

CSCL Assay Methodologies

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Adiponectin, Total (Acrp30)

Dates of Use: 2004 – current

Adiponectin is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Quantikine Human Adiponectin/Acrp30 Immunoassay kit from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The laboratory inter-assay CV is 8.8% at a mean concentration of 12477 ng/mL.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Adiponectin, High Molecular Weight

Dates of Use: Proposed

High molecular weight adiponectin is measured in serum or plasma using a sandwich enzyme-linked immunosorbent assay (ELISA) kit that uses two kinds of anti-human adiponectin monoclonal antibodies (Adiponectin (Multimeric) EIA Kit, ALPCO Diagnostics, Salem, NH 03079). Specimens are first pretreated with proteases for the selective measurement of multimeric adiponectin as follows: to measure total adiponectin fraction, specimens are treated with SDS-containing acid buffer to convert multimeric adiponectin mainly to a dimer form; to measure high molecular adiponectin fraction, specimens are incubated with a protease that selectively digests the low molecular adiponectin fraction and the middle-molecular adiponectin fraction, and the remaining fraction is treated with SDS-containing acid buffer to convert it to a dimer form. Resulting adiponectin species of the same sample are measured on the same ELISA plate, and intensity of color is read on a plate reader at 492 nm. The CV of the method is <15%.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma

Minimum volume needed: 125uL

Sample volume used: 20uL (for total and high molecular weight adiponectin concentrations);

10uL (for high molecular weight adiponectin concentration only)

Dead volume: 50uL

Albumin

Dates of Use: 2006 – current

Albumin is measured in serum or plasma on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a bromcresol purple method (Roche Diagnostics, Indianapolis, IN 46250). At the reaction pH, bromcresol purple binds selectively with albumin causing a color change that is measured photometrically. The laboratory inter-assay CV is 2.6% at a level of 3.14 g/dL and 2.5% at a level of 4.01 g/dL.

Sample type: Serum/EDTA plasma/lithium-heparin plasma

Minimum volume needed: 150uL

Sample volume used: 2.2uL

Dead volume: 75uL

Albumin, glycated

Dates of Use: 2009 - current

This is a complex method by Asahi Kasei Pharma adapted to the Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation). The assay requires separate measurements of total albumin (bromocresol purple) and glycated albumin (enzymatic method utilizing ketoamine oxidase and an albumin-specific protease) measured in serum or plasma. The glycated albumin result is expressed as a percentage of total albumin. Glycated albumin (%) inter-day CV is 1.8% (mean 56.0%), 2.1% (mean 22.7%).

Sample type: Serum/EDTA plasma/lithium-heparin plasma

Minimum volume needed: 150uL

Sample volume used: 7uL

Dead volume: 75uL

Albumin, urine

Dates of Use: 2005 - current

Albumin is measured in urine using kit reagents on the ProSpec nephelometric analyzer (Dade Behring GMBH, Marburg, Germany D-35041). In this reaction, rabbit-derived anti-human albumin is incubated with the urine specimen, forming an immunocomplex between the antibody and the albumin in the specimen and resulting in an increase in light scatter. The intensity of light scatter is directly proportional to the concentration of albumin which is determined by comparing its light scatter to that observed using known standards in a calibration curve. The laboratory inter-assay CV is 3.2%.

Sample type: Urine, (untreated) random or timed

Minimum volume needed: 350uL (unless sample is in 2mL vial, then 250uL)

Sample volume used: 100uL

Dead volume: 200uL (unless sample is in 2mL vial, then 100uL)

Amyloid A, serum (SAA)

Dates of Use: 2004 - current

Serum amyloid A is measured in plasma or serum using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) kit from BioSource International, Inc. (Camarillo, CA). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 3.8-10.8% for the serum or plasma assay. Data from our lab give an inter-assay CV of 14.4% (mean: 13075 ng/mL) on controls assayed over 9 days.

Sample type: Serum/plasma

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

1,5 Anhydroglucitol

Dates of Use: 2009 - current

1,5AG is measured in serum or plasma on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation). First the sample is pretreated by glucokinase (GK) to convert glucose to glucose 6-phosphate in the presence of adenosine triphosphate (ATP), pyruvate kinase (PK) and phosphoenol pyruvate (PEP). The purpose of this reaction is to alter glucose so it can not react in the primary assay for 1,5-AG. Then pyranose oxidase oxidizes the second hydroxyl of 1,5-anhydroglucitol. The amount of hydrogen peroxide generated in this reaction is directly related to serum 1,5-AG concentrations and is detected by colorimetry using peroxidase. The interassay CV is 5%.

Sample type: Serum/EDTA plasma/lithium-heparin plasma

Minimum volume needed: 150uL

Sample volume used: 6uL

Dead volume: 75uL

Apolipoprotein A-1 (ApoA1)

Dates of Use: 2004

ApoA1 is measured in serum by rate nephelometry using the Immage[®] Immunochemistry System (Beckman Coulter, Fullerton, CA 92834-3100). The lower limit of detection is 25 mg/dL. Reference range is 95 – 180 mg/dL for males and 100 – 200 mg/dL for females. CV of the method is 3.8%.

Apolipoprotein A-1 (ApoA1)

Dates of Use: Proposed

ApoA1 is measured in serum or plasma by rate nephelometry using the Siemens ProSpec Nephelometer. The laboratory inter-assay CV is 4.0%.

Sample type: Serum/heparin plasma

Minimum volume needed: 275uL (unless sample is in 2mL vial, then 175uL)

Sample volume used: 10uL

Dead volume: 200uL (unless sample is in 2mL vial, then 100uL)

Apolipoprotein B (ApoB)

Dates of Use: 2002

ApoB is measured in serum by rate nephelometry using the Immage[®] Immunochemistry System (Beckman Coulter, Fullerton, CA 92834-3100). The lower limit of detection is 35 mg/dL. Reference range is 50 – 150 mg/dL for males and 50 – 170 mg/dL for females. CV of the method is 6.8%.

Ascorbic Acid

Dates of Use: 2004

Plasma samples will be collected and stabilized with a solution of metaphosphoric acid and dithiothreitol as described by Margolis et al.⁴ and subsequently stored at -70°C. This collection and storage method prevents artifactual oxidation and loss of ascorbic acid for at least 2 years. The stored samples will be analyzed by an HPLC-based method with electrochemical detection as described by Dhariwal et al.⁵. The assay has interassay coefficients of variation of less than 8% and a detection limit (< 1.0 mg/liter) well below typical human values. Proficiency testing will be performed routinely with reference materials obtained from the NIST.

B-type natriuretic peptide (BNP)

Dates of use: 2007 – current

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) (Siemens Diagnostics, Deerfield, IL) BNP assay is a fully automated two-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of two monoclonal antibodies measured in EDTA plasma. The first antibody, in the Lite Reagent, is an acridinium ester labeled monoclonal antibody, in the Solid Phase, is a biotinylated monoclonal mouse anti-human antibody specific to the C-terminal portion of BNP, which is coupled to streptavidin magnetic particles.

Sample type: EDTA plasma collected in plastic blood collection tubes (BNP is unstable in glass containers)

Minimum volume needed: 325uL

Sample volume used: 120uL

Dead volume: 150uL

B-type natriuretic peptide, N-terminal pro (NT-proBNP)

Dates of use: 2010-current

NT-proBNP is measured in serum or plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample reacts with a biotinylated monoclonal NT-proBNP-specific antibody and a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of NT-proBNP in the sample. The Roche reported CV is 2.4% at a level of 126 pg/mL, 1.3% at a level of 2,410 pg/mL, and 2.7% at a level of 33,606 pg/mL.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 275uL

Sample volume used: 15uL

Dead volume: 200uL

Bicarbonate

Dates of Use: 2006 - current

Bicarbonate is measured in serum or plasma using Roche CO2-L reagent on the Modular P Chemistry analyzer. In this reaction, bicarbonate is converted to oxaloacetate in the presence of phosphoenolpyruvate (PEP) and phosphoenolpyruvate carboxylase (PEPC). Then, oxaloacetate is converted to malate in the presence of an NADH analog which is simultaneously converted to an NAD analog. The rate of disappearance of NADH analog is measured at 415 nm, and it is directly proportional to the amount of bicarbonate in the specimen. The laboratory inter-assay CV is 5.0%.

Sample type: Serum/heparin plasma/lithium-heparin plasma

Minimum volume needed: 150uL

Sample volume used: 2uL

Dead volume: 75uL

Bilirubin

Dates of Use: 2006 - current

Total bilirubin is measured in serum or plasma using a diazonium salt/ion colorimetric assay (Roche Diagnostics, Indianapolis, IN 46250) and read on the Roche Modular P Chemistry Analyzer (Roche Diagnostics). The reference range is 0.2 – 1.3 mg/dL with an inter-assay CV of 3.1%. Direct bilirubin is measured in serum on the Roche Modular P Chemistry Analyzer using a colorimetric assay based on the diazo Jendrassik-Grof procedure (Roche Diagnostics). The reference range is 0 – 0.2 mg/dL with an inter-assay CV of 4.7%.

Sample type: Serum/EDTA plasma/lithium-heparin plasma

Minimum volume needed: 150uL

Sample volume used: 3uL

Dead volume: 75uL

C-Peptide

Dates of use: Proposed

C-Peptide is measured in serum or EDTA or heparin plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample reacts with a biotinylated monoclonal C-peptide-specific antibody and a monoclonal C-peptide-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added, and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of C-peptide in the sample. The Roche reported CV is 4.6% at a level of 1.82 ng/mL and 1.3% at a level of 24.1 ng/mL.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 250uL

Sample volume used: 20uL

Dead volume: 200uL

C-reactive Protein, High Sensitive (hsCRP)

Dates of Use: 2006 and prior

hsCRP (highly sensitive C-reactive protein) is measured in serum or plasma using a latex-particle enhanced immunoturbidimetric assay kit (Kamiya Biomedical Company, Seattle, WA 98188) and read on the Roche/Hitachi 911 (Roche Diagnostics, Indianapolis, IN 46250). The reference range is 0 – 0.5 mg/dL. The inter-assay CV in our laboratory is 4.5%.

C-reactive Protein, High Sensitive (hsCRP)

Dates of Use: 2006 - current

hsCRP is measured in serum or plasma using a latex-particle enhanced immunoturbidimetric assay kit (Roche Diagnostics, Indianapolis, IN 46250) and read on the Roche Modular P Chemistry analyzer (Roche Diagnostics). The reference range is 0 – 5 mg/L. The inter-assay CV in our laboratory is 4.5%.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 150uL

Sample volume used: 10uL

Dead volume: 75uL

C-terminal cross-link telopeptide type 1 collagen (CTX, ICTP)

Dates of Use: 2009 - current

ICTP, a bone marker assay, is measured in serum or EDTA-anticoagulated plasma using the UniQ ICTP EIA kit from Orion Diagnostica (Espoo, Finland). This kit measures the C-terminal telopeptide of type I collagen by competitive immunoassay. Briefly, samples or standards are incubated with peroxidase-labeled ICTP and unlabeled rabbit antiserum in microplate wells coated with goat anti-rabbit antibodies. The wells are washed, then a substrate solution containing tetramethylbenzidine is added and color in the wells develops in inverse proportion to the amount of ICTP in the sample. The color is read on a SpectraMax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA 94089). The concentration of ICTP in the sample is determined by preparing a standard curve from standards of known concentration. Our laboratory inter-assay CV on 5 samples is 8.9% at a mean concentration of 24.8 ug/L. The kit insert reports inter-assay CVs of 6.4% at mean concentration 3.2 ug/L, 7.5% at mean concentration 7.1 ug/L, and 6.4% at mean concentration 28.2 ug/L.

Sample type: Serum/EDTA plasma; do not use heparin plasma or citrate plasma

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

CagA IgG

Dates of use: 2008-current

CagA protein IgG antibodies are detected using the CagA IgG ELISA kit (ALPCO Diagnostics, Salem, NH) and measured in serum or plasma. CagA proteins are produced by some strains of *Helicobacter pylori* (*H. pylori*). Diluted serum samples are incubated with recombinant CagA protein immobilized on microtiter wells. Rabbit anti-human IgG conjugated to horseradish peroxidase is added to the wells and binds to surface-bound antibodies in the second incubation. After unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of stop solution terminates the reaction and provides the appropriate pH for color development. The optical densities of the standards, controls and samples are measured using a microplate reader at 450nm. Optical density is directly proportional to antibody in the sample. This is a semi-quantitative assay, so no CV is available.

*Sample type: Serum/plasma**Minimum volume needed: 125uL**Sample volume used: 10uL**Dead volume: 50uL***Calcium**

Dates of Use: 2006 - current

Calcium is measured in serum or plasma on a Roche Modular P Chemistry Analyzer. (Roche Diagnostics Corporation) using a colorimetric method. Calcium reacts with o-cresolphthalein complexone in the presence of 8-hydroxquinoline to form a purple chromophore which is measured photometrically. The laboratory inter-assay CV is 2.3% (mean 9.58 mg/dL)

*Sample type: Serum/heparin plasma/lithium-heparin plasma**Minimum volume needed: 150uL**Sample volume used: 7uL**Dead volume: 75uL***Carcinoembryonic antigen**

Dates of use: 2007

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) CEA assay is a two-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of two antibodies in serum. The first antibody in the LITE Reagent is a purified polyclonal rabbit anti-CEA antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-CEA antibody covalently coupled to paramagnetic particles.

*Sample type: Serum**Minimum volume needed: 175uL**Sample volume used: 60uL**Dead volume: 50uL*

Carotenoids and Tocopherols

Dates of Use: Myron Gross' lab

Carotenoids and tocopherols will be measured simultaneously by a high performance liquid chromatography (HPLC)-based assay as described by Bieri et al. with several modifications within our laboratory for the analysis of tocopherols and with calibration by the methods of Craft et al. A description of the method and the modifications has been reported by Gross et al. The analytes that are quantitated by this method include lycopene, alpha-carotene, beta-carotene, beta-cryptoxanthin, zeaxanthin plus lutein, and alpha-tocopherol and gamma-tocopherol. Between- and within-day CVs are <10% for all analytes. Quality control procedures include the routine analysis of reference sera from National Institutes of Standards and Technology (NIST)

Chlamydia pneumonia (C. pneumonia) IgG

Dates of Use: 2003 – 2004

Serum IgG antibodies to *Chlamydia pneumoniae* (*C. pneumoniae*) were detected using a microimmunofluorescent antibody (MIF) assay employing a two stage sandwich procedure for the qualitative and semi-quantitation detection of IgG antibodies to *C. pneumoniae* (Focus Technologies, Cypress, CA). Positive reactions appear as bright apple-green fluorescent elementary bodies with a background matrix of yolk sac. Fluorescence is graded as follows: 2 to 4+: moderate to intense apple-green fluorescence; 1+: definite, but dim fluorescence; negative: no fluorescence. A positive test is one in which fluorescence is 1+ or greater. This is not a quantitative assay, so no CV is available.

