NHANES 2001-2002 Public Release Data File
Laboratory 40 - Standard Biochemistry Profile, Follicle Stimulating Hormone, and Luteinizing Hormone

Update: Correction for Serum Creatinine for NHANES 2001-2002 is <u>not</u> necessary.

(1) Documentation File Update- September 2006

(2) Documentation File Name- Laboratory 40 - Standard Biochemistry Profile

(3) Survey Years Included in this File Release-2001-2002

(4) Component Description

4.1 Standard Biochemistry Profile
This battery of measurements are used in the diagnosis and treatment of certain liver, heart, and kidney diseases, acid-base imbalance in the respiratory and metabolic systems, other diseases involving lipid metabolism and various endocrine disorders as well as other metabolic or nutritional disorders.

4.1.1 Alanine Aminotransferase (ALT)

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 asparate aminotransferase (AST).

4.1.2 Albumin

Albumin measurements are used in the diagnosis and treatment of numerous diseases primarily involving the liver or kidneys.

4.1.3 Alkaline Phosphatase (ALP)

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.

4.1.4 Aspartate Aminotransferase (AST)

AST measurements are used in the diagnosis and treatment of certain types of liver and heart disease. Elevated levels of the transaminases can signal myocardial infarction, hepatic disease, muscular dystrophy, or organ damage.

4.1.5 Bicarbonate (HCO_3)

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.

4.1.6 Blood Urea Nitrogen (BUN)

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.

4.1.7 Calcium

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, pseudo-hypoparathyroidism, chronic renal failure, rickets, infantile tetany, and steroid therapy.

4.1.8 Cholesterol

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

4.1.9 Creatinine

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.

4.1.10 Gamma Glutamyl Transaminase (GGT)

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.

4.1.11 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.

4.1.12 Iron

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) .

4.1.13 Lactate Dehydrogenase (LDH)

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.

4.1.14 Phosphorus

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, kidney diseases, and vitamin D imbalance.

4.1.15 Sodium, Potassium, and Chloride

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.

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.

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.

4.1.16 Total Bilirubin

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 .

4.1.17 Total Protein

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 .

4.1.18 Triglycerides

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.

4.1.19 Uric Acid

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.

4.2 Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)

Serum FSH and LH levels and questionnaire data on menstrual history will be used to classify women according to menopausal status. This information and data on exogenous hormone use are important for evaluating women's risk for certain health conditions such as cardiovascular disease and osteoporosis (see reproductive health questionnaire section).

(5) Sample Description:

5.1 Eligible Sample

Participants aged 12 year and older are tested.

(6) Description of the Laboratory Methodology

6.1 Coulston Foundation Biochemistry Profile method

The 21 analytes described in this method constitute the routine biochemistry profile. The analyses are performed with a Hitachi Model 704 multichannel analyzer (Boehringer Mannheim 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.

6.1.1 Alanine Aminotransferase (ALT)

α-Ketoglutarate reacts with L-alanine in the presence of ALT to form L-glutamate plus pyruvate. The indicator reaction utilizes the pyruvate for a kinetic determination of NADH consumption. As a group the transaminases catalyze the interconversion of amino acids and α-ketoacids by transfer of amino groups.

6.1.2 Albumin

At the reaction pH, the bromcresol purple (BCP) in the Boehringer Manneheim Diagnostics (BMD) albumin system reagent binds selectively with albumin. This reaction is based on a modification of a method described by Doumas. 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 with the increased pH. In addition, the use of a sample blank eliminates background spectral interferences not completely removed by bichromatic analyses.

6.1.3 Alkaline Phosphatase (ALP)

p-Nitrophenylphosphate is hydrolyzed in the presence of magnesium ions by phosphatase to phosphate and p-nitrophenol. The rate of p-nitrophenol liberation is proportional to the alkaline phosphatase activity and can be measured photometrically.

6.1.4 Aspartate Aminotransferase (AST)

α-Ketoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate. The indicator reaction utilizes the oxaloacetate for a kinetic determination of NADH consumption. As a group the transaminases catalyze the interconversion of amino acids and a-ketoacids by transfer of amino groups.

