

NHANES 1999-2000 Public Data Release (June 2002)

Laboratory 18 – Standard Biochemistry Profile, Follicle Stimulating Hormone, and Luteinizing Hormone

1 Description

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.

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 aspartate aminotransferase (AST).

Albumin

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

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.

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.

Bicarbonate (HCO₃)

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.

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.

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.

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.

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.

Gamma Glutamyltransaminase (g-GT)

g-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.

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.

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).

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.

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.

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.

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.

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.

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.

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.

FSH/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).

Laboratory

Blood and urine specimens are collected on participants aged one year and older at the mobile examination centers (MECs). . Hematological profiles are complete for all participants, and specified laboratory tests are performed upon each specimen based on the participant's age at time of interview and sex.

The laboratory component of NHANES includes the collection, processing, storage, and shipping of blood, urine and other biological and environmental specimens. The blood collection procedure consists of administering a questionnaire to screen for conditions that excludes the participants from the blood draw and determines fasting status, a blood draw, and collecting specimens for special studies. .

Eligible Sample Exclusion Criteria

- Participants aged 12 years and older who do not meet any of the exclusion criteria were eligible for the Standard Biochemistry Profile
- Women age 35-60 years are eligible for the FSH and LH tests.

Exclusion Criteria

- Hemophiliacs
- Participants who received chemotherapy within last 4 weeks
- The presence of the following on both arms: rashes, gauze dressings, casts, edema, paralysis, tubes, open sores or wounds, withered arms or limbs missing, damaged, sclerosed or occluded veins, allergies to cleansing reagents, burned or scarred tissue, shunt or IV.

Data Collection Methods

The MEC phlebotomist draws blood from the participant's arm. In the laboratory, the blood is processed, stored, and shipped to the Coulston Foundation for analysis.

Examination Protocol

Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Vials are stored under appropriate refrigerated (4-8 degrees Centigrade) conditions until they are shipped to Coulston Foundation for testing. The analytical methods are described in the Analytic methodology section.

Staff

The NHANES 1999-2000 laboratory staff consists of medical technologists and phlebotomists. The medical technologists hold baccalaureates in medical technology. The American Society for Clinical Pathologists or a similar organization certifies the medical technologists and the phlebotomists. All laboratory staff completes comprehensive training in standardized laboratory procedures before they begin working in the MEC. The MEC phlebotomists complete comprehensive training in pediatric phlebotomy techniques, including instruction by a pediatric nurse practitioner.

Data Collection Forms

Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Each chapter in the LPM specifies the procedure to be used for preparation of the participant, specimen collection, labeling, processing, and preservation, and conditions for specimen transport that are appropriate for that method.

Analytic Methodology

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.

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.

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.

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.

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 α -ketoacids by transfer of amino groups.

Bicarbonate (HCO₃)

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.

Blood Urea Nitrogen (BUN)

Urea is hydrolyzed by urease to form CO_2 and ammonia. The ammonia formed then reacts with α -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.

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 compleximetric 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.

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 formed is proportional to the cholesterol concentration and can be measured photometrically.

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.

Gamma Glutamyltransaminase (g-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.

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.

Iron

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

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.

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), utilizing a sample blanked endpoint method.

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
C _t	=	ion concentration in test solution
C _i	=	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:

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

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 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.

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

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.

Triglycerides

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.

Uric Acid

Uric acid is oxidized by the specific enzyme uricase to form allantoin and H₂O₂. 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.

Follicle Stimulating Hormone and Luteinizing Hormone

IMx Ultrasensitive FSH and LH is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of human hormone in serum or plasma on the IMx analyzer.

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

At menopause ovarian function is diminished, with concomitant decrease in estradiol secretion. FSH and LH then increase significantly in response to diminished feedback inhibition of gonadotropin release.

Analytic Notes

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.

LBXSIR:

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

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

LBXFSH and LBXLH:

These tests are performed only on females aged 35-60 years.