Chloride (see Electrolytes)

See the entry "Electrolytes."

Cholesterol

Dates of Use: 2000 and prior

Total cholesterol is measured in EDTA plasma using a cholesterol oxidase method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche COBAS FARA centrifugal analyzer. This method incorporates cholesterol esterase and peroxidase in the reagent and monitors cholesterol oxidation at 500 nm upon conversion of 4-aminoantipyrine to quinoneimine. This enzymatic method is standardized with a serum standard prepared in our laboratory and frozen at -70°C. The assigned value of this standard is set by replicate Abell-Kendall cholesterol analysis performed by a CDC/NHLBI Cholesterol Reference Method Laboratory Network laboratory. The calibration of this assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends reference range of <200 mg/dL. The laboratory CV is 1.6%.

Cholesterol

Dates of Use: Nov 2000 – Spring 2006

Total cholesterol is measured in EDTA plasma using a cholesterol oxidase method (Roche Diagnostics Corporation, Indianapolis, IN 46250) on a Roche Hitachi 911 (Roche Diagnostics Corporation). This method incorporates cholesterol esterase and peroxidase in the reagent and monitors cholesterol oxidation at 500 nm upon conversion of 4-aminoantipyrine to quinoneimine. This enzymatic method is standardized with a serum standard prepared in our laboratory and frozen at -70°C. The assigned value of this standard is set by replicate Abell-Kendall cholesterol analysis performed by a CDC/NHLBI Cholesterol Reference Method Laboratory Network laboratory. The calibration of this assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends reference range of <200 mg/dL. The laboratory CV is 1.6%.

Cholesterol

Dates of Use: Spring 2006 - current

Total cholesterol is measured in serum or EDTA plasma using a cholesterol oxidase method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation). This method incorporates cholesterol esterase and peroxidase in the reagent and monitors cholesterol oxidation at 500 nm upon conversion of 4-aminoantipyrine to quinoneimine. This enzymatic method is standardized with a serum standard prepared in our laboratory and frozen at -70°C. The assigned value of this standard is traceable to replicate Abell-Kendall cholesterol analysis performed by a CDC/NHLBI Cholesterol Reference Method Laboratory Network laboratory. The calibration of this assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends reference range of <200 mg/dL. The laboratory CV is 1.6%.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 150uL

Sample volume used: 2uL

Dead volume: 75uL

Cholesteryl Ester Transfer Protein (CETP) Activity

Dates of Use: Fall 2002 – Spring 2003

CETP activity is determined in plasma using a CETP Activity Kit (Roar Biomedical, Inc., New York, NY) that includes donor (without apo-A1) and acceptor particles. Incubation of donor and acceptor with a CETP source results in the CETP mediated transfer of fluorescent neutral lipid from donor molecule to a VLDL acceptor molecule. The fluorescence of the reaction increases as the fluorescent neutral lipid is removed from the core of the donor molecule to the acceptor. CETP activity is reported in nmol/mL/hr. The laboratory CV for this assay is 13.3%.

Cholesteryl Ester Transfer Protein (CETP) Mass

Dates of Use: Fall 2002 – Spring 2003

CETP mass is measured in serum by a sandwich enzyme immunoassay (Wako CETP Test; Wako Chemical USA, Inc., Richmond, VA) method. Serum samples are pretreated with detergent to release CETP from lipoproteins. The monoclonal antibody coated to the solid phase and the horse radish peroxidase (HRP)-labeled monoclonal antibody reacts with the released CETP. The activity of HRP bound to the solid phase is proportional to the CETP mass in the sample. The laboratory CV for this assay is 11.1%. The reference range is reported to be 1.92 ± 0.57 ug/mL.

Creatinine

Dates of use: 2004-2007

Creatinine is measured using the Jaffe rate reaction on the Beckman Synchron CX3 Clinical System. In this method, creatinine reacts with picrate in an alkaline solution to form a red creatinine-picrate complex. The rate of the color development 25.6 seconds after sample injection is measured at 520 nm and 560 nm. The rate difference between the two wavelengths is proportional to the concentration of creatinine. The laboratory CV for urine samples is 3.26% at a concentration of 17mg/dL and 2.6% at a concentration of 160mg/dL. The laboratory CV for serum samples is 6.10% at a concentration of 1.0mg/dL and 2.60% at a concentration of 6.0mg/dL.

Creatinine

Dates of Use: Acute Care Lab

Creatinine is measured in plasma or serum by rate reflectance spectrophotometry using thin film adaptation of the creatine amidinohydrolase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY 14650). The reference range in adult females is 0.4-1.1 mg/dL and in adult males is 0.5-1.2 mg/dL. The laboratory CV is 2.2%.

Creatinine

Dates of Use: 2006 - current

Creatinine is measured in serum or EDTA plasma by the Roche enzymatic method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry Analyzer. (Roche Diagnostics Corporation). In this enzymatic method, creatinine is converted to creatine by creatinase. Creatine is then acted upon by creatinase to form sarcosine and urea. The sarcosine is converted to hydrogen peroxide which reacts with a chromophore to produce a colored product that is measured colorimetrically. This method has the advantage over the Jaffe method in that it is not susceptible to interferences from no-creatinine chromogens. The method is calibrated using a National Institute of Standards and Technology (NIST) standard traceable to reference material SRM 909b (Isotope Dilution Mass Spectroscopy (IDMS)). The laboratory CV is 2.3%.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 150uL

Sample volume used: 4uL

Dead volume: 75uL

Cryopreservation of lymphocytes

Dates of use: 1999 - current

Isolation of mononuclear cells from whole blood is performed utilizing the 8 mL Vacutainer CPT (Cell Preparation Tube with Sodium Citrate) (Becton Dickinson and Company, Franklin Lakes, NJ). Centrifugation of the tube yields a layer of mononuclear cells that are transferred under sterile conditions for final separation and freezing steps. The final cell suspension is divided into cryovials that are placed in a controlled rate freezer to gradually lower the temperature of the suspension to -80°C in preparation for final storage in liquid nitrogen.

Sample type: whole blood collected in 8mL Vacutainer CPT

Minimum volume needed: 4mL collection; 8mL collection (1 or 2 tubes) (preferred) (yield is dependent on volume)

Sample volume used: n/a

Dead volume: none

Cystatin C

Dates of Use: 2008 - current

Cystatin C is determined nephelometrically in serum or plasma using the Dade Behring BN100 (Dade Behring Inc, Deerfield, IL 60060) nephelometer. A solution of polystyrene particles coated with antibodies specific to human cystatin C is incubated with diluted specimen. A reaction occurs between the bound antibody and cystatin C in the specimen, resulting in particle aggregation and an increase in light absorbance. The concentration of cystatin C of the test specimen is determined by comparing its absorbance change to that observed in a calibration curve. The reference range is 0.53 – 0.95 mg/L. Our inter-assay laboratory CV is 4.7%.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 300uL (unless sample is in 2mL vial, then 200uL)

Sample volume used: 30uL

Dead volume: 200uL (unless sample is in 2mL vial, then 100uL)

Cytomegalovirus (CMV) IgG

Dates of Use: Summer 2002 – Winter 2003

Serum IgG antibodies to cytomegalovirus (CMV) are detected using an indirect enzyme immunoassay (Diamedix Immunosimplicity[®] CMV IgG Test Kit, Diamedix Corporation, Miami, Florida). EU/mL (ELISA) of each specimen is determined by comparison to absorbance values obtained on predetermined standards. EU/mL of <8.0 is interpreted as negative for anti-CMV IgG; EU/mL of 8.0-9.9 obtained on two determinations is equivocal for anti-CMV IgG; and EU/mL of ≥ 10.0 is interpreted as positive for anti-CMV IgG. This is a semi-quantitative assay, so no CV is available.

Deoxypyridinoline (DPD)

Dates of Use: 2009 - current

Deoxypyridinoline, a bone marker assay, is measured in urine using the MicroVue DPD EIA kit from Quidel Corporation (San Diego, CA 92121). This kit measures DPD crosslinks by a competitive immunoassay. Briefly, samples or standards are incubated with DPD purified from bovine bone (conjugated to alkaline phosphatase) in microplate wells coated with murine monoclonal anti-DPD. The wells are washed, then a substrate solution containing p-nitrophenyl phosphate is added and color in the wells develops in inverse proportion to the amount of DPD in the sample. The color is read on a SpectraMax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA 94089). The concentration of DPD in the sample is determined by preparing a standard curve from standards of known concentration, and the concentration is adjusted for variation in urine concentration by dividing the result by the creatinine concentration of the sample. Our laboratory inter-assay CV on 5 samples is 0.9% at a mean concentration of 14.0 nmol/L, and 8.5% at a mean concentration of 106.7 nmol/L. The kit insert reports inter-assay CVs of 8.4% at mean concentration 10.7 nmol/L, 4.3% at mean concentration 30.0 nmol/L, and 5.5% at mean concentration 174.7 nmol/L.

Sample type: Urine

Minimum volume needed: 125uL

Sample volume used: 20uL

Dead volume: 50uL

DNA Isolation

Dates of use: 1994 - current

DNA is isolated from whole blood or packed cells that are frozen at -70°C . The DNA extraction and purification method uses sodium dodecylsulfate cell lysis followed by a salt precipitation method for protein removal using commercial Puregene® reagents (formerly Gentra Systems, Inc., Minneapolis, MN 55447; currently Qiagen Instrument Service, Germantown, MD 20874). DNA is quantitated using the NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitation by Picogreen analysis (Molecular Probes, Eugene, OR) is also available. A mean yield of 200 μg or 40 μg DNA/mL packed cell is obtained, and DNA is of high quality (mean purity $A_{260}/A_{280}=1.77$) and high molecular weight as determined by gel electrophoresis.

Sample type: Whole blood collected in ACD, EDTA or heparin tubes; frozen packed cells from EDTA or Na citrate tubes; buffy coats and saliva or other body fluids are also acceptable but may give lower yields

Minimum volume needed: 3-10 mL whole blood; 2-5 mL packed cells

(soluble) E-Selectin

Dates of use: 2004 - 2005

sE-Selectin (soluble E-Selectin) is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Parameter kit from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 5.7-8.8% for the serum or plasma assay. The inter-assay CV range in our laboratory is 8.5%.

(soluble) E-Selectin

Dates of use: 2006 - current

sE-Selectin (soluble E-Selectin) is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Quantikine kit from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 7.3-8.7% for the serum or plasma assay.

Sample type: Serum/heparin plasma/citrate plasma (EDTA plasma not recommended because sE-Selectin is a Ca²⁺-dependent molecule)

Minimum volume needed: 125uL

Sample volume used: 20uL

Dead volume: 50uL

Electrolytes

Dates of use: 2006 - current

Sodium, potassium, and chloride electrolytes are measured in serum or plasma by ion-selective electrode (ISE) method on the Roche Modular P (Mod P) Chemistry analyzer (Roche Diagnostics Corporation). A dilution of the sample is prepared by the analyzer and aspirated into the electrode chamber. The chamber consists of three separate ion selective membrane cartridges, sandwiched in sequence. Each membrane is composed of a material that preferentially attracts one of the three electrolytes. When the specimen ions accumulate on one side of the membrane, and the internal reference ions accumulate on the other side of the membrane, an electrical potential (EMF) develops across the membrane. The EMF is proportional to the concentration of the electrolytes in the specimen. The EMF value is compared to those obtained from the daily calibration, and the concentration is reported. The Mod P has two identical ISE modules. They are typically run simultaneously; and if one fails, the other can serve as a backup. The laboratory interassay CVs for sodium, potassium, and chloride are 1.0%.

Sample type: Serum/lithium-heparin plasma

Minimum volume needed: 150uL

Sample volume used: 15uL

Dead volume: 75uL

Endothelin-1 (ET-1)

Dates of use: 2004 - current

ET-1 is measured in serum or EDTA plasma using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) QuantiGlo kit from R & D Systems (Minneapolis, MN). A monoclonal antibody specific for ET-1 is pre-coated onto a microplate. Any ET-1 present in the sample is bound by the immobilized antibody. After washing, an enzyme-linked monoclonal antibody specific for ET-1 is added and binds to the immobilized, bound ET-1. Following a wash, an enhanced luminol/peroxide substrate solution is added and light is produced in proportion to the amount of ET-1 bound in the initial step. The intensity of the light is measured on a luminometer (BioTek Synergy HT Multi-Detection Microplate Reader, BioTek, Winooski, VT). The inter-assay CV range reported in the kit insert is 8.9 to 4.6% at concentrations ranging from 1.76 to 86.8 pg/mL.

Sample type: Serum/EDTA plasma (heparin plasma and citrate plasma not recommended)

Minimum volume needed: 200uL

Sample volume used: 100uL

Dead volume: 50uL

Estradiol

Dates of Use: Proposed

Estradiol is measured in Serum or EDTA plasma using the Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN). In the Elecsys immunoassay, the patient sample reacts with a biotinylated monoclonal estradiol-specific antibody and a monoclonal estradiol-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of estradiol in the sample. This method has been standardized via isotope dilution-gas chromatography/mass spectrometry (ID-GC/MS). The inter-assay CV in our laboratory is 6.2% at a mean concentration of 34.8 pg/mL, 2.3% at a mean concentration of 132 pg/mL, and 3.6% at a mean concentration of 3009 pg/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 250uL

Sample volume used: 35uL

Dead volume: 200uL

Factor VIII

Dates of use: Coagulation Lab

Factor VIII is measured on the Stago STA-R Instrument (Diagnostica Stago, Parsippany, NJ 07054) and employs the principle whereby the clotting time of patient plasma is compared to the clotting times of standard plasma that has a known factor VIII activity. Clotting times for these plasmas are determined using an activated partial thromboplastin time (APTT) reagent for factor VIII. The laboratory CV is 5.2%.