6.1.5 Bicarbonate (HCO_3)

Bicarbonate reacts with phosphoenolpyruvate (PEP) in the presence of PEPC to produce oxaloacetate and phosphate. The resultant consumption of NADH causes a decrease in absorbance in the UV range (320 to 400 nm). The rate of change in absorbance is directly proportional to the concentration of bicarbonate in the sample being assayed.

6.1.6 Blood Urea Nitrogen (BUN)

Urea is hydrolyzed by urease to form CO_2 and ammonia. The ammonia formed then reacts with a-ketoglutarate and NADH in the presence of GLDH to yield glutamate and NAD^+. The decrease in absorbance due to consumption of NADH is measured kinetically.

6.1.7 Calcium

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

O-Cresolphthalein complexone offers a rapid, specific, and sensitive method for the quantitative determination of calcium in serum. This method and other complexiметric methods for the determination of calcium, which are derived from the work of Schwarzenbach, are less tedious than the classic permanganate reference procedures. In 1966, Connerty and Briggs devised a manual photometric method using o-cresolphthalein complexone with protein precipitation to release bound calcium and 8-hydroxyquinoline to mask the interference by magnesium. Sarkar and Chauhan introduced a direct determination of serum calcium in 1967 and modified by Baginski et al in 1973. Others have adapted this method for use with automated analyzers.

6.1.8 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 a 0-quinone imine dye. The intensity of the color formed is proportional to the cholesterol concentration and can be measured photometrically.

6.1.9 Creatinine

This method, which uses the Jaffe reaction, is based on the work of Popper, Seeling, and Wuest. This modification resulted in higher sensitivity and better precision when compared to the original Jaffé method. 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.

6.1.10 Gamma Glutamyltransaminase (-GT)

The g-GT activity at both normal and abnormal levels measured with g-glutamyl-p-nitroanilide and its carboxy derivative was identical. The procedure described below is based on the studies of Persijn and van der Slik using the readily soluble L-g-glutamyl-3-carboxy-4-nitroanilide as the substrate for g-GT activity determinations. The rate of 5-amino-2-nitrobenzoate liberation is proportional to g-GT activity and can be measured photometrically.

6.1.11 Glucose

The glucose hexokinase method described here, based on the work of Schmidt and Peterson and Young, has long been recognized as the most specific method for the determination of glucose. Hexokinase catalyzes the phosphorylation of glucose by ATP; G-6-P is oxidized to 6-phosphogluconate in the presence of NADP by the enzyme glucose-6-phosphate dehydrogenase. No other carbohydrate is oxidized.

The amount of NADPH formed during the reaction is equivalent to the amount of D-glucose in the specimen and can be measured photometrically by the increase in absorbance.

6.1.12 Iron

Fe^{3+} is separated from transferrin by means of guanidin-ium chloride in the weakly acidic pH range and reduced to Fe^{2+} with ascorbic acid. Fe^{2+} then forms a colored complex with ferrozine.

6.1.13 Lactate Dehydrogenase (LDH)

The LD reaction proceeds as follows: NAD and lactate are converted in equimolar amounts at the same rate. The rate at which NADH is formed is determined by an increase in absorbance and is directly proportional to enzyme activity.

6.1.14 Phosphorus

Inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form ammonium phosphomolybdate with a formula of $(NH_4)_3\,[PO_4\,(MoO_3)_{12}]$. The ammonium phosphomolybdate is quantified in the ultraviolet range (340 nm), utilizing a sample blanked endpoint method.

