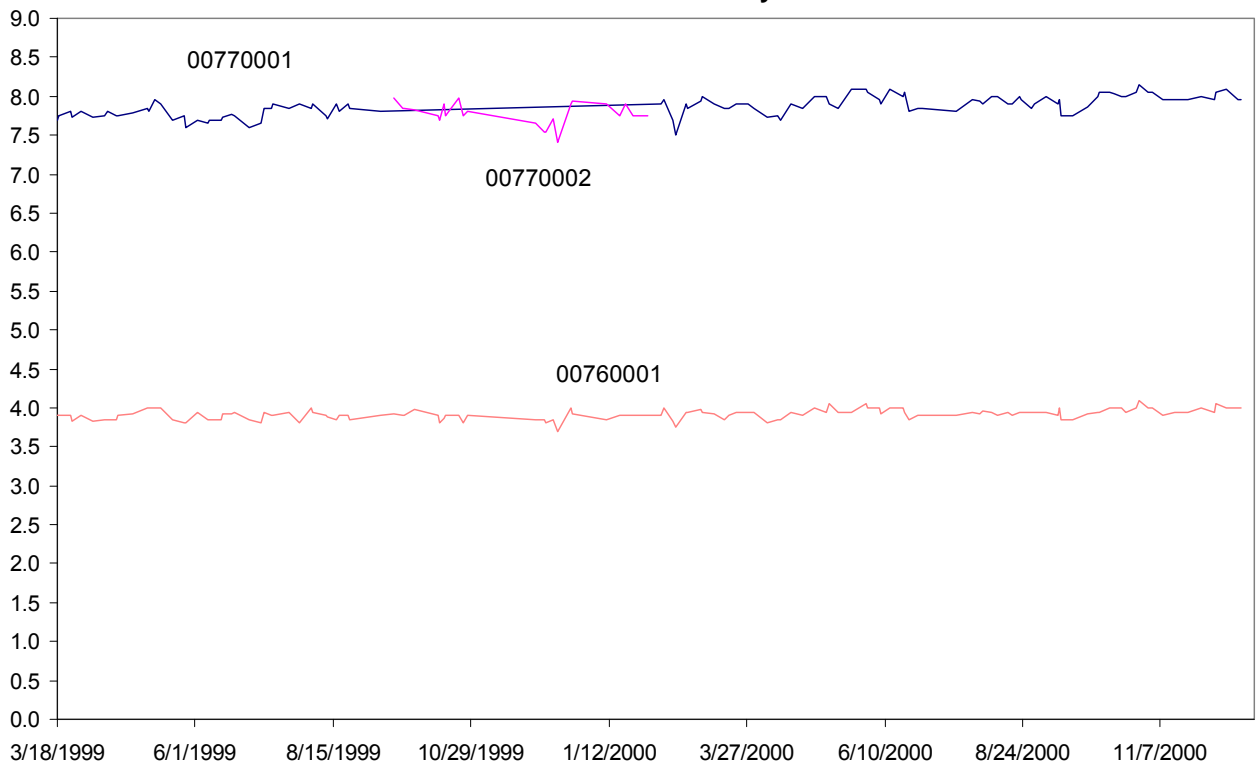


u. Uric Acid

Summary Statistics for Uric Acid by Lot

| Lot      | N   | Start Date | End Date   | Mean | Standard Deviation | Coefficient of Variation |
|----------|-----|------------|------------|------|--------------------|--------------------------|
| 00770001 | 113 | 3/18/1999  | 12/21/2000 | 7.88 | 0.13               | 1.60                     |
| 00760001 | 136 | 3/18/1999  | 12/21/2000 | 3.92 | 0.07               | 1.70                     |
| 00770002 | 23  | 9/17/1999  | 2/2/2000   | 7.78 | 0.14               | 1.83                     |

1999-2000 Uric Acid Quality Control



REFERENCES

1. Procedural insert: Hitachi 917 ALT/IFCC. Indianapolis: Roche Diagnostics.
2. Procedural insert: Hitachi 917 Albumin/BCP. Indianapolis: Roche Diagnostics.
3. Procedural insert: Hitachi 917 Alk Phos/AMP. Indianapolis: Roche Diagnostics.
4. Procedural insert: Hitachi 917 AST/IFCC. Indianapolis: Roche Diagnostics.
5. Procedural insert: Hitachi 917 Bicarbonate. Indianapolis: Roche Diagnostics.
6. Procedural insert: Hitachi 917 BUN. Indianapolis: Roche Diagnostics.



7. Procedural insert: Hitachi 917 Calcium/CAPS Roche Diagnostics.
8. Procedural insert: Hitachi 917 Cholesterol HP. Indianapolis: Roche Diagnostics.
9. Procedural insert: Hitachi 917 Creatinine. Indianapolis: Roche Diagnostics.
10. Procedural insert: Hitachi 917  $\gamma$ -GT. Indianapolis: Roche Diagnostics.
11. Procedural insert: Hitachi 917 Glucose/HK. Indianapolis: Roche Diagnostics.
12. Procedural insert: Hitachi 917 Iron. Indianapolis: Roche Diagnostics.
13. Procedural insert: Hitachi 917 LDH. Indianapolis: Roche Diagnostics.
14. Procedural insert: Hitachi 917 Phosphorus. Indianapolis: Roche Diagnostics.
15. Procedural insert: Hitachi 917 ISE/Na,K,Cl. Indianapolis: Roche Diagnostics.
16. Procedural insert: Hitachi 917 Bilirubin/DPD. Indianapolis: Roche Diagnostics.
17. Procedural insert: Hitachi 917 Total Protein/Biuret. Indianapolis: Roche Diagnostics.
18. Procedural insert: Hitachi 917 Triglycerides/GPO. Indianapolis: Roche Diagnostics.
19. Procedural insert: Hitachi 917 Uric Acid/PAP. Indianapolis: Roche Diagnostics.

#### Additional Sources

Glick MR, and Ryder KW. Interferographs: Users Guide to Interferences in Clinical Chemistry Instruments, Indianapolis: Science Enterprises, Inc.; 1987, p. 132.

Parameter Procedural inserts: Hitachi 917. Indianapolis: Roche Diagnostics.

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