Fatty Acid Profile, Plasma

Dates of use: 1990 - current

Fatty acids are found free and in the phospholipid, cholesterol ester and triglyceride fractions within lipoprotein particles in plasma. For the extraction of plasma phospholipid fatty acids from plasma, the method previously described by Cao et al is used (Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clin Chem;52:2265-72). In summary, 0.3 mL of EDTA plasma is mixed with 0.7 volume of 0.9% saline and 50 uL of 2 g/L 17:0 standard (diheptadecanoyl phosphatidylcholine) is added to monitor the extraction efficiency. Lipids are extracted from plasma with a mixture of chloroform:methanol (2:1, v/v), and cholesterol, triglycerides and phospholipid subclasses are separated on a silica thin-layer chromatography (TLC) plate in a solvent mixture of petroleum ether, diethyl ether, and glacial acetic acid (80:20:1, v/v/v). The phospholipids stay at the origin during TLC whereas the cholesterol esters, being less polar, migrate with the solvent front. The band of phospholipids is harvested for the formation of methyl esters. Fatty acid methyl esters are prepared with 1.5 mL of 14% boron trifluoride in methanol, incubated at 80°C for 90 minutes, and extracted with petroleum ether. The final product is dissolved in heptane and injected onto a capillary Varian CP7420 100-m column with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a HP6890A autosampler. The GC is configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation software. Adequate separation of fatty acid methylesters is obtained over a 50-min period with an initial temperature of 190°C for 25 minutes. The temperature is increased to 240°C at a rate of 2°C/min and held for 5 minutes. Fatty acid methylesters from 12:0 through 24:1n9 are separated, identified and expressed as percent of total. The following CVs were obtained on 20 blind duplicates: linoleic acid (LA), 2.6%; alpha-linolenic acid (ALA), 2.4%; arachidonic acid (AA), 2.4%; eicosapentaenoic acid (EPA), 3.3%; docosapentaenoic acid (DPA), 2.9% and docosahexaenoic acid (DHA), 2.7%.

Sample type: EDTA plasma

Minimum volume needed: 300uL

Sample volume used: 200-300uL

Dead volume: none

Fatty Acid Profile, Whole blood

Dates of use: 1990 - current

Fatty acid profile is measured in EDTA anticoagulated whole blood that has been frozen at -70°C using the method previously described by Cao et al (Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clin Chem;52:2265-72). The erythrocytes are washed with cold distilled water and centrifuged at 20,000g at 10 °C for 20 min to form a tight pellet. Supernatant is removed and the above procedure is repeated 4 times until supernatant is clear. Erythrocyte ghosts are brought to a volume of 2 mL with distilled water and gently sonicated on ice. Lipids are extracted from the erythrocyte membranes with a mixture of chloroform:methanol (2:1, v/v), and 25 µL of 2 g/L 17:0 standard (diheptadecanoyl phosphatidylcholine) is added to the filtered chloroform to monitor the extraction efficiency. Samples with <60% recovery are considered unacceptable. Tubes of chloroform are evaporated to dryness under nitrogen for the formation of methylesters. Fatty acid methyl esters are prepared with 1.5 mL of 14% boron trifluoride in methanol, incubated at 80 °C for 90 minutes, and extracted with petroleum ether. The final product is dissolved in heptane and injected onto a capillary Varian CP7420 100-m column with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a HP6890A autosampler. The GC is configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation software. Adequate separation of fatty acid methylesters is obtained over a 50-min period with an initial temperature of 190 °C for 25 minutes. The temperature is increased to 240 °C at a rate of 2 °C/min and held for 5 minutes. Fatty acid methylesters from 12:0 through 24:1n9 are separated, identified and expressed as percent of total. The following CVs were obtained on 20 blind duplicates: linoleic acid (LA), 2.6%; ALA, 2.4%; arachidonic acid (AA), 2.4%; EPA, 3.3%; docosapentaenoic acid (DPA), 2.9% and DHA, 2.7%.

Sample type: whole blood

Minimum volume needed: 500uL

Sample volume used: 500uL

Dead volume: none

Fatty Acids, Prep for RBC membrane

Dates of use: 2002 - 2005

Red blood cells are separated from EDTA plasma at the Field Centers, an equal volume of acid citrate-dextrose preservative solution is added, and the mixture is sent to the Collaborative Studies Clinical Laboratory for fatty acid analysis. The RBC are lysed, the fatty acids are extracted from the RBC ghosts and quantitated by gas-liquid chromatography. Quantitation is achieved by measuring peak area relative to an internal standard. The fatty acid profile includes approximately 29 fatty acids from 12:0 through 24:1ω9. Our laboratory CV is 6% for most fatty acids analyzed.

Ferritin

Dates of use: 2006-current

Ferritin is measured in serum or plasma using Roche reagents on the Roche/Hitachi Modular P instrument (Roche Diagnostics, Indianapolis IN). This is a turbidimetric antibody method in which ferritin antibody bound to latex forms an antigen-antibody complex with ferritin in the sample. Turbidity measured at 700 nm is directly proportional to the concentration of ferritin. The laboratory CV for this assay is 9.9% at 42.2 ng/mL and 13.8% at 51.2 ng/mL.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 150uL

Sample volume used: 4uL

Dead volume: 75uL

Fetuin-A

Dates of use: proposed

Fetuin-A is measured in serum, plasma or urine using the two-site sandwich technique of the Fetuin-A ELISA kit from ALPCO Diagnostics (Salem, NH). A polyclonal goat anti-human Fetuin-A antibody is pre-coated onto a microplate. Any Fetuin-A present in the sample is bound by the immobilized antibody. After washing, a horseradish peroxidase (HRP) conjugated polyclonal anti-human Fetuin-A antibody is added and binds to the immobilized, bound Fetuin-A. Following a wash, a substrate solution is added and color develops in proportion to the amount of Fetuin-A bound in the initial step. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 6.8% at a concentration 32.4 ng/mL and 5.7% at a concentration of 123.7 ng/mL.

Sample type: Serum/plasma/urine

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Fibrinogen

Dates of use: Coagulation Lab

Fibrinogen is measured using the Clauss clotting method, and the clot is detected using electromagnetic viscosity detection on the Stago STA-R Instrument (Diagnostica Stago, Parsippany, NJ 07054). The laboratory CV is <5.2%.

Fibroblast Growth Factor 21 (FGF-21)

Dates of use: Proposed

FGF-21 is measured in serum or plasma using an enzyme-linked immunosorbent assay (ELISA) (Human FGF-21 ELISA, BioVendorUSA, Candler, NC 28715). A sample containing FGF-21 is incubated in a microtiter well coated with polyclonal anti-human FGF-21 antibody; any FGF-21 contained in the sample is immunologically bound to the well. After washing, biotin-labelled polyclonal anti-human FGF-21 antibody is added and incubated. After a second wash, streptavidin-HRP conjugate is added and incubated. After a final wash, tetramethylbenzidine substrate solution is added, and color develops in proportion to the concentration of FGF-21 in the sample. This color is detected in a spectrophotometric plate reader and concentration is determined from a standard curve. The inter-assay CV, as reported in the kit insert, is 3.9%.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma

Minimum volume needed: 175uL

Sample volume used: 75uL

Dead volume: 50uL

Fibroblast Growth Factor 23 (FGF 23) C-terminus

Dates of use: 2009 - current

FGF-23 is measured in EDTA anticoagulated plasma using a two site enzyme-linked immunosorbent assay (ELISA) (Human FGF-23 (C-Term) ELISA kit (2nd generation), Immotopics, Inc., San Clemente, CA 92673). A sample containing FGF-23 is incubated in a microtiter well simultaneously with two antibodies that recognize epitopes of the C-terminal portion of FGF-23: a biotinylated capture antibody and an HRP-conjugated detection antibody. FGF-23 contained in the sample is immunologically bound by the capture antibody and the detection antibody to form a sandwich complex. After washing, the immobilized sandwich complex is incubated with substrate solution and measured in a spectrophotometric plate reader and concentration is determined from a standard curve. The reported inter-assay CV is 4.7%.

Sample type: EDTA plasma (serum not recommended “due to variable lability of the molecule”)

Minimum volume needed: 200uL

Sample volume used: 100uL

Dead volume: 50uL

Fibroblast Growth Factor 23 (FGF-23) Intact

Dates of use: 2009 - current

FGF-23 is measured in EDTA anticoagulated plasma using a two site enzyme-linked immunosorbent assay (ELISA) (Human Intact FGF-23 ELISA kit, Immotopics, Inc., San Clemente, CA 92673). A sample containing FGF-23 is incubated simultaneously with the immobilized capture antibody and the HRP conjugated detection antibody in a microtiter well. Intact FGF-23 contained in the sample is immunologically bound by the capture antibody and the detection antibody to form a sandwich complex. After washing, the immobilized sandwich complex is incubated with substrate solution and measured in a spectrophotometric plate reader and concentration is determined from a standard curve. The inter-assay CV is 6.1%.

Note: specimens from end-stage kidney disease patients have very high FGF-23 concentrations and will not dilute out accurately. Immotopics said specimens from these types should be assayed using the C-terminus kit.

Sample type: EDTA plasma (serum not recommended “due to variable lability of the molecule”)

Minimum volume needed: 250uL

Sample volume used: 150uL

Dead volume: 50uL

Folate

Dates of use: 2002 – 2004

Folate was measured in serum on the Hitachi 911 (Roche Diagnostics, Indianapolis, IN 46250) using the CEDIA[®] Folate enzyme immunoassay (Boehringer Mannheim).

Folate

Dates of use: 2007 – current

The ADVIA[®] Centaur (Siemens Diagnostics, Deerfield, IL) Folate assay is a competitive immunoassay using direct chemiluminescent technology. Folate in the patient sample competes with acridinium ester-labeled folate in the LITE Reagent for a limited amount of biotin-labeled folate-binding protein. Biotin-labeled folate binding protein binds to avidin which is covalently coupled to paramagnetic particles in the Solid Phase. In the ADVIA[®] Centaur Folate assay, the sample is pretreated to release the folate from endogenous binding proteins.

Sample type: Serum

Minimum volume needed: 375uL

Sample volume used: 170uL

Dead volume: 150uL

Fructosamine

Dates of use: 2009 - current

Fructosamine is measured in serum or plasma on the Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a colorimetric assay based on the ability of ketoamines to reduce nitrotetrazolium-blue (NBT) to formazan in an alkaline solution. The rate of formation of formazan is directly proportional to the concentration of fructosamine. Uricase serves to eliminate uric acid interference and detergent eliminates matrix effects. The rate of reaction is measured photometrically at 546 nm. The laboratory inter-assay CV is 3%.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 150uL

Sample volume used: 13uL

Dead volume: 75uL

Gadolinium

Dates of use: 2009 - current

Gadolinium-DTPA is measured in serum or urine using the FIT-GFR Gd-DTPA kit (BioPAL, Inc. Worcester, MA). Following intravenous administration of Gd-DTPA, sequential blood and urine samples are collected from a subject, Gd-DTPA concentrations are measured, and GFR is calculated. Sample containing gadolinium is incubated with an HRP-conjugated Gd-DTPA antibody and rabbit anti-Gd-DTPA antibody (antiserum). The Gd-DTPA in the sample and the antibody compete for binding sites of the goat anti-rabbit Gd-DTPA coated microplate wells. After washing, the bound antibody complexes are incubated with substrate solution and the color of the reaction is measured in a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) and concentration is determined from a standard curve. The laboratory inter-assay CV is <20%.

Sample type: Serum/urine (EDTA plasma cannot be used because DTPA is also a chelating agent)

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Ghrelin

Dates of use: 2008 - current

Ghrelin is measured in serum or plasma using the sandwich ELISA technique of the Human Ghrelin (total) ELISA kit from Millipore (St Charles, MO). Our inter-assay laboratory CV is 12.4% at a mean concentration of 310 pg/mL and 7.4 at a mean concentration of 1861pg/mL.

Sample type: Serum/EDTA plasma/heparin plasma

Note: Active ghrelin is very unstable. Samples should be processed quickly and on ice. Pefabloc or AEBSF may be added to blood collection tubes and the serum or plasma acidified with addition of hydrochloric acid. Untreated samples may exhibit ~30% less total ghrelin than treated samples.

Minimum volume needed: 125uL

Sample volume used: 20uL

Dead volume: 50uL

Glucose

Dates of use: Acute Care Lab

Serum glucose is measured by rate reflectance spectrophotometry using thin film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY 14650) at the Collaborative Studies Clinical Laboratory at Fairview University Medical Center (Minneapolis, MN). The adult reference range is 60-115 mg/dL. The laboratory CV is 1.1%.

Glucose

Dates of use: 2006 - current

Glucose is measured in serum or plasma by the Roche hexokinase method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation). In this enzymatic method, glucose is converted to glucose-6-phosphate by hexokinase in the presence of ATP. Glucose-6-phosphate dehydrogenase then converts the G-6-P to gluconate-6-P in the presence of NADP. The resulting increase in absorbance as NADP is reduced to NADPH is measured at 340 nm. The method is calibrated against Standard Reference Material 965a from National Institute of Standards and Technology (NIST) traceable to the NIST definitive method for glucose (Isotope Dilution Mass Spectroscopy (IDMS)). The laboratory CV is 1.6%

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 150uL

Sample volume used: 2uL

Dead volume: 75uL

Glucagon-like Peptide

Dates of Use: Proposed

Biologically active glucagon-like peptide-1 (GLP-1) (7–36, 7–37) will be assayed using the Glucagon-Like Peptide-1 (Active) ELISA kit from Millipore/Linco Research (Billerica, MA). Blood samples should be collected with a DPP-IV inhibitor. This assay uses monoclonal capture antibody that binds specifically to the N-terminal region of active GLP-1 and an anti GLP-1-alkaline phosphatase detection conjugate that binds to the captured GLP-1; quantification of bound detection conjugate is determined by adding methyl umbelliferyl phosphate, which in the presence of alkaline phosphatase forms the fluorescent product umbelliferone. The fluorescence is quantified using a BioTek Synergy HT plate reader (BioTek Instruments, Inc; Winooski, VT). . This assay exhibits 100% cross-reactivity with both GLP-1 (7-36 amide) and GLP-1 (7-37). The interassay CVs reported for this method range from 7-13%.

*Sample type: Plasma**Minimum volume needed: 200uL**Sample volume used: 100uL**Dead volume: 50uL***HDL-Cholesterol**

Dates of use: 2006 - current

HDL-cholesterol is measured in EDTA plasma or serum using the cholesterol oxidase cholesterol method (Roche Diagnostics) after precipitation of non-HDL-cholesterol with magnesium/dextran. This method is standardized as described for the cholesterol assay; and calibration of the assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends reference range of >40 mg/dL. The laboratory CV is 2.9%.