6.1.15 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 measurements 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 in concentration of the test ion in the test solution and the internal filling solution. The EMF develops according to the Nernst equation for a specific ion in solution:

(1) $E = E_o + x \ln$

Where:

E	**=**	**electrode EMF**
E_o	**=**	**standard EMF**
R	**=**	**constant**
T	**=**	**temperature**
n	**=**	**charge of the ion**

F	**=**	**Faraday's constant**
ln	**=**	**natural logarithm (base e)**
f	**=**	**activity coefficient**
Ct	**=**	**ion concentration in test solution**
Ci	**=**	**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 Roche/Hitachi ISE Modules where the sample is diluted 1:31, the ionic strength, and therefore, the activity coefficient are essentially constant. (For the Roche/Hitachi 736 ISE Module the sample is diluted 1:16). The concentration of the test ion in the internal filling solution is also constant. These constants may be combined into the E_o term. The value of E_o is also specific for the type of reference electrode used. Equation (1) can be rewritten to reflect these conditions:

(2) $E = E'_o + S \times \ln (Ct)$

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 measurements are used in the diagnosis and treatment of aldosteronism (excessive secretion of the hormone aldosterone), diabetes insipidus (chronic excretion of large amounts of dilute urine, accompanied by extreme thirst), adrenal hypertension, Addison's disease (caused by destruction of the adrenal glands), dehydration, inappropriate antidiuretic hormone secretion, or other diseases involving electrolyte imbalance. Potassium measurements are used to monitor electrolyte balance in the diagnosis and treatment of disease conditions characterized by low or high blood potassium levels. Chloride measurements are used in the diagnosis and treatment of electrolyte and metabolic disorders such as cystic fibrosis and diabetic acidosis.

6.1.16 Total Bilirubin

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

6.1.17 Total Protein

Colorimetric assay
- Sample and addition of R1 (blank reagent)

- Addition of R2 (biuret reagent) and start of the reaction: Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents autoreduction of copper. The color intensity is directly proportional to the protein concentration that can be determined photometrically.

Plasma proteins are synthesized predominantly in the liver, plasma cells, lymph nodes, and the spleen and in bone marrow. In the course of disease the total protein concentration and also the percentage represented by individual fractions can significantly deviate from normal values.

6.1.18 Tiglycerides

The following method, while based on Wahlefeld's work, uses lipase taken from a microorganism to promote rapid and complete hydrolysis of triglycerides to glycerol with subsequent oxidation to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, the peroxide reacts with 4-aminophenazone and 4-chlorophenol in a Trinder reaction to a colorimetric endpoint.

6.1.19 Uric acid

Uric acid is oxidized by the specific enzyme uricase to form allantoin and H_2O_2. The intensity of the red color formed is proportional to the uric acid concentration.

The test described here is the colorimetric method developed by Town, et al. The sample is initially incubated with a reagent mixture containing ascorbate oxidase and a clearing system. In this test system it is important that any ascorbic acid present in the sample is eliminated in the preliminary reaction; this precludes any ascorbic acid interference with the subsequent POD indicator reaction. Upon addition of the starter reagent, oxidation of uric acid by uricase begins.

6.2 Collaborative Laboratory Services Biochemistry Profile method

The 21 analytes described in this method constitute the routine biochemistry profile. The analyses are performed with a Beckman Synchron LX20. 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.

6.2.1 Alanine Aminotransferase (ALT)

The LX20 uses an enzymatic rate method to measure ALT activity in serum or plasma. In the reaction, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample.

6.2.2 Albumin

The method used to measure the albumin concentration on the LX20 is a bichromatic digital endpoint method. In the reaction, the albumin combines with Bromcresol Purple (BCP) reagent to form a complex. The system monitors the change in absorbance at 600 nm. The change in absorbance is directly proportional to the concentration of albumin in the sample.

6.2.3 Alkaline Phosphatase (ALP)

The LX system uses an enzymatic rate using a 2-Amino-2-Methyl-1-Propanol (AMP) buffer to measure ALP activity in serum or plasma. In the reaction, the ALP catalyzes the hydrolysis of the colorless organic phosphate ester substrate, p-Nitrophenylphosphate, to the yellow colored product p-Nitrophenol and phosphate. This reaction occurs at an alkaline pH of 10.3. The system monitors the rate of change in absorbance at 410 nm over a fixed-time interval. This rate of change in absorbance is directly proportional to the ALP activity in the serum.

6.2.4 Aspartate Aminotransferase (AST)

The LX20 uses an enzymatic rate method to measure the AST activity in serum or plasma. In the reaction, the AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the AST activity in the sample.

6.2.5 Bicarbonate (HCO_3)

The LX20 system uses indirect (or diluted) ISE methodology to measure the total CO_2 level in serum, plasma or urine. The system measures the rate of pH change as Co_2 ions diffuse across a membrane. The electrode used for CO_2 determination is actually a pH electrode with the tip covered by a silicone rubber membrane and lowers the pH of a bicarbonate solution between the tip of the membrane and the tip of the pH electrode. The rate of pH change is directly proportional to the carbon dioxide (CO_2) in the sample.

6.2.6 Blood Urea Nitrogen (BUN)

The LX20 modular chemistry side uses the Jaffe rate method (kinetic alkaline picrate) to determine the concentration of creatinine in serum, plasma, or urine. A precise volume of sample is introduced into a reaction cup containing an alkaline picrate solution. Absorbance readings are taken at both 520 nm and 560 nm. Creatinine from the sample combines with the reagent to produce a red color complex. The observed rate measurement at 25.6 seconds after sample introduction has been shown to be a direct measure of the concentration of the creatinine in the sample.

6.2.7 Calcium

The LX20 system uses indirect (or diluted) ISE methodology to measure calcium concentration in serum, plasma, or urine. The system determines calcium concentration by measuring calcium ion activity in solution. When the sample buffer mixture contacts the electrode, calcium ions complex with the ionophore at the electrode surface. Changes in potential develop at the electrode surface as the reaction occurs. These changes in potential are referenced to a sodium reference electrode. The reference signal is used in calculating the analyte concentrations based on the Nernst equation.

6.2.8 Cholesterol

The LX uses the timed-endpoint method to measure the cholesterol concentration in serum or plasma. In the reaction, the cholesterol esterase hydrolyzes cholesterol esters to free cholesterol and fatty acids. The free cholesterol is oxidized to cholesten-3-one and hydrogen peroxide by cholesterol oxidase. Peroxidase catalyzes the reaction of hydrogen peroxide with 4-aminoantipyrine and phenol to produce a colored quinoneimine product. The system monitors the change in absorbance and 520 nm at a fixed-time interval. The change in absorbance is directly proportional to the concentration of cholesterol in the sample.

6.2.9 Creatinine

The LX20 modular chemistry side uses the Jaffe rate method (kinetic alkaline picrate) to determine the concentration of creatinine in serum, plasma, or urine. A precise volume of sample is introduced into a reaction cup containing an alkaline picrate solution. Absorbance readings are taken at both 520 nm and 560 nm. Creatinine from the sample combines with the reagent to produce a red color complex. The observed rate measurement at 25.6 seconds after sample introduction has been shown to be a direct measure of the concentration of the creatinine in the sample.

6.2.10 Gamma Glutamyltransaminase (-GT)

The LX uses an enzymatic rate method to determine the GGT activity in serum or plasma. In the reaction, the GGT catalyzes the transfer of a gamma-glutamyl group from the colorless substrate, gamma-glutamyl-p-nitroaniline, to the acceptor, glycylglycine with production of the colored product, p-nitroaniline. The system monitors the rate of change in absorbance at 410 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the activity of GGT in the sample.

6.2.11 Glucose

On the Modular Chemistry side of the LX20, glucose concentration in biologic fluids is determined by the oxygen rate method employing a Beckman Oxygen electrode. A precise volume of sample is introduced in a reaction cup containing an electrode that responds to oxygen concentration. Electronic circuits determine the rate of

oxygen consumption, which is directly proportional to the concentration of glucose in the sample.

6.2.12 Iron

The method used to measure the iron concentration is a timed-endpoint method. In the reaction, iron is released from transferrin by acetic acid and is reduced to the ferrous state by hydroxylamine and thioglycolate. The ferrous ion is immediately complexed with the FerroZine Iron Reagent. The system monitors the change in absorbance at 560 nm at a fixed-time interval. This change in absorbance is directly proportional to the concentration of iron in the sample

6.2.13 Lactate Dehydrogenase (LDH)

The LX20 with LD reagent (using lactate as substrate) utilizes an enzymatic rate method to measure LD activity in biological fluids. In the reaction, the LD catalyzes the reversible oxidation of L-Lactate to Pyruvate with the concurrent reduction of ß-Nicotinamide Adenine Dinucleotide (NAD) to ß-Nicotinamide Adenine Dinucleotide (reduced form) (NADH). The system monitors the rate of change in absorbance at 340 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the activity of LD in the sample.