*Sample type: EDTA plasma (preferred)/serum**Minimum volume needed: 525uL**Sample volume used: 500uL**Dead volume: none*

HDL-Cholesterol, Direct

Dates of Use: 2006 - current

HDL-cholesterol is measured directly in serum or EDTA plasma using the Roche HDL-Cholesterol 3rd generation direct method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation). In this method, a magnesium/dextran sulfate solution is first added to the specimen to form water-soluble complexes with non-HDL cholesterol fractions. HDL-cholesterol esters are converted to HDL-cholesterol by PEG-cholesterol esterase. The HDL-cholesterol is acted upon by PEG-cholesterol oxidase, and the hydrogen peroxide produced from this reaction combines with 4-aminoantipyrine and HSDA under the action of peroxidase to form a purple/blue pigment that is measured at 600nm. When the cholesterol measuring enzymes are modified with PEG, they are preferentially more reactive with HDL-cholesterol than the other cholesterol fractions, thus the reaction is specific for HDL-cholesterol. This method is standardized against the designated CDC reference method; and calibration of the assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends reference range of >40 mg/dL. The laboratory CV is 2.9%.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 150uL

Sample volume used: 2uL

Dead volume: 75uL

HDL Subfractions

Dates of Use: 2002 – 2004

HDL subfraction concentrations are determined in plasma using the Lipoprint HDL System (Quantimetrix Corporation, Redondo Beach, CA). The HDL subfractions are separated by high resolution polyacrylamide gradient gel electrophoresis on the basis of their molecular size. The gels are scanned by a densitometer, and a public domain software program (NIH Image Version 1.62) identifies 8 major HDL subfractions according to their migration distance (Rf) relative to the albumin fraction. The percent and concentration (mg/dL) of each fraction is calculated. The laboratory CVs for the 8 major fractions range from 6.3 to 21.8%. The software also allows for the option of isolating three HDL subfractions. Laboratory CVs for the three fractions are 9.2, 7.2 and 17.4%, respectively.

Heat Shock Protein 60, Anti Human (Hsp60)

Dates of Use: 2003 - 2004

Anti-human Hsp60 antibodies (IgG, IgA and IgM) are measured by an ELISA assay (StressXpress™ Anti-Human Hsp60 (total) ELISA Kit; Stressgen, Victoria, BC, Canada). Anti-human Hsp60 antibodies bind to recombinant human Hsp60 coated to the solid phase. The captured anti-human Hsp60 antibodies are detected with hydrogen peroxidase conjugated goat polyclonal antibody specific for human IgG, IgA and IgM antibodies. The reported kit sensitivity is 2.88 ng/mL. The laboratory CV for this assay is 18.8%.

Helicobacter pylori (H. pylori) IgG

Dates of Use: 2002 – 2004

Serum IgG antibodies to *H. pylori* antigen are detected using an indirect enzyme immunoassay (Diamedix Immunosimplicity® *H. pylori* IgG Test Kit, Diamedix Corporation, Miami, Florida). The Index Value of each specimen is determined by comparison to absorbance value obtained on a cut-off calibrator. Index Value of <0.90 is interpreted as no detectable antibodies to *H. pylori*; Index Value of 0.90 - 1.09 obtained on two determinations is equivocal for IgG antibodies to *H. pylori*; and Index Value of ≥ 1.10 is interpreted as positive for *H. pylori* antibody. This is a semi-quantitative assay, so no CV is available.

Helicobacter pylori (H. pylori) IgG

Dates of use: 2008 - current

Helicobacter pylori (*H. pylori*) IgG antibodies are detected in serum or plasma using the HpG Screen ELISA kit (ALPCO Diagnostics, Salem, NH). Diluted serum samples are incubated with partially purified *H. pylori* antigens immobilized on microtiter wells. Rabbit anti-human IgG conjugated to horseradish peroxidase is added to the wells and binds to surface-bound antibodies in the second incubation. After unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of stop solution terminates the reaction and provides the appropriate pH for color development. The optical densities of the standards, controls and samples are measured using a microplate reader at 450nm. Optical density is directly proportional to antibody in the sample. This is a semi-quantitative assay, so no CV is available.

Sample type: Serum/plasma

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Helicobacter pylori (H. pylori) CagA IgG (see CagA IgG)

See entry "CagA IgG."

Hematocrit

Dates of use: Hematology Lab

Hematocrit is determined by an automated method on the Coulter LH 750 analyzer (Beckman Coulter, Inc. Fullerton, CA 92834). Within the LH750, each cell is suspended in an isotonic electrolyte solution (LH 700 series diluent). An electrical pulse is generated when a cell passes through an aperture in the analyzer. These electrical pulses can be counted and sized. Hematocrit is a calculated value, so no laboratory CV is available.

Hemoglobin, free

Dates of use: Acute Care

Free hemoglobin (plasma hemoglobin) is measured in plasma on the Vitros Analyzer (Johnson and Johnson Clinical Diagnostics, Inc., Rochester, NY). Absorbance of the free hemoglobin is measured against an oxyhemoglobin standard solution at 575 nm. The laboratory CV is <10%.

Hemoglobin, glycated (HbA_{1c})

Dates of use: 2007 - current

HbA_{1c} is measured in EDTA whole blood on the Tosoh HPLC G7 Glycohemoglobin Analyzer (Tosoh Medics, Inc., San Francisco CA 94080) using an automated high performance liquid chromatography method. This method is calibrated utilizing standard values derived by the National Glycohemoglobin Standardization Program (NGSP). Reference range is 4.3-6.0% with a laboratory CV range of 1.4-1.9%.

Sample type: EDTA-anticoagulated whole blood

Minimum volume needed: 50uL (1mL in a 3mL vacutainer preferred)

Sample volume used: 5uL

Dead volume: 1mL

Hemoglobin, glycated (HbA_{1c}) from Prep vials

Dates of use: 2007 - current

HbA_{1c} is measured in EDTA blood that is diluted at the Field Centers in a sample preparation vial containing an aqueous solution of EDTA and potassium cyanide (HbA_{1c} Sample Preparation Kit, Bio-Rad, Hercules, CA 94547). Specimen from the prep vial is analyzed on the Tosoh A_{1c} 2.2 Plus Glycohemoglobin Analyzer (Tosoh Medics, Inc., San Francisco, CA 94080) or currently the Tosoh G-7 using an automated high performance liquid chromatography method. Reference range is 4.3-6.0% with a laboratory CV range of 1.4-1.9%.

Sample type: EDTA-anticoagulated whole blood

Minimum volume needed: 300uL

Sample volume used: 5uL

Dead volume: 300uL

Hepatitis A antibodies (HAVAB)

Dates of use: 2002 – 2003

Total serum antibodies to hepatitis A virus (anti-HAV) were detected using the IM[®]x HAVAB qualitative microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL). The rate of each specimen is determined and compared to a cutoff rate obtained on each run by a calibrator, as described by the manufacturer. Values greater than the Cutoff rate are considered nonreactive by the criteria of the IMx HAVAB assay; values less than the Cutoff rate are considered reactive by the criteria of the IMx HAVAB assay. This is a semi-quantitative assay, so no CV is available.

Hepatitis A virus, total antibodies

Dates of use: 2009 – current

Hepatitis A virus antibodies are measure in serum or plasma. The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) HAV Total assay is a fully automated, competitive immunoassay using direct, chemiluminescent technology. The assay consists of three reagent addition and incubation steps. First, the sample is pretreated with Ancillary Reagent containing cysteine. Next, HAV antigen is added from the ancillary well (Antigen Reagent). Lite Reagent and Solid Phase are then added. The Lite Reagent contains monoclonal mouse antibody to HAV antigen labeled with acridinium ester and biotinylated Fab fragment of a monoclonal mouse antibody to HAV antigen. The Solid Phase contains streptavidin covalently coupled to paramagnetic particles. After the final incubation, the immuno-complex formed is washed with Wash 1 prior to initiation of the chemiluminescent reaction.

Sample type: Serum/EDTA plasma/lithium-heparin plasma/sodium-heparin plasma

Minimum volume needed: 150uL

Sample volume used: 30uL

Dead volume: 50uL

Hepatitis B core antigen, total antibodies

Dates of use: 2009 - current

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) HBc Total assay is a two wash antigen sandwich immunoassay in which antigens are bridged by antibody measured in serum or plasma. The Solid Phase contains a preformed complex of streptavidin coated microparticles and biotinylated recombinant HBc antigen and is used to capture anti-HBc in the patient sample. The Lite Reagent contains recombinant HBc antigen labeled with acridinium ester and is used to detect anti-HBc in the sample. Solid Phase and Chaotrope Reagent are added to the sample, followed by Ancillary Reagent and Lite Reagent. Antibody-antigen complexes will form if anti-HBc antibodies (IgM and IgG) are present in the sample.

Sample type: Serum/EDTA plasma/lithium-heparin plasma/sodium-heparin plasma

Minimum volume needed: 175uL

Sample volume used: 60uL

Dead volume: 50uL

Hepatitis B surface antigen

Dates of use: 2009 – current

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) HBsAg assay is a sandwich immunoassay using direct, chemiluminometric technology measured in serum or plasma. Non-magnetic latex particles are added from the ancillary well. The Lite Reagent, packaged in a ReadyPack ancillary reagent pack, contains a biotinylated anti-HBs mouse monoclonal capture antibody and an acridinium-ester labeled anti-HBs mouse monoclonal antibody. HBsAg in the sample complexes with the antibodies and streptavidin-coated magnetic latex particles in the Solid Phase capture the HBsAg-antibody complexes.

Sample type: Serum/EDTA plasma/lithium-heparin plasma/sodium-heparin plasma

Minimum volume needed: 270uL

Sample volume used: 120uL

Dead volume: 150uL

Hepatitis B surface antigen, antibodies

Dates of use: 2009 – current

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) Anti-HBs assay is a sandwich immunoassay using direct, chemiluminometric technology measured in serum or plasma. Purified human sourced HBsAg (subtypes Ad and Ay) are covalently coupled to magnetic latex particles in the Solid Phase. In the Lite reagent, the purified HBsAg (subtypes Ad and Ay) is labeled with acridinium ester. Non-magnetic latex particles are added from the ancillary well. The sample is incubated simultaneously with Lite Reagent, Solid Phase and Ancillary Reagent. Antibody-antigen complexes form if anti-HBs are present in the sample.

Sample type: Serum (preferred)/EDTA plasma (preferred)/sodium- or lithium-heparin plasma

Minimum volume needed: 225uL

Sample volume used: 110uL

Dead volume: 50uL

Hepatitis C virus IgG (HCV)

Dates of use: 2009 – current

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) HCV assay is an indirect two wash sandwich immunoassay measure in serum or plasma. The sample is incubated with Solid Phase containing recombinant and synthetic peptide HCV antigens. Antigen-antibody complexes will form if anti-HCV antibody is present in the sample. Lite Reagent containing monoclonal anti-human IgG labeled with acridinium ester is used to detect anti-HCV IgG in the sample.

Sample type: Serum/EDTA plasma/lithium-heparin plasma/sodium-heparin plasma

Minimum volume needed: 125uL

Sample volume used: 20uL

Dead volume: 50uL

Hepcidin

Dates of Use: Proposed

Hepcidin is measured in Serum or EDTA plasma using the Hepcidin ELISA from DRG International (Mountainside, NJ). This is a competitive immunoassay that uses a monoclonal antibody directed towards an antigenic site of the bioactive Hepcidin 25 molecule. Hepcidin in the sample competes with a hepcidin-biotin conjugate for binding sites of the capture antibody. Sample quantitation is accomplished by the use of a streptavidin-peroxidase solution and tetramethylbenzidine. The optical density of the wells is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) at 450nm. The reported CVs for this assay range are 7.4% at a mean concentration of 34.3 ng/mL and 11.4% at a mean concentration of 6.1 ng/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 100uL

Sample volume used: 10uL

Dead volume: 50uL

Herpes simplex Virus (HSV) IgG

Dates of use: 2002 – 2003

Serum IgG antibodies to herpes simplex virus (HSV) type 1 and type 2 are detected using an indirect enzyme immunoassay (Diamedix Immun simplicity[®] HSV 1 & 2 Test Kit, Diamedix Corporation, Miami, Florida). EU/mL (ELISA) of each specimen is determined by comparison to absorbance values obtained on a predetermined calibrator. EU/mL of <16.0 is interpreted as negative for anti-HSV 1 & 2 IgG; EU/mL of 16.0-19.9 EU/mL obtained on two determinations is equivocal for anti-HSV 1 & 2 IgG; and ≥20.0 EU/mL is interpreted as positive for anti-HSV 1 & 2 IgG. This is a semi-quantitative assay, so no CV is available.

Homocysteine, total (tHcy)

Dates of use: Biochemical Genetics

Plasma tHcy is measured by a fluorescence polarization immunoassay (IMx Homocysteine Assay, Axis Biochemicals ASA, Oslo, Norway) using the IMx Analyzer (Abbott Diagnostics, 100 Abbott Park Rd, Abbott Park, Illinois 60064). The method is based on the enzymatic conversion of free homocysteine to S-adenosyl-L-homocysteine, which is subsequently detected by a competitive immunoassay. The assay range is 0.5-50 umol/L with a laboratory CV range of 3.8 – 5.1%. The reference range on fasting plasma is 4.0 - 12.0 μmol/L.

Homocysteine, total (tHcy)

Dates of use: Biochemical Genetics

tHcy is measured in serum or plasma using Liquid chromatography/tandem mass spectrometry (Water's 2795 Liquid Chromatogram equipped with an autosampler and column oven). Samples are injected into a Rapa/Tacr column and, using post column splitting, into the ionization source of a Micromass Quattro tandem mass spectrometer. Data is obtained using the MassLynx software with automated data processing by QuanLynx software. The laboratory CV at 7.93 is 6.67% and at 22.0 is 3.96%.

Sample type: EDTA plasma (preferred)/lithium-heparin plasma/sodium-heparin plasma/serum (if normal range specified for serum)

Minimum volume needed: 200uL

Sample volume used: 100uL

Dead volume: 50uL

Hyaluronic acid

Dates of use: 2007

Hyaluronic acid is measured using the enzyme-linked binding protein assay of the Hyaluronic acid (HA) test kit (Corgenix Inc., Westminster, CO). The kit insert reports inter-assay CVs of 5.7-7.0%.

Insulin

Dates of Use: 2002 – 2003

Insulin is determined in serum by an immunoenzymatic sandwich assay using Access[®] Ultrasensitive Insulin Reagent Packs on the Access[®] Immunoassay System (Beckman Instruments, Inc.). The assay range is 0.03-300 mU/L with a laboratory CV of 4.9%. The reference range on fasting serum is <20 mU/L.

Insulin

Dates of Use: 2004 and prior

Insulin is determined by a radioimmunoassay method using the Linco Human Insulin Specific RIA Kit (Linco Research, Inc., St. Charles, MO 63304). This assay utilizes ¹²⁵I-labeled Human Insulin and a Human Insulin antiserum to determine the level of insulin. The lower limit of sensitivity is 2 U/L with a laboratory CV of 4.9%. The reference range on fasting serum is <20 mU/L.

Insulin

Dates of Use: 2007 - 2009

Insulin is measured in serum using a solid phase two site enzyme-linked immunosorbent assay (ELISA) from Mercodia (Uppsala, Sweden). It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. Specimen, control, or standard is pipetted into the sample well followed by the addition of peroxidase-conjugated anti-insulin antibodies. During incubation, insulin present in the sample binds to anti-insulin antibodies bound to the sample well, while the peroxidase-conjugated anti-insulin antibodies will also bind to the insulin at the same time. After washing to remove unbound enzyme-labeled antibodies, 3,3',5,5'-tetramethylbenzidine (TMB)-labeled substrate is added and binds to the conjugated antibodies. Acid is added to the sample well to stop the reaction, and the colorimetric endpoint is read on a microplate spectrophotometer set to the appropriate light wavelength. The Mercodia Insulin ELISA kit is calibrated against 1st International Reference Preparation 66/304. The laboratory CV is 8.4%.