6.2.14 Phosphorus

The LX system uses a timed-rate method to determine the concentration of phosphorus in serum, plasma and urine. In the reaction, inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a colored phosphomolybdate

6.2.15 Sodium, Potassium, and Chloride

The LX system utilizes indirect (or diluted) I.S.E. methodology to determine the concentration of sodium in biological fluids. The LX determines sodium ion concentration by measuring electrolyte activity in solution. When the sample/buffer mixture contacts the electrode, sodium ions undergo an ion exchange in the hydrated outer layer of the glass electrode. As the ion exchange takes place, a change in voltage (potential) is developed at the face of the electrode. The potential follows the Nernst equation and allows the calculation of sodium concentration in a solution.

The LX system uses indirect (or diluted) I.S.E. methodology to measure potassium in biological fluids. The system determines potassium ion concentration by measuring electrolyte activity in solution. The potassium electrode consists of valinomycin membrane. The voltage (potential) change that takes place within the membrane follows the Nernst equation and allows the calculation of potassium concentration in solution.

The LX system uses indirect (or diluted) I.S.E. methodology to determine chloride concentration in biological fluids. Chloride is measured using an Ag/AgCl electrode. At the face of the electrode, solid AgCl dissolves to the extent as to saturate the solution around the tip with silver (Ag+) and Chloride (Cl-) ions until

equilibrium is established. The product of the ion concentrations in solution, at equilibrium, with an excess of the slightly soluble AgCl is defined as the solubility product constant (Ksp). When chloride sample is added, the Ksp of the solution at the tip is disrupted as AgCl precipitates out of solution. To reestablish the equilibrium, Ag+ ions are generated from the tip causing a change in the potential. According to the Nernst equation, this change is proportional to the concentration of chloride in the sample.

6.2.16 Total Bilirubin

The LX20 uses a timed-endpoint Diazo method to measure the concentration of total bilirubin in serum or plasma. In the reaction, bilirubin reacts with diazo reagent in the presence of caffeine, benzoate, and acetate as accelerators to form azobilirubin. The system monitors the change in absorbance at 520 nm at a fixed-time interval. This change in absorbance is directly proportional to the concentration of total bilirubin in the sample.

6.2.17 Total Protein

The LX20 uses a timed rate biuret method to measure the concentration of total protein in serum or plasma. Proteins in the sample combine with the reagent producing alkaline copper-protein chelate. The rate change in absorbance is monitored by a detector at 545 nm. The observed rate of chelate formation is directly proportional to the total protein concentration in the sample.

6.2.18 Tiglycerides

The LX uses a timed-endpoint method to determine the concentration of triglycerides in serum or plasma. Triglycerides in the sample are hydrolyzed to glycerol and free fatty acids by the action of lipase. A sequence of three coupled enzymatic steps using glycerol kinase (GK), glycerophosphate oxidase (GPO), and horseradish peroxidase (HPO) causes the oxidative coupling of 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) with 4-aminoantipyrine to form a red quinoneimine dye. The system monitors the change in absorbance at 520 nm for a fixed-time interval. The change in absorbance is directly proportional to the concentration of triglycerides in the sample.

6.2.19 Uric acid

The LX20 uses a timed endpoint method to measure the concentration of uric acid in serum, plasma or urine. Uric acid is oxidized by uricase to produce allatoin and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzene sulfonate (DCHBS) in a reaction catalyzed by peroxidase to produce a colored product. The system monitors the change in absorbance at 520 nm at a fixed time interval. The change in absorbance is directly proportional to the concentration of uric acid in the sample.

6.3 Coulston Foundation Follicle Stimulating Hormone (FSH)

Human follicle stimulating hormone (FSH, follitropin) is a glycoprotein of approximately 30,000 daltons which, like luteinizing hormone (LH, lutropin), human chorionic gonadotropin (hCG) and thyroid stimulating hormone (TSH, thyrotropin), consists of two noncovalently associated subunits designated alpha and beta. The alpha subunit of FSH contains 92 amino acids and is very similar to the alpha subunits of LH, hCG, and TSH. The beta subunit of FSH is unique and confers its immunological and functional specificity.

6.4 Coulston Foundation Luteinizing Hormone (LH)

The IMx LH assay is based on the Microparticle Enzyme Immunoassay (MEIA) technology. The IMx LH reagents and sample are added to the reaction cell in the following sequence:

The probe/electrode assembly delivers the sample and Anti-beta LH Coated Microparticles to the incubation well of the reaction cell. The LH binds to the Anti-beta FSH Coated Microparticles forming an antibody-antigen complex. An aliquot of the reaction mixture containing the antibody-antigen complex bound to the microparticles is transferred to the glass fiber matrix. The microparticles bind irreversibly to the glass fiber matrix. The matrix is washed with the Wash Buffer to remove unbound materials.

The Anti-alpha LH subunit specific Alkaline Phosphatase conjugated is dispensed onto the matrix and binds with the antibody-antigen complex. The matrix is washed to remove unbound materials. The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.

6.5 Collaborative Laboratory Services Follicle Stimulating Hormone (FSH)

The Access FSH assay is a paramagnetic particle, chemiluminescent, two-step enzyme immunoassay for the quantitative determination of hFSH in human serum using the Access Immunoassay System. A sample is added to a reaction vessel with paramagnetic particles coated with goat anti-mouse: mouse anti-hFSH complexes and Tris buffered saline with protein. The serum hFSH binds to the immobilized mouse anti-hFSH on the solid phase. Separation in a magnetic field and washing removes materials not bound to the solid phase. Alkaline phosphatase conjugated goat anti-hFSH is then added and binds to the previously bound hFSH on the particles. A second separation and wash step removes unbound conjugate. A chemiluminescent substrate, Lumi-Phos 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The photon production is proportional to the amount of hFSH in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

6.6 Collaborative Laboratory Services Luteinizing Hormone (LH)

The Access LH assay is a paramagnetic particle, chemiluminescent, two-step enzyme immunoassay for the quantitative determination of hLH in human serum using the Access Immunoassay System. A sample is added to a reaction vessel with paramagnetic particles coated with goat anti-mouse: mouse anti-hLH complexes and Tris buffered saline with protein. The serum hLH binds to the immobilized mouse anti-hLH on the solid phase. Separation in a magnetic field and washing removes materials not bound to the solid phase. Alkaline phosphatase conjugated goat anti-hLH is then added and binds to the previously bound hLH on the particles. A second separation and wash step removes unbound conjugate. A chemiluminescent substrate, Lumi-Phos 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The photon production is proportional to the amount of hLH in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

(7) Laboratory Quality Control and Monitoring
The NHANES quality control and quality assurance protocols (QA/QC) meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed quality control and quality assurance instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.

(8) Data Processing and Editing

Specimens were processed, stored and shipped to Coulston Foundation, Alamogordo, New Mexico and to Collaborative Laboratory Services in Ottumwa, Iowa. Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the Description of the Laboratory Methodology section.

(9) Data Access:

All data are publicly available.

(10) Analytic Notes for Data Users:

The analysis of NHANES 2001-2002 laboratory data must be conducted with the key survey design and basic demographic variables. The NHANES 2001-2002 Household Questionnaire Data Files contain demographic data, health indicators, and other related information collected during household interviews. They also contain all survey design variables and sample weights for these age groups. The phlebotomy file includes auxiliary information such as the conditions precluding

venipuncture. The household questionnaire and phlebotomy files may be linked to the laboratory data file using the unique survey participant identifier SEQN.

10.1 Coulston Foundation at Alamagordo, New Mexico performed testing in 2001 and Collaborative Laboratory Services at Ottumwa, Iowa performed testing in 2002.