Insulin

Dates of Use: 2009 - current

Insulin is measured in serum or plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample reacts with a biotinylated monoclonal insulin-specific antibody and a monoclonal insulin-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of insulin in the sample. The method has been standardized using the 1st IRP WHO Reference Standard 66/304 (NIBSC). The laboratory CV is 6.2% at a level of 19.2 μU/mL and 5.2% at a level of 173.3 μU/mL.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 275uL

Sample volume used: 20uL

Dead volume: 200uL

Insulin-Like Growth Factor 1 (IGF-1)

Dates of Use: Proposed

IGF-1 (insulin-like growth factor 1) is measured in serum or plasma by a quantitative sandwich immunoassay technique using the enzyme-linked immunosorbent assay (ELISA)

Quantikine® Human IGF-1 Kit from R & D Systems (Minneapolis, MN). Serum or plasma samples are pretreated to release IGF-1 from binding proteins prior to being analyzed by the ELISA technique. Color developed in proportion to the amount of IGF-1 bound in the ELISA assay is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA). Inter-assay CV is reported to be <8.3% at various levels of samples. Reference range reported by R & D Systems using this kit is 40-258 ng/mL in serum and 40-174 ng/mL in EDTA plasma.

Sample type: Serum/EDTA plasma/heparin plasma (citrate plasma not recommended)

Minimum volume needed: 125uL

Sample volume used: 20uL

Dead volume: 50uL

Insulin-Like Growth Factor Binding Protein 2 (IGFBP-2)

Dates of Use: Proposed

IGF-binding protein 2 (IGFBP-2) is measured in serum or EDTA plasma using the Active IGFBP-2 ELISA kit from Diagnostic Systems Laboratory (Webster, TX) (now owned by Beckman Coulter). In the assay, standards, controls and samples are incubated with anti-IGFBP-2 antibody in plate wells that have been coated with anti-IGFBP-2 antibody. After incubation and washing, tetramethylbenzidine is added to the wells. An acidic stop solution terminates the reaction. The optical density of each well is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) at 450nm and 620nm. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of IGFBP-2 in the sample and concentration is determined from a standard curve. The interassay CVs are 5.7% at a mean concentration of 1.3 ng/mL, 3.4% at a mean concentration of 3.1 ng/mL and 2.5% at a mean concentration of 6.3 ng/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 100uL

Sample volume used: 10uL

Dead volume: 50uL

Insulin-Like Growth Factor Binding Protein 3 (IGFBP-3)

Dates of Use: Proposed

IGF-binding protein 3 is measured in serum or EDTA plasma using the Active IGFBP-3 ELISA kit from Diagnostic Systems Laboratory (Webster, TX) (now owned by Beckman Coulter). In the assay, Standards, Controls and unknowns are incubated in plate wells coated with anti-IGFBP-3 polyclonal antibody. After incubation and washing, an HRP-labeled anti-IGFBP-3 polyclonal antibody is added. After a second incubation and washing step, tetramethylbenzidine is added to the wells. An acidic stop solution terminates the reaction. The optical density of the wells is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) at 450nm and 620nm. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of IGFBP-3 in the sample and concentration is determined from a standard curve. The interassay CVs are 11.4% at a mean concentration of 5.64 ng/mL, 10.4% at a mean concentration of 25.13 ng/mL and 8.2% at a mean concentration of 65.55 ng/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 100uL

Sample volume used: 10uL

Dead volume: 50uL

(soluble) Intercellular Adhesion Molecule-1 (sICAM-1)

Dates of Use: 2004 - 2007

sICAM-1 is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Parameter kit from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 6.0-10.1% for the serum or plasma assay. The inter-assay CV range in our laboratory is 7.8%.

(soluble) Intercellular Adhesion Molecule-1 (sICAM-1)

Dates of Use: 2008 - current

sICAM-1 is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Quantikine kit from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 4.4-6.8% for serum or plasma. The inter-assay CV range in our laboratory is 7.8%.

Sample type: Serum/EDTA plasma/heparin plasma (citrate plasma not recommended)

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Interleukin-1 (IL-1)

Dates of Use: Proposed

IL-1 α and IL-1 β are both measured in serum or plasma using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) kit, both available from R & D Systems (Minneapolis, MN). A monoclonal antibody specific for either IL-1 α or IL-1 β is pre-coated onto a microplate. Any IL-1 α or IL-1 β present in the sample is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for IL-1 α or IL-1 β is added and will bind to the immobilized, bound IL-1 α or IL-1 β . Following the addition of substrate solution, color develops in proportion to the amount of IL-1 α or IL-1 β bound in the initial step. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). Software supplied with the spectrophotometer calculates the amount of IL-1 α or IL-1 β contained in each specimen using a standard line prepared with each assay. Commercially obtained controls and in-house controls are run daily with inter-assay CVs of 3.4-4.3% for IL-1 α and 4.1-8.4% for IL-1 β .

IL-1 α *Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma**Minimum volume needed: 300uL**Sample volume used: 200uL**Dead volume: 50uL*

IL-1 β (for the Quantikine high sensitivity assay; Quantiglo and Quantkine assays are also available)

*Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma**Minimum volume needed: 250uL**Sample volume used: 150uL**Dead volume: 50uL***Interleukin-2 soluble receptor α (IL-2 sR α)**

Dates of Use: 2004 - current

IL-2 sR α is measured by ultra-sensitive ELISA (Quantikine Human IL-2 sR α Immunoassay; R&D Systems, Minneapolis, MN). This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The assay plate is pre-coated with a monoclonal antibody specific for IL-2 sR α and an anti-IL-2 sR α polyclonal antibody is used as the sandwich antibody. The amount of IL-2 sR α is determined colorimetrically. The lower detection level is 10 pg/ml and the detection range is 78.1 – 5000 pg/ml. The assay CV ranges from 4.6-7.2%.

*Sample type: Serum/EDTA plasma/heparin plasma**Minimum volume needed: 150uL**Sample volume used: 50uL**Dead volume: 50uL*

Interleukin-6 (IL-6)

Dates of Use: 2004 – current

IL-6 is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) QuantiKine High Sensitivity kit from R & D Systems (Minneapolis, MN). A monoclonal antibody specific for IL-6 is pre-coated onto a microplate. Any IL-6 present in the sample is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for IL-6 is added and will bind to the immobilized, bound IL-6. Following substrate and amplifier additions, color develops in proportion to the amount of IL-6 bound in the initial step. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). Software supplied with the spectrophotometer calculates the amount of IL-6 contained in each specimen using a standard line prepared with each assay. Commercially obtained controls and in-house controls are run daily with inter-assay CV's of 4.9-6.5%.

Sample type: serum/EDTA plasma/citrate plasma/urine; heparin plasma not recommended

Minimum volume needed: 200uL

Sample volume used: 100uL

Dead volume: 50uL

Interleukin-10 (IL-10)

Dates of Use: Proposed

IL-10 is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Quantikine high sensitivity IL-10 Immunoassay kit from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 8.1-15.6% for the serum or plasma assay.

Sample type: Serum/EDTA plasma/citrate plasma (heparin plasma not recommended)

Minimum volume needed: 300uL

Sample volume used: 200uL

Dead volume: 50uL

Inulin

Dates of use: 2009 - current

Inulin is measured in serum of plasma using the FIT-GFR Inulin kit (BioPAL, Inc. Worcester, MA). Following intravenous administration of inulin, sequential blood and urine samples are collected from a subject, inulin concentrations are measured, and GFR is calculated. Sample containing inulin is incubated with a rabbit anti-inulin antibody (antiserum). The inulin in the sample and the antibody compete for binding sites on the coated microplate wells. After washing, goat anti-rabbit IgG-HRP antibody is added and incubated. After a second wash, an HRP substrate is added and the color of the reaction is measured in a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) and concentration is determined from a standard curve. According to the kit insert, inter-assay CVs are < 12%.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma/urine

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Iron

Dates of use: 2006-current

Iron is measured in serum using Roche reagents on the Roche/Hitachi Modular P instrument (Roche Diagnostics, Indianapolis IN). This method for iron measurement utilizes the FerroZine® reagent without deproteinization. In an acidic environment (pH <2) iron is liberated from transferrin (binding protein). Lipemic specimens are also cleared during this step. Ascorbate reduces these released Fe³⁺ ions to Fe²⁺ ions which then react with FerroZine® to form a colored complex. The color intensity of this complex is directly proportional to the iron concentration, and this final product is measured as an endpoint reaction at 570 nm (secondary wavelength = 700 nm). The laboratory CV for this assay is 3.7% at 91.1 ug/dL and 1.8% at 227.4 ug/dL.

Sample type: Serum/heparin plasma

Minimum volume needed: 150uL

Sample volume used: 15uL

Dead volume: 75uL

F2-isoprostanes (8-isoprostanes)

Dates of use: Myron Gross' lab

Free F2-isoprostanes (a collection of isomers) are measured in EDTA plasma by a gas chromatography-mass spectrometry (GC-MS)-based method as described by Morrow (Morrow JD, Chen Y, Brame CJ, et al. The isoprostanes: unique prostaglandin-like products of free-radical-initiated lipid peroxidation. *Drug Metab Rev* 1999;31:117-39. Morrow JD. The isoprostanes: their quantification as an index of oxidant stress status in vivo. *Drug Metab Rev* 2000;32:377-85. Morrow JD, Roberts LJ, 2nd. Mass spectrometric quantification of F2-isoprostanes as indicators of oxidant stress. *Methods Mol Biol* 2002;186:57-66.) and Gross (Gross M, Steffes M, Jacobs DR, Yu X, Lewis L, Lewis CE, Loria C. Plasma F2-Isoprostanes and coronary artery calcification: the CARDIA study. *Clin Chem* 2005;51:125-31.). The isoprostanes from a participant's sample are extracted with 4 deuterium-labeled 8-isoprostaglandin F2 alpha, used as an internal standard. Unlabeled purified F2-isoprostane is used as a standard for the F2-isoprostanes measured in the samples. This F2-isoprostane standard co-migrates and, as expected, has the same molecular weight as the F2-isoprostanes measured in the samples. Thus, the F2-isoprostane standard is a good marker for the assay of F2-isoprostanes by GC-MS that measures a well-defined set of F2-isoprostane isomers. This laboratory has measured over 10,000 samples and this method has an analytical variation of 10% in control pools at 3 concentrations. All samples will be analyzed within one year of collection, consistent with our long-term studies and previous results showing levels of plasma F2-isoprostanes to be stable for >60 months when stored at -70°C.

Sample type: EDTA plasma

Minimum volume needed: 1.1mL

Sample volume used: 1.1mL

Dead volume: none

LDL-Cholesterol Calculated

Dates of Use: 1985 - current

LDL-cholesterol is calculated in plasma specimens having a triglyceride value <400 mg/dL using the formula of Friedewald et al. (Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502). The NCEP program recommends reference range of <100 mg/dL.

LDL Subfractions

Dates of Use: 2002 – 2004

LDL subfraction concentrations are determined in plasma using the Lipoprint HDL System (Quantimetrix Corporation, Redondo Beach, CA). The LDL subfractions are separated by high resolution polyacrylamide gradient gel electrophoresis on the basis of their molecular size. A typical Lipoprint profile consists of 1 VLDL band, 3 Midbands, up to 7 LDL bands and 1 HDL band. The gels are scanned by a densitometer, and a public domain software program (NIH Image Version 1.62) identifies fractions by their mobility (Rf) using VLDL as the starting reference point and HDL as the leading reference point. The percent and concentration (mg/dL) of each fraction is calculated. Reported CV's range from 1.2 – 7.3% for HDL, LDL and VLDL fractions, 2.9 – 11.1% for midband subfractions, and 1.7 – 17.9% for LDL subfractions.

Leptin

Dates of Use: 2002 - 2005

Leptin is measured in serum or plasma using a Human Leptin Radioimmunoassay kit from LINCO Research (St. Charles, Missouri). The inter-assay CV range reported in the kit insert is 8.8-12.6%.

Leptin

Dates of Use: Proposed

Leptin is measure in serum or plasma and is the quantitative sandwich enzyme immunoassay technique of the Human Leptin Quantikine Immunoassay Kit by R&D Systems, Inc. (Minneapolis, MN). The kit insert reports an inter-assay CV of 3.5-5.4%.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

(soluble) Leptin Receptor

Dates of Use: Proposed

(soluble) Leptin receptor is measured in serum or EDTA plasma using the Quantikine Human Leptin sR Immunoassay from R & D Systems (Minneapolis, MN). This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for the soluble leptin receptor has been pre-coated onto microplate wells. Standards and samples are added and any soluble leptin receptor present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for soluble leptin receptor is added. Following a wash to remove any unbound antibody-enzyme reagent, tetramethylbenzidine is added to the wells and color develops in proportion to the amount of soluble leptin receptor bound in the initial step. The color development is stopped and the intensity of the color is read on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA). A standard curve is generated, and soluble leptin receptor present in the samples is determined directly from this curve. The reported interassay CV of this method is 8.6% at a mean concentration of 2.34 ng/mL, 5.3% at a mean concentration of 6.95 ng/mL and 6.7% at a mean concentration of 14.3 ng/mL.

Sample Type: Serum/EDTA Plasma

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

Lipoprotein (a) (Lp(a))

Dates of Use: 2006 - current

Total Lp(a) mass is determined in serum or EDTA plasma by immunoprecipitin analysis using the SPQ™ Antibody Reagent System of DiaSorin (DiaSorin Inc., Stillwater, MN 55082-0285). Turbidity produced by the antigen-antibody complexes is measured using the Roche Modular P Chemistry Analyzer. (Roche Diagnostics Corporation). The laboratory inter-assay CV is 9.3% at a level of 50.4 mg/dL and 11.7% at a level of 20.4 mg/dL.

Sample type: Serum/EDTA plasma

Minimum volume needed: 150uL

Sample volume used: 4uL

Dead volume: 75uL

Lymphocyte transformation

Dates of use: 1999 - current

The primary purpose of lymphocyte transformation (or immortalization) is to provide unlimited quantities of DNA for future analyses. Cryopreserved lymphocytes are incubated with Epstein-Barr Virus (EBV) and cyclosporin A (CSA) for 21 days; EBV infects B lymphocytes, resulting in pleomorphic cell formation, alteration of growth regulation and cell immortalization while CSA inhibits T cell growth. The success rate in our laboratory (defined as the number of cultures attempted minus the number of cultures with a failure to produce LCLs divided by the number of cultures attempted) is 99.7%, 99.4%, 99.3% for the EGEN, GEN3, and CARDIA studies, respectively.