10.2 LBXSTR:

This value was obtained from the standard battery of biochemical assessments. Use of the laboratory test result from the reference method (LBXTR), rather than the (LBXSTR) value, is generally recommended. For most analyses, the appropriate variable to use is (LBXTR). The value from the biochemistry profile (LBXSTR) should not be used routinely.

10.3 LBXSCH:

This value was obtained from the standard battery of biochemical assessments. Use of the laboratory test result from the reference method (LBXTC), rather than the (LBXSCH) value, is generally recommended. For most analyses of serum cholesterol, the appropriate variable to use will be (LBXTC). The (LBXSCH) value from the biochemistry profile should not be used routinely

10.4 LBXSGL

This value was obtained from the standard battery of biochemical assessments. Use of the laboratory test result from the reference method (LBXGLU), rather than the (LBXSGL) value, is generally recommended. These serum glucose values(LBXSGL) reported in this release should not be used to determine undiagnosed diabetes or prediabetes. Instead, plasma glucose values (LBXGLU) should be used based on the reference analytic method of this analyte. Use the special weights included in this data file when analyzing data.

10.5 Adjustment of Data Between the Laboratories:

The distributions of sample person results were compared between the Coulston Foundation Laboratory (2001) and the Collaborative Laboratory Services (2002). The means of the sample person distributions for the two labs were compared using a weighted t-test. Five tests including Alkaline Phosphatase, Creatinine, Lactate Dehydrogenase, Phosphorus, and Total Bilirubin had significantly ($p<0.05$) different means. A cross-over study between the two labs was performed to establish linear regression equations to convert Coulston Foundation Laboratory values to the Collaborative Laboratory Services values. The regression equations were applied to the five tests and a weighted t-test was done after regression. The weighted t-test revealed that no significant differences ($p<0.05$) between the two labs after regression.

10.6 Correction for Serum Creatinine for NHANES 2001-2002 is <u>not</u> necessary:

Serum creatinine is not standardized in many laboratories. The National Kidney Disease Education Program is attempting to have all laboratories standardize serum creatinine to reference methods (Myers, GL, *et al*. Recommendations for Improving Serum Creatinine Measurement: A Report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin. Chem. 2006; 5-18). Equations for estimating glomerular filtration rate (GFR) from standardized creatinine have been published (Stevens LA, *et al*. N Engl J Med. 2006 Jun 8;354(23):2473-83). Serum creatinine assays on 194 stored specimens from NHANES 2001-2002 were used to determine if serum creatinine needed to be adjusted when compared to a method traceable to a "gold" standard reference method. The Cleveland Clinic Foundation (CCF) laboratory analyzed the serum creatinine specimens using a Roche coupled enzymatic assay (creatininase, creatinase, sarcosine oxidase, kits # 1775677 and 1775766) performed on a Roche P Module instrument. The Roche method calibrators were traceable to an isotope dilution mass spectrometric method for serum creatinine using standard references methods (NIST SRM 967) and confirmed by analysis of CAP LN-24 linearity set based on NIST assigned values. Serum creatinine by the Roche method was then compared to the original NHANES 2001-2002 measurements which used the Jaffe kinetic alkaline picrate method performed on a Beckman LX-20 analyzer and a Roche Hitachi 917 analyzer. There were no significant differences in results between these two measurements. The comparison of values revealed the mean (SD) serum creatinine at NHANES, CCF, and their difference were 0.982 (0.316), 0.987 (0.325), and 0.005 (0.060) mg/dL, respectively (paired t-test, p=0.28). The regression of CCF (Y) on NHANES (X) had an intercept and slope of -0.005 (0.014) and 1.010 (0.013) mg/dL and a correlation of 0.983. The difference between the methods was within the analytical error of the NHANES method (CV of 4.4% at 0.68 mg/dL or 0.030 mg/dL and a CV of 2.9% at 1.26 mg/dL or 0.037 mg/dL). Thus, <u>no correction is necessary for serum creatinine values in NHANES 2001-2002.</u>