Sample type: cryopreserved lymphocytes

Minimum volume needed:

Sample volume used:

Dead volume:

Macrophage Colony Stimulating Factor (M-CSF)

Dates of Use: Proposed

Human M-CSF concentration is measured in serum or plasma using the quantitative sandwich enzyme immunoassay technique of the Human M-CSF Quantikine Immunoassay Kit by R&D Systems, Inc. (Minneapolis, MN). The kit insert reports an inter-assay CV of 3.1-5.2%.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma (Note: fresh serum/plasma is recommended, however samples frozen at $\leq -20^{\circ}\text{C}$ are acceptable)

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

Magnesium

Dates of Use: 2006 - current

Magnesium is measured in serum, plasma and/or urine using a Roche colorimetric method on the Modular P Chemistry analyzer. In alkaline solution, magnesium forms a purple complex with xylidyl blue, a diazonium salt. The magnesium concentration is measured photometrically via the decrease in the xylidyl blue absorbance. The laboratory inter-assay CV is 2.8%.

Sample type: Serum/lithium-heparin plasma, urine

Minimum volume needed: 150uL

Sample volume used: 4uL

Dead volume: 75uL

Matrix Metalloproteinase-1 (MMP-1)

Dates of use: 2010 - current

Pro-MMP-1 is measured in serum or heparin plasma using the quantitative sandwich enzyme immunoassay technique of the Human pro-MMP-1 Quantikine Immunoassay kit from R & D Systems (Minneapolis, MN). This assay is specific for pro-MMP-1 and will not detect active MMP-1 or TIMP-1-bound MMP-1.

According to the kit insert, the inter-assay CV is 7.7-11.7%.

Sample type: Serum/heparin plasma (Note: EDTA plasma and citrate plasma cannot be used because of their chelating properties.)

Minimum volume needed: 150uL

Sample volume used: 100uL

Dead volume: 50uL

Matrix Metalloproteinase-2 (MMP-2)

Dates of use: 2010 - current

MMP-2 is measured in serum or heparin plasma using the quantitative sandwich enzyme immunoassay technique of the Human MMP-2 Quantikine Immunoassay kit from R & D Systems (Minneapolis, MN). This assay measures free MMP-2 but not total MMP-2. According to the kit insert, the inter-assay CV is 5.6-9.8%.

Sample type: Serum/heparin plasma (Note: EDTA plasma and citrate plasma are not recommended.)

Minimum volume needed: 125uL

Sample volume used: 20uL

Dead volume: 50uL

Matrix Metalloproteinase-3 (MMP-3)

Dates of Use: Proposed

MMP-3, also known as stromelysin-1, is measured in serum or heparin plasma by a solid-phase sandwich ELISA (Human MMP-3 (total) Quantikine Immunoassay, R&D Systems, Minneapolis, MN) using a polyclonal antibody specific for both the pro- and active forms of MMP-3. According to the kit insert, the inter-assay CV is 7.0-8.6%.

Sample type: Serum/heparin plasma (Note: EDTA plasma and citrate plasma cannot be used because of their chelating properties.)

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

Matrix Metalloproteinase-9 (MMP-9)

Dates of use: 2005 - current

MMP-9 is measured in serum or heparin plasma by a solid-phase sandwich ELISA (Human MMP-9 (total) Quantikine Immunoassay, R&D Systems, Minneapolis, MN) using a polyclonal antibody specific for both the pro- and active forms of MMP-9. According to the kit insert, the inter-assay CV is 6.9-7.9%.

Sample type: Serum/platelet-free heparin plasma (Note: EDTA plasma and citrate plasma cannot be used because of their chelating properties.)

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Microalbumin, urine

Dates of use: Chavers' lab

Urine microalbumin is measured using a solid-phase, non-competitive, double-antibody fluorescent immunoassay as described by Chavers et al (Chavers BM, Simonson J, Michael AF. A solid-phase fluorescent immunoassay for the measurement of human urinary albumin. *Kidney Int.* 1984; 25: 576-8). Urine specimen albumin antigen reacts with albumin antibody that is covalently attached to polyacrylamide beads. This resulting solid-phase antibody complex is then reacted with fluorescein-labeled antibody. Unattached fluorescent antibody and other proteins are removed by centrifugation. The fluorescence of the stable solid-phase double-antibody complex is measured with a fluorometer and is directly proportional to the amount of urine albumin present. The standard line calibration material is human serum albumin with a range of 0.5 to 20 µg/mL.

Monocyte chemotactic protein 1 (MCP-1)

Dates of use: 2004 - current

MCP-1 is measured in serum or plasma using the quantitative sandwich enzyme immunoassay technique of the Human CCL2/MCP-1 Quantikine Immunoassay kit from R & D Systems (Minneapolis, MN). Our laboratory inter-assay CV is 4.7% at a mean concentration of 334 pg/mL.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma/urine

Minimum volume needed: 225uL

Sample volume used: 125uL

Dead volume: 50uL

Myeloperoxidase (MPO)

Dates of Use: Proposed

MPO is measured in serum or plasma using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Quantikine kit from R & D Systems (Minneapolis, MN). A monoclonal antibody specific for MPO is pre-coated onto a microplate. Any MPO present in the sample is bound by the immobilized antibody. After washing, an enzyme-linked polyclonal antibody specific for MPO is added and binds to the immobilized, bound MPO. Following a wash, a substrate solution is added and color develops in proportion to the amount of MPO bound in the initial step. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 9.4 to 6.5% at concentrations ranging from 19.0 to 69.2 ng/mL.

Sample type: Serum (preferred)/EDTA or heparin plasma may be used if collected on ice and centrifuged an additional time at 10,000xg for 10 min for complete platelet removal.

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

N-terminal cross-linking telopeptide of type 1 collagen (NTX-1) Dates of Use: 2009 - current
NTX-1, a bone marker assay, is measured in serum or EDTA-anticoagulated plasma using the Osteomark NTx kit from Wampole Laboratories (Princeton, NJ 08540). This kit measures cross-linked N-telopeptides of type I collagen by a competitive-inhibition enzyme-linked immunosorbent assay. Briefly, samples or standards are incubated with murine monoclonal anti-NTx (conjugated to horseradish peroxidase) in microplate wells coated with synthetic NTx antigen. The wells are washed, then a substrate solution containing tetramethylbenzidine is added and color in the wells develops in inverse proportion to the amount of NTx in the sample. The color is read on a SpectraMax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA 94089). The concentration of NTx in the sample is determined by preparing a standard curve from standards of known concentration. Our laboratory inter-assay CV on 8 samples is 8.2% at a mean concentration of 28.0 nmBCE/L (nanomoles Bone Collagen Equivalents per liter). The kit insert reports an inter-assay CV of 6.9% at an unreported mean concentration.

Sample type: serum/EDTA plasma

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

N-terminal pro-B-type natriuretic peptide (NT-proBNP) (see *B-type natriuretic peptide...*)
See the entry "B-type natriuretic peptide, N-terminal pro."

Osteocalcin (OCN) Dates of Use: 2009 - current
Osteocalcin, a bone marker assay, is measured in serum or plasma using the MicroVue Osteocalcin EIA kit from Quidel Corporation (San Diego, CA 92121). This kit measures intact (*de novo*) osteocalcin by a competitive immunoassay. Briefly, samples or standards are incubated with murine monoclonal anti-osteocalcin antibody in microplate wells coated with osteocalcin (purified from human bone). The wells are washed, then goat anti-mouse IgG antibody (conjugated to alkaline phosphatase) is added to the microplate wells. The wells are washed, then a substrate solution containing p-nitrophenyl phosphate is added and color in the wells develops in inverse proportion to the amount of osteocalcin in the sample. The color is read on a SpectraMax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA 94089). The concentration of osteocalcin in the sample is determined by preparing a standard curve from standards of known concentration. Our laboratory inter-assay CV on 4 samples is 4.9% at a mean concentration of 6.0 ng/mL, and 3.6% at a mean concentration of 22.1 ng/mL. The kit insert reports inter-assay CVs of 10.0% at mean concentration 6.2 ng/mL, 4.8% at mean concentration 7.4 ng/mL, and 8.0% at mean concentration 16.5 ng/mL.

Sample type: serum/EDTA plasma (processing should be done at 2-8°C to prevent proteolysis of osteocalcin)

Minimum volume needed: 125uL

Sample volume used: 25uL

Dead volume: 50uL

Oxidized LDL (oxLDL)

Dates of Use: Proposed

Oxidized LDL is measured in serum or plasma using the Oxidized LDL ELISA enzyme immunoassay kit from Mercodia AB (Uppsala, Sweden). The ELISA is a two-site enzyme immunoassay using the direct sandwich technique where two antibodies recognize different antigenic determinants on the oxidized apolipoprotein B molecule. The inter-assay CV, according to the kit insert, is 4.0-6.2%.

Sample type: EDTA plasma (preferred)/serum/heparin plasma

Minimum volume needed: 125uL

Sample volume used: 25uL

Dead volume: 50uL

Parathyroid Hormone, Intact (PTH)

Dates of Use: Proposed

PTH, intact is measured in serum or EDTA plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample reacts with a biotinylated monoclonal PTH-specific antibody and a monoclonal PTH-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of PTH in the sample. The Roche reported CV is 6.5% at a concentration of 26.7 pg/mL, 3.9% at a concentration of 52.5 pg/mL, and 3.0% at a concentration of 261pg/mL.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 300uL

Sample volume used: 50uL

Dead volume: 200uL

Phosphorus

Dates of Use: 2006 - current

Phosphorus is measured in serum or plasma on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a colorimetric method. The method is based on the reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate without reduction. An accelerator is added to increase the rate of reaction and sample blanking yields more precise results. The laboratory inter-assay CV is 2.2% (mean 5.87 mg/dL).

Sample type: serum/EDTA plasma/lithium-heparin plasma

Minimum volume needed: 150uL

Sample volume used: 4uL

Dead volume: 75uL

Placental growth factor (PIGF)

Dates of Use: 2005

PIGF is measured in serum or plasma using the quantitative sandwich enzyme immunoassay technique of the Human PIGF Quantikine Immunoassay kit from R & D Systems (Minneapolis, MN). According to the kit insert, the inter-assay CV is 10.9-11.8%

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma/urine

Minimum volume needed: 200uL

Sample volume used: 100uL

Dead volume: 50uL

Plasminogen Activator Inhibitor-1 (PAI-1)

Dates of Use: Coagulation Lab

Plasminogen Activator Inhibitor-1 (PAI-1) is measured using the Asserachrom PAI-1 kit (Diagnostica Stago). This method is an enzyme immunoassay (EIA) procedure for the quantitative determination of PAI-1 by the sandwich technique, also known as ELISA. Blood must be collected in 0.109 M trisodium citrate anticoagulant in a non-wettable tube to prevent platelet activation. Perform centrifugation for 15 minutes at 4°C at 3000 g to minimize platelet alteration and to achieve maximum removal of the platelets. The inter-assay CV range reported in the kit insert is 6.5-8.7%. Data from our lab give an inter-assay CV of 13.2% (mean, 15.0 ng/mL) and 8.8% (mean, 74.4 ng/mL).

Potassium (see *Electrolytes*)

See the entry "Electrolytes."

Protein Carbonyl Content

Dates of Use: 2008

Protein carbonyls are quantitated in plasma or serum using the enzyme-linked immunosorbent assay (ELISA) technique in an assay kit from Zenith Technology Corp. Ltd. (Dunedin, New Zealand). Protein carbonyls are derivatized from a plasma or serum sample, then used in the ELISA procedure. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The expected inter-assay CV range reported in the kit insert is 5-15%.

Progesterone

Dates of Use: Proposed

Progesterone is measured in serum or EDTA plasma using the Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN). In the Elecsys immunoassay, the patient sample reacts with a biotinylated monoclonal progesterone-specific antibody and a monoclonal progesterone-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of progesterone in the sample. This method has been standardized via isotope dilution-gas chromatography/mass spectrometry (ID-GC/MS). The inter-assay CV in our laboratory is 5.4% at a mean concentration of 1.57 ng/mL, 4.1% at a mean concentration of 12 ng/mL and 5.5% at a mean concentration of 30.2 ng/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 250uL

Sample volume used: 30uL

Dead volume: 200uL

Prohepcidin

Dates of Use: Proposed

Prohepcidin is measured in serum or EDTA plasma using the Hecpidin Prohormone EIA from DRG International (Mountainside, NJ). This is a competitive immunoassay that uses a polyclonal antibody directed towards an antigenic site on the hepcidin prohormone molecule (28-47 aa). Prohepcidin in the sample competes with a hepcidin prohormone-biotin conjugate for binding sites of the capture antibody. After washing, tetramethylbenzidine is added to the wells. An acidic stop solution terminates the reaction. The optical density of the wells is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) at 450nm. The intensity of color developed is inversely proportional to the concentration of prohepcidin in the sample and the concentrations are determined by a standard curve. The reported CVs for this assay are 4.8% at a mean concentration of 432 ng/mL and 9.7% at a mean concentration of 110 ng/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 100uL

Sample volume used: 10uL

Dead volume: 50uL

Pyridinium (PYD)

Dates of Use: 2009 - current

Pyridinium (PYD), a bone marker assay, is measured in urine using the Metra PYD EIA kit from Quidel Corporation (San Diego, CA 92121). This kit measures PYD crosslinks by a competitive immunoassay. Briefly, samples or standards are incubated with murine monoclonal anti-pyridinium crosslinks antibody (conjugated to alkaline phosphatase) in microplate wells coated with PYD purified from bovine bone. The wells are washed, then a substrate solution containing p-nitrophenyl phosphate is added and color in the wells develops in inverse proportion to the amount of PYD in the sample. The color is read on a SpectraMax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA 94089). The concentration of PYD in the sample is determined by preparing a standard curve from standards of known concentration, and the concentration is adjusted for variation in urine concentration by dividing the result by the creatinine concentration of the sample. Our laboratory inter-assay CV on 5 samples is 8.6% at a mean concentration of 95.7 nmol/L, and 6.7% at a mean concentration of 426.6 nmol/L. The kit insert reports inter-assay CVs of 11.2% at mean concentration 66 nmol/L, 5.8% at mean concentration 160 nmol/L, and 3.9% at mean concentration 407 nmol/L.

*Sample type: urine**Minimum volume needed: 125uL**Sample volume used: 20uL**Dead volume: 50uL***RNA, total**

Dates of Use: Proposed

Total RNA is extracted from freshly-collected EDTA-anticoagulated whole blood using the 5 Prime Manual PerfectPure RNA blood kit (Gaithersburg, MD 20878). Briefly, red blood cells are lysed to separate them from the white blood cells and to remove contaminating proteins and heme. The white blood cells are lysed with a detergent/salt solution, while endogenous RNase activity is also eliminated. The lysate is then passed through a column to remove debris. The cleaned lysate is applied to a column, where the RNA is bound to a column filter. The bound RNA is washed and treated with DNase to remove any remaining contaminants. Finally, the RNA is eluted from the filter using water. Expected yields using this method are 0.3-0.7 micrograms RNA per million cells or 1.2-7 micrograms of RNA per milliliter of whole blood. In our laboratory, previous yields from cultured cells using the similar 5 Prime Manual PerfectPure RNA Cell and Tissue kit averaged 4.5 micrograms RNA per million cells \pm 2.0 micrograms (SD) for 44 samples isolated over 4 days.

*Sample type: EDTA-anticoagulated whole blood**Minimum volume needed: 3mL**Sample volume used: 3mL or more (yield dependent on volume)**Dead volume: none*

Remnant-like Particle-Cholesterol (RLP-C)

Dates of Use: 2002 – 2004

RLP-C is measured using the RLP[®]-Cholesterol Immunoseparation Assay (POLYMEDCO, Inc., Cortlandt Manor, NY) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). This method uses immunoseparation techniques to isolate a population of remnant lipoproteins that have the same physical and chemical properties of β -VLDL—these particles are referred to as remnant-like particles (RLP). The remnant particles are separated from LDL, Lp(a), nascent VLDL and HDL by mouse monoclonal antibodies to human apolipoproteins A-1 and B-100 conjugated to sepharose-4B beads. The remnant particles present in the unbound fraction are measured by assaying the cholesterol (remnant lipoprotein cholesterol) by an enzymatic assay using the Roche Cobas Mira centrifugal analyzer (Roche Diagnostics, Indianapolis, IN 46250). The laboratory CV range for this assay is 5.5% - 9.3%.

Resistin

Dates of Use: Proposed

Resistin is measured in serum or EDTA plasma using the Quantikine Human Resistin Immunoassay from R & D Systems (Minneapolis, MN). The R&D Systems assay uses two monoclonal antibodies directed against human resistin; a capture antibody, coated on the plate wells, and an enzyme-linked detection antibody. The ALPCO kit also uses a monoclonal capture antibody, however, the detection antibody is a polyclonal antibody. We have extensive experience using assays from R & D Systems, and we have found excellent assay performance and have been able to duplicate the CVs stated in the kit insert. The interassay CVs are 8.2% at a mean concentration of 0.61 ng/mL, 9.2% at a mean concentration of 2.28 ng/mL and 7.8% at a mean concentration of 4.76 ng/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

Retinol Binding Protein-4 (RBP-4)

Dates of Use: Proposed

Retinol binding protein 4 (RBP-4) is measured in serum or EDTA plasma using the Retinol-binding protein (RBP)/RBP4 ELISA Kit from ALPCO Diagnostics (Salem, NH). RBP/RBP4 in samples and standards are allowed to bind to polyclonal rabbit anti-RBP/RBP4 antibodies that are immobilized to the plate wells. A peroxidase-conjugated anti-RBP/RBP4 antibody is used for detection and quantification, with tetramethylbenzidine as a peroxidase substrate. Optical density at 450nm is read on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA). A standard curve is generated, and RBP/RBP4 present in the samples is determined directly from this curve. The reported interassay CV of this method is 9.8% at a mean concentration of 4.4 ng/mL and 9.7% at a mean concentration of 6.9 ng/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 100uL

Sample volume used: 20uL

Dead volume: 50uL

Sex Hormone Binding Globulin (SHBG)

Dates of Use: Proposed

Sex hormone binding globulin (SHBG) is measured in serum or EDTA plasma using the Sex Hormone Binding Globulin ELISA from ALPCO Diagnostics (Salem, NH). This is a sandwich assay that uses a monoclonal antibody specific for SHBG coated on the microwell plate and another monoclonal antibody specific for a different region of SHBG conjugated to horseradish peroxidase (HRP). SHBG from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with HRP conjugate. After a second washing step, tetramethylbenzidine and hydrogen peroxide substrate is added. The enzymatic reaction is terminated by addition of an acidic stop solution. The absorbance is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA). The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of SHBG in the sample and concentration is determined from a standard curve. The interassay CVs reported for this assay range are 7.2% at a mean concentration of 194 nmol/L and 11.6% at a mean concentration of 3.8 nmol/L.

*Sample type: Serum/EDTA Plasma**Minimum volume needed: 100uL**Sample volume used: 10uL**Dead volume: 50uL***Sodium (see *Electrolytes*)**

See the entry "Electrolytes."

Superoxide dismutase (SOD) activity

Dates of use: 2006

Superoxide dismutase (SOD) activity is measured using the Cayman Chemical (Ann Arbor, MI) superoxide dismutase assay kit. Briefly, sample is incubated with xanthine oxidase and hypoxanthine (to generate the superoxide ion) and a tetrazolium salt (to generate a light-absorbing compound). Reduction of the appearance of the light-absorbing compound is a measure of the SOD activity present in the sample. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. According to the kit insert, extracellular SOD accounts for the majority of the SOD activity in serum, so SOD3 is measured using this assay.

Superoxide dismutase (SOD)mass

Dates of use: 2006

SOD mass is measured using the Bender MedSystems (Vienna, Austria) human Cu/ZnSOD ELISA. Briefly, Cu/ZnSOD present in the sample binds to antibody bound to a microwell and HRP-conjugated monoclonal anti-Cu/ZnSOD is added and binds to Cu/ZnSOD captured by the first antibody. A substrate solution is added and reacts with HRP to form a colored product proportional to the amount of Cu/ZnSOD present in the sample. Absorbance of the colored product is read on a microplate spectrophotometer (SpectraMax, Molecular Devices, Sunnyvale, CA) at 450nm. Sample concentration of SOD is determined using a standard curve prepared with standards run on the plate. The antibody used in the test is a monoclonal murine antibody to human Cu/ZnSOD. The kit manufacturer does not have data available regarding cross-reactivity of the antibody to extracellular SOD, other than to note that the literature reports the measurement of Cu/ZnSOD in serum and other body fluids (Porstmann T et al. Clin Chim Acta 1988;171:1-10. Porstmann T et al. Hum Genet 1990;85:362-6).

Tartrate-resistant acid phosphatase (TRAP5b)

Dates of Use: 2009 - current

TRAP5b, a bone marking assay, is measured in serum or plasma using the Metra TRAP5b Assay kit from Quidel Corporation (San Diego, CA 92121). This kit measures tartrate-resistant acid phosphatase isoform 5b (TRAP5b) by a 2-step direct capture EIA. Briefly, samples or standards are incubated in microplate wells coated with two different monoclonal antibodies, Trk49 and Trk62 (both of which are generated with immunization of purified TRAP5b from human bone cells). The wells are washed, then a substrate solution containing 2-chloro-4-nitrophenyl phosphate is added and color in the wells develops in proportion to the amount of TRAP5b in the sample. The color is read on a SpectraMax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA 94089). The concentration of TRAP5b in the sample is determined by preparing a standard curve from standards of known concentration. Our laboratory inter-assay CV on 8 samples is 3.3% at a mean concentration of 3.5 U/L, and 3.1% at a mean concentration of 6.6 U/L. The kit insert reports inter-assay CVs of 3.0% at mean concentration 3.8 U/L and 2.0% at mean concentration 7.4 U/L.

Sample type: serum/EDTA plasma/heparin plasma

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

Testosterone

Dates of Use: Proposed

Testosterone is measured in serum or EDTA plasma using the Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN). In the Elecsys immunoassay, the patient sample reacts with a biotinylated monoclonal testosterone-specific antibody and a monoclonal testosterone-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of either estradiol, progesterone or testosterone in the sample. Each method has been standardized via isotope dilution-gas chromatography/mass spectrometry (ID-GC/MS). The inter-assay CV is 7.4% at a mean concentration of 0.24 ng/mL, 2.2% at a mean concentration of 2.75 ng/mL and 1.7% at a mean concentration of 7.01 ng/mL.

Sample type: Serum/EDTA Plasma

Minimum volume needed: 300uL

Sample volume used: 50uL

Dead volume: 200uL

Thyroid Peroxidase, antibody (anti-TPO)

Dates of Use: Proposed

Thyroid peroxidase antibody (anti-TPO) is measured in serum or plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a competition immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample is mixed with anti-TPO-antibodies labeled with a ruthenium complex. Biotinylated TPO and streptavidin-coated microparticles are added during the second incubation. The anti-TPO antibodies in the sample compete with the ruthenium-labeled anti-TPO antibodies for the biotinylated TPO antigen. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is inversely proportional to the amount of anti-TPO in the sample. The Roche reported CVs are 24.4 at a concentration of 12.4 IU/mL, 9.2% at a concentration of 109 IU/mL, and 7.1% at a concentration of 308 IU/mL.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 275uL

Sample volume used: 20uL

Dead volume: 200uL

Thyroid-stimulating hormone (TSH)

Dates of use: 2006 – current

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) is a third-generation assay that employs anti-FITC monoclonal antibody covalently bound to paramagnetic particles, an FITC-labeled anti-TSH capture monoclonal antibody, and a tracer consisting of a proprietary acridinium ester and an anti-TSH mAb antibody conjugated to bovine serum albumin for chemiluminescent detection. This assay is measured in serum or plasma.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 325uL

Sample volume used: 120uL

Dead volume: 150uL

Thyroid-stimulating hormone (TSH, thyrotropin)

Dates of Use: Proposed

Thyroid-stimulating hormone is measured in serum or plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample is mixed with a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added, and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically, and unbound material is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of TSH in the sample. The Roche reported CVs are 3.3% at a concentration of 0.91 μ IU/mL and 3.6% at a concentration of 3.96 μ IU/mL.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 300uL

Sample volume used: 50uL

Dead volume: 200uL

Thyroxine, free (FT4)

Dates of use: 2006 – current

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) Free Thyroxine (FT4) assay is a competitive immunoassay measured in serum using direct chemiluminescent technology. Free T4 in the patient sample competes with acridinium ester-labeled T4 in the LITE Reagent for limited amount of polyclonal rabbit anti-T4 antibody which is covalently coupled to paramagnetic particles in the Solid Phase.

Sample type: Serum

Minimum volume needed: 150uL

Sample volume used: 35uL

Dead volume: 50uL

Thyroxine, free (FT4)

Dates of Use: Proposed

Thyroxine (free) is measured in serum or EDTA plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a competition immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, patient sample is mixed with T4-specific antibody labeled with a ruthenium complex. Biotinylated T4 and streptavidin-coated microparticles are added during the second incubation. The still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound material is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is inversely proportional to the amount of T4 in the sample. The Roche reported CVs are 3.5% at a concentration of 0.68 ng/dL, 3.3% at a concentration of 1.64 ng/dL, and 6.6% at a concentration of 3.95 ng/dL.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 275uL

Sample volume used: 15uL

Dead volume: 200uL

Thyroxine, total (T4)

Dates of use: 2006 – current

The ADVIA® Centaur (Siemens Diagnostics, Deerfield, IL) T4 assay is a competitive immunoassay measured in serum using direct chemiluminescent technology. T4 in the patient sample competes with T4 which is covalently coupled to paramagnetic particles in the Solid Phase for limited amount of acridinium ester-labeled monoclonal mouse anti-T4 antibody in the LITE Reagent.

Sample type: Serum

Minimum volume needed: 150uL

Sample volume used: 35uL

Dead volume: 50uL

Tissue inhibitor of matrix metalloproteinase-1 (TIMP-1)

Dates of use: 2010 – current

TIMP-1 is measured in serum or plasma using the quantitative sandwich enzyme immunoassay technique of the Human TIMP-1 Quantikine Immunoassay kit from R & D Systems (Minneapolis, MN). According to the kit insert, the inter-assay CV is 3.9-4.9%

Sample type: Serum/EDTA plasma/heparin plasma (Note: citrate plasma is not recommended.)

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Tissue inhibitor of matrix metalloproteinase-2 (TIMP-2)

Dates of use: 2010 - current

TIMP-2 is measured in serum or plasma using the quantitative sandwich enzyme immunoassay technique of the Human TIMP-2 Quantikine Immunoassay kit from R & D Systems (Minneapolis, MN). According to the kit insert, the inter-assay CV is 5.7-7.3%

Sample type: Serum/EDTA plasma/heparin plasma (Note: citrate plasma is not recommended.)

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Transferrin receptor

Dates of use: 2007-current

Transferrin receptor is measured in serum or plasma using Roche reagents on the Roche/Hitachi Modular P instrument (Roche Diagnostics, Indianapolis IN). This assay is an automated homogeneous immunoturbidimetric assay in which microparticles coupled with specific anti-sTfR monoclonal antibodies react with the sTfR in the serum sample to form an antigen-antibody complex. Following agglutination, this is measured turbidimetrically. The laboratory CV for this assay is 4.2% at 1.59 mg/L and 3.7% at 2.68 mg/L.

Sample type: Serum/heparin plasma

Minimum volume needed: 150uL

Sample volume used: 3uL

Dead volume: 75uL

Transforming growth factor beta 1 (TGF β 1)

Dates of use: Proposed

Transforming growth factor beta 1 (TGF β 1) is measured in serum, EDTA plasma, or urine using the quantitative sandwich enzyme immunoassay technique of the Human TGF β 1 Quantikine Immunoassay kit from R & D Systems (Minneapolis, MN). Prior to quantitation, latent TGF β 1 is activated to immunoreactive TGF β 1 by treating urine with an acidic buffer, then neutralizing the sample. The activated sample is then assayed by ELISA. A monoclonal antibody specific for TGF β 1 is pre-coated onto a microplate. Any TGF β 1 present in the sample is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for TGF β 1 is added and will bind to the immobilized, bound TGF β 1. Following addition of substrate, color develops in proportion to the amount of TGF β 1 bound in the initial step. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 5.7-8.4% for the urine assay.

Sample type: Serum/platelet-poor EDTA plasma/urine

Minimum volume needed: 150uL (serum/plasma), 200uL (urine)

Sample volume used: 40uL (serum/plasma), 100uL (urine)

Dead volume: 50uL

Triglyceride

Dates of Use: 2000 and prior

Triglyceride is measured in EDTA plasma using Triglyceride GB reagent (Roche Diagnostics, Indianapolis, IN 46250) on the Roche COBAS FARA centrifugal analyzer at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). This assay performs an automated glycerol blank by taking a spectrophotometric reading after endogenous glycerol has reacted and before lipase is added to release the glycerol from the triglyceride. This method is calibrated with a frozen serum standard prepared in our laboratory and frozen at -70°C. We have assigned this calibrator by comparison to CDC reference materials. The accuracy and precision The NCEP program recommends reference range of <150 mg/dL. The laboratory CV is 4.0%.

Triglyceride

Dates of Use: Nov 2000 – Spring 2006

Triglyceride is measured in EDTA plasma using Triglyceride GB reagent (Roche Diagnostics, Indianapolis, IN 46250) on the Roche Hitachi 911 (Roche Diagnostics Corporation). This assay performs an automated glycerol blank by taking a spectrophotometric reading after endogenous glycerol has reacted and before lipase is added to release the glycerol from the triglyceride. This method is calibrated with a frozen serum standard prepared in our laboratory and frozen at -70°C. We have assigned this calibrator by comparison to CDC reference materials. The accuracy and precision The NCEP program recommends reference range of <150 mg/dL. The laboratory CV is 4.0%.

Triglyceride

Dates of Use: 2006 - current

Triglyceride is measured in serum or EDTA plasma using Triglyceride GB reagent (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry Analyzer. (Roche Diagnostics Corporation). This assay performs an automated glycerol blank by taking a spectrophotometric reading after endogenous glycerol has reacted and before lipase is added to release the glycerol from the triglyceride. This method is calibrated with a frozen serum standard prepared in our laboratory and frozen at -70°C. We have assigned this calibrator by comparison to CDC reference materials. The NCEP program recommends reference range of <150 mg/dL. The laboratory CV is 4.0%.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 150uL

Sample volume used: 2 µL

Dead volume: 75 µL

Triiodothyronine (T3)

Dates of Use: Proposed

Triiodothyronine (T3) is measured in serum or EDTA plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a competition immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). Bound T3 is released from the binding proteins in the sample by 8-anilino-1-naphthalene sulfonic acid (ANS). In the first incubation, T3 in the patient sample reacts with T3-specific antibody labeled with a ruthenium complex. Biotinylated T3 and streptavidin-coated microparticles are added during the second incubation. The still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound material is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is inversely proportional to the amount of T3 in the sample. The Roche reported CVs are 5.4% at a concentration of 0.79 ng/mL, 4.7% at a concentration of 1.87 ng/mL, and 5.4% at a concentration of 3.31 ng/mL.

Sample type: EDTA plasma (preferred)/serum

Minimum volume needed: 275uL

Sample volume used: 20uL

Dead volume: 200uL

Troponin I

Dates of Use: Proposed

Troponin I is measured in serum or EDTA or heparin plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample reacts with a biotinylated monoclonal anti-cardiac troponin I antibodies and two monoclonal anti-cardiac troponin I antibodies labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added, and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of Troponin I in the sample. The Roche reported CV is 5.3% at a level of 0.322 µg/L and 2.7% at a level of 17.6 µg/L.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 250uL

Sample volume used: 30uL

Dead volume: 200uL

Troponin T

Dates of Use: Proposed

Troponin T is measured in serum or EDTA, citrate, or heparin plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample reacts with a biotinylated monoclonal troponin T-specific antibody and a monoclonal troponin T-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added, and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of Troponin T in the sample. The Roche reported CV is 3.4% at a level of 0.040 µg/L and 1.5% at a level of 6.04 µg/L.

Sample type: serum/EDTA plasma/heparin plasma

Minimum volume needed: 250uL

Sample volume used: 15uL

Dead volume: 200uL

Tumor Necrosis Factor- α (TNF- α)

Dates of Use: 2004 - current

TNF- α is measured in serum or plasma using the quantitative sandwich enzyme immunoassay technique of the Human TNF- α QuantiGlo immunoassay from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a Packard luminometer (Packard Instrument Company, Meriden, CT incorporated by PerkinElmer, Boston, MA) or BioTek plate reader. The inter-assay CV range is 4.6 – 9.1%.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma

Minimum volume needed: 200uL

Sample volume used: 100uL

Dead volume: 50uL

Tumor Necrosis Factor- α (TNF- α)

Dates of Use: Alternate Method

TNF- α is measured in serum or plasma using the quantitative sandwich enzyme technique of the enzyme immunoassay Human TNF- α high sensitivity Quantikine immunoassay from R & D Systems (Minneapolis, MN). A monoclonal antibody specific for TNF- α is pre-coated onto a microplate. Any TNF- α present in the sample is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for TNF- α is added and will bind to the immobilized, bound TNF- α . Following substrate and amplifier additions, color develops in proportion to the amount of TNF- α bound in the initial step. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). Software supplied with the spectrophotometer calculates the amount of TNF- α in each specimen using a standard line prepared with each assay. Commercially obtained controls and in-house controls are run daily with inter-assay CV's of 4.6-7.4%.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma

Minimum volume needed: 300uL

Sample volume used: 200uL

Dead volume: 50uL

(soluble) Tumor Necrosis Factor receptor 1 (sTNF R1)

Dates of Use: Proposed

sTNF R1 is measured in serum or plasma using an ultra-sensitive ELISA assay (Quantikine Human sTNF RI Immunoassay; R&D Systems, Minneapolis, MN). A monoclonal antibody specific for sTNF-R1 is coated on the assay plate and a polyclonal anti-TNF-R1 antibody is used as the sandwich assay. The amount of receptor is determined by a colorimetric reaction. The laboratory CV for this assay is 5%. The lower detection level is 1-3 pg/ml and the detection range is 7.8 – 500 pg/mL. The normal range for TNF-R1 in serum is 479 to 1966 pg/mL.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma/urine

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

(soluble) Tumor Necrosis Factor Receptor II (sTNF RII)

Dates of Use: 2008 - current

Soluble tumor necrosis factor receptor II (sTNF RII) is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) QuantiKine kit from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 3.5-5.1% for the serum or plasma assay. Our laboratory CV is 3.7% at a mean concentration of 2455pg/mL.

Sample type: Serum/EDTA plasma/heparin plasma/citrate plasma/urine

Minimum volume needed: 150uL

Sample volume used: 50uL

Dead volume: 50uL

Unbound iron binding capacity (UIBC)

Dates of use: 2006-current

Unbound iron binding capacity (UIBC) is measured in serum or plasma using Roche reagents on the Roche/Hitachi Modular P instrument (Roche Diagnostics, Indianapolis IN). This method for UIBC measurement is a modification of the method of Goodwin, utilizing the FerroZine® reagent without deproteinization. Serum is added to an alkaline buffer/reductant solution containing a known concentration of iron to saturate the available transferrin binding sites in the specimen. Reduced excess iron then reacts with the FerroZine® reagent. The UIBC is equal to the difference measured in the concentrations of the added iron and the excess unbound iron. This is an endpoint reaction with sample blank, measured at 570 nm (secondary wavelength = 700 nm). The laboratory CV is 6.9% at 172.9 ug/dL and 3.3% at 222.4 ug/dL.

*Sample type: Serum/heparin plasma**Minimum volume needed: 150uL**Sample volume used: 8uL**Dead volume: 75uL***Urea Nitrogen**

Dates of Use: 2006 - current

Urea nitrogen is measured in serum or plasma using the Roche Urea/BUN reagent on the Roche Modular P Chemistry analyzer (Roche Diagnostics Corporation). In this reaction, urea is hydrolyzed by urease to form CO₂ and ammonia. The ammonia produced reacts with alpha-ketoglutarate and NADH in the presence of glutamate dehydrogenase to yield glutamate and NAD⁺. The decrease in absorbance as NADH is converted to NAD⁺ is measured kinetically at 340 nm. The rate of NADH oxidation is directly proportional to the concentration of urea. The laboratory interassay CV is 4.4%.

*Sample type: serum/EDTA plasma/lithium-heparin plasma**Minimum volume needed: 150uL**Sample volume used: 3uL**Dead volume: 75uL***Uric Acid**

Dates of Use: 2006 - current

Uric acid is measured in serum or plasma using an enzymatic colorimetric assay kit (Roche Diagnostics, Indianapolis, IN 46250) and read on the Roche Modular P Chemistry analyzer (Roche Diagnostics). The reference range is 3.4 – 7.0 mg/L. The inter-assay CV range in our laboratory is 1.9%.

*Sample type: serum/EDTA plasma/lithium-heparin plasma**Minimum volume needed: 150uL**Sample volume used: 5uL**Dead volume: 75uL*

(soluble) Vascular Cell Adhesion Molecule-1 (sVCAM-1)

Dates of Use: 2004 - 2007

sVCAM-1 (soluble vascular cell adhesion molecule-1) is measured in plasma or serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Parameter kit from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 8.9-10.2% for the serum or plasma assay.

Sample type: serum/EDTA plasma/heparin plasma

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

(soluble) Vascular Cell Adhesion Molecule-1 (sVCAM-1)

Dates of Use: 2008 - current

sVCAM-1 (soluble vascular cell adhesion molecule-1) is measured in serum or plasma using the quantitative sandwich enzyme technique of the enzyme immunoassay Human sVCAM-1 Quantikine assay from R & D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 8.9-10.2% for the serum or plasma assay. Our laboratory CV is <10%.

Sample type: serum/EDTA plasma/heparin plasma

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Vascular Endothelial Growth Factor (VEGF)

Dates of Use: Proposed

VEGF is measured in serum or plasma using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) Quantikine kit from R & D Systems (Minneapolis, MN). A monoclonal antibody specific for VEGF is pre-coated onto a microplate. Any VEGF present in the sample is bound by the immobilized antibody. After washing, an enzyme-linked polyclonal antibody specific for VEGF is added and binds to the immobilized, bound VEGF. Following a wash, a substrate solution is added and color develops in proportion to the amount of VEGF bound in the initial step. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California). The inter-assay CV range reported in the kit insert is 8.8 to 6.2% at concentrations ranging from 64 to 1003 pg/mL.

Sample type: serum/EDTA plasma/heparin plasma/citrate plasma

Minimum volume needed: 125uL

Sample volume used: 10uL

Dead volume: 50uL

Vitamin B6 (Pyridoxal-5'-Phosphate)

Dates of Use: 2000 - 2004

Vitamin B6 was measured in serum by a radioenzymatic assay from ALPCO Diagnostics (American Laboratory Products Company, Ltd. Windham, NH). This method is based on the decarboxylation of ³H-tyrosine; excess ³H-tyrosine is measured by liquid scintillation counting.

Vitamin B12

Dates of Use: 2004

Vitamin B12 was measured in serum on the Hitachi 911 (Roche Diagnostics, Indianapolis, IN 46250) using the CEDIA[®] Vitamin B₁₂ enzyme immunoassay (Boehringer Mannheim).

Vitamin B12

Dates of use: 2007-2009

The ADVIA[®] Centaur (Siemens Diagnostics, Deerfield, IL) Vitamin B12 (VB12) assay is a competitive immunoassay using direct chemiluminescent technology measured in serum or plasma. VB12 in the patient sample competes with acridinium ester-labeled VB12 in the LITE Reagent for a limited amount of intrinsic factor, which is covalently coupled to paramagnetic particles in the Solid Phase. The ADVIA[®] Centaur VB12 assay uses Releasing agent (sodium hydroxide) and DTT to release the VB12 from endogenous binding proteins in the sample and cobinamide to prevent rebinding after the Solid Phase is added to the sample.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 325uL

Sample volume used: 120uL

Dead volume: 150uL

Vitamin D2 and D3 (25-OH)

Dates of Use: Biochemical Genetics

25-OH Vitamin D2 (ergocalciferol, present in small amounts from food of plant origin) and D3 (cholecalciferol, obtained from foods of animal origin and UV light) are measured in serum or plasma using Liquid chromatography/tandem mass spectrometry (Water's 2795 Liquid Chromatogram equipped with an autosampler and column oven). Samples are injected into a C18 Water's XTerra column and, using post column splitting, into the ionization source of a Micromass Quattro tandem mass spectrometer. Data is obtained using the MassLynx software with automated data processing by QuanLynx software. Concentrations of D2, D3 and total vitamin D are reported. The laboratory CV is 3.7, 5.6 and 19.7% at total vitamin D concentrations of 165, 57 and 8 ug/L, respectively.

Sample type: Serum (preferred)/EDTA plasma/lithium-heparin plasma/sodium-heparin plasma

Minimum volume needed: 300uL

Sample volume used: 200uL

Dead volume: 50uL

Vitamin D (1,25-OHD)

Dates of Use: 2010-current

1,25 dihydroxy vitamin D (1,25 vitD) is measured in serum or plasma using an enzyme immunoassay (EIA) from Immunodiagnostic Systems, Inc (Fountain Hills, AZ 85269). Serum or plasma samples are delipidated by incubating sample with a solution of dextran sulfate and magnesium chloride. The delipidated sample is then incubated with monoclonal antibody to 1,25 vitD linked to solid phase particles in suspension with vitamin D binding protein inhibitor. After elution, the immunopurified sample is dried and reconstituted in assay buffer prior to the EIA. Reconstituted eluates and calibrators of known concentration are incubated with a highly-specific sheep anti-1,25 vitD antibody. A portion of this is incubated in microplate wells that are coated with a specific anti-sheep antibody. 1,25 vitD linked to biotin is then added, and the plate incubated before aspiration and washing. Horseradish peroxidase-labeled avidin is added and binds selectively to complexed biotin and, following a further wash step, color is developed using tetramethylbenzidine and hydrogen peroxide. The absorbances of the stopped reaction mixtures are read in a spectrophotometric plate reader (SpectraMax 250; Molecular Devices, Sunnyvale, CA 94089), the color intensity being inversely proportional to the concentration of 1,25 vitD. Inter-assay CVs for this assay are <11%.

Sample type: Serum/EDTA plasma/heparin plasma

Minimum volume needed: 525uL

Sample volume used: 500uL

Dead volume: none

von Willebrand**Dates of Use: Coagulation Lab**

von Willebrand antigen is determined using an in-house enzyme-linked immunosorbent assay (ELISA). Rabbit anti-human von Willebrand factor (Dako Corporation, Carpinteria, CA) is used to coat wells of a microplate. Samples of sodium citrate plasma are added to the wells and any von Willebrand antigen present will bind to the antibody on the microwell wall. Peroxidase-conjugated rabbit anti-human von Willebrand antibody (Dako Corporation, Carpinteria, CA) is added and will bind the immobilized von Willebrand antigen. Orthophenylenediamine (Dako Corporation, Carpinteria, CA) serves as the substrate for cleavage by the peroxidase. The intensity of the color is measured on a KC Junior Plate Reader (Biotek Instruments, Inc., Winooski, VT). The laboratory CV is 4.7%.

White Blood Cell Count**Dates of Use: Hematology Lab**

White blood cell count is determined by an automated method on the Coulter LH 750 analyzer (Beckman Coulter, Inc. Fullerton, CA 92834). Within the LH750, each cell is suspended in an isotonic electrolyte solution (LH 700 series diluent). An electrical pulse is generated when a cell passes through an aperture in the analyzer. These electrical pulses can be counted and sized. The laboratory CV is <1.7% (for the range 9.0-11.0 x 10⁹/L).