NHANES 2001-2002 Public Release Dataset
June 2007
Laboratory 11 – C-reactive protein (CRP), Fibrinogen, Bone Alkaline Phosphatase and Urinary N-telopeptides

This is being updated to add Urinary N-telopeptides.

(1) Documentation File Date- February, 2007

(2) Documentation File Name- Laboratory 11 – C-reactive protein (CRP), Fibrinogen, Bone Alkaline Phosphatase and Urinary N-telopeptides

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

(4) Component Description

4.1 C-reactive protein

C-reactive protein is considered one of the best measures of the acute phase response to an infectious disease or other cause of tissue damage and inflammation. It is used to correct the iron status measures, which are affected by inflammation. It can also be used to measure the body's response to inflammation from chronic conditions, such as arthritis, and environmental exposures to agents such as tobacco smoke.

4.2 Fibrinogen

Fibrinogen is an essential blood-clotting factor and is involved in a range of other functions, including platelet aggregation and smooth muscle proliferation. A growing body of evidence has identified fibrinogen as an important risk factor for cardiovascular disease, the major cause of death in the U.S. The objective of including this measure was to provide data on laboratory, clinical, and socio-demographic correlates of fibrinogen levels. Of particular importance in NHANES, the data can be used to study the relationship between fibrinogen levels and clinically measured lower extremity arterial blood flow as assessed by the Ankle-Brachial Index in the Lower Extremity Disease component.

4.3 Bone alkaline phosphatase and N-telopeptides

Evaluation of bone mineral status can utilize measures of total bone mineral content and bone mineral density. Serum bone alkaline phosphatase is a marker of bone formation and urinary N-telopeptides are markers of bone resorption.

(5) Sample Description:

5.1 Eligible Sample

C-reactive protein (CRP)

Participants aged 3 years and older were tested.

5.2 Fibrinogen

Participants aged 40 years and older were tested.

5.3 Bone alkaline phosphatase

Participants aged 8 years and older were tested.

5.4 N-telopeptides

Participants aged 8 years and older were tested, but data was released for twenty years and older. The data for 8-19 years is available in the Research Data Center at the National Center for Health Statistics.

(6) Description of the Laboratory Methodology

6.1 C-reactive protein
This method quantified C-reactive protein (CRP) by latex-enhanced nephelometry. Particle-enhanced assays were based on the reaction between a soluble analyte and the corresponding antigen or antibody bound to polystyrene particles. For the quantification of CRP, particles consisting of a polystyrene core and a hydrophilic shell were used in order to link anti-CRP antibodies covalently. A dilute solution of test sample was mixed with latex particles coated with mouse monoclonal anti-CRP antibodies. CRP present in the test sample forms an antigen-antibody complex with the latex particles.

An automatic blank subtraction was performed. CRP concentrations were calculated by using a calibration curve. Data reduction of the signals was performed by using a storable logit-log function for the calibration curve performed data reduction of the signals. These assays were performed on a Behring Nephelometer for quantitative CRP determination.

6.2 Fibrinogen

On the STA-Compact, the Clauss clotting method determined the fibrinogen concentration in plasma quantitatively. This test method involves measuring the rate of fibrinogen to fibrin conversion in diluted sample under the influence of excess thrombin. Since under these conditions the fibrinogen content was rate limiting, the clotting time can be used as a measure of the concentration of the fibrinogen and in fact, the clotting time is inversely proportional to the level of fibrinogen in the plasma.

Clot detection by the STA-Compact involved an electromagnetic-mechanical system. The oscillation of a steel ball within the cuvette with the thrombin and diluted plasma was monitored by the STA-Compact. When the oscillation of the steel ball was stopped by clot formation, the sensor registered the time in seconds. The time was translated into fibrinogen concentration from a fibrinogen standard curve, stored on the STA Compact.

6.3 Bone alkaline phosphatase

There were two methods used to measure Bone Alkaline Phosphatase in NHANES 2001-2002.

For NHANES 2001, the HybritechTandem-MP Ostase ImmunoEnzymetric assay was used for quantitative measurement of Bone Alkaline Phosphatase (BAP), an indicator of osteoblastic activity, in human serum. The Hybritech BAP was measured using a Bio Tek EL 808 Automated Microtiter Plate Reader or the Rosys Plato 3301.

The Hybritech Ostase assay was a solid phase, monoclonal antibody immnumoenzymetric assay. Samples containing BAP were reacted with a biotin-labeled, BAP-specific monoclonal antibody. The reaction was performed in a plastic well strip (solid phase) coated with strepavidin and enclosed in a plastic frame. Following the formation of a solid phase/capture antibody/BAP complex, the microplate was washed to remove unbound BAP and was then incubated with an enzyme substrate. The amount of substrate turnover was determined colorimetrically by measuring the absorbance of the quenched reaction at 405 nm in a microplate reader. The absorbance was proportional to the concentration of BAP present in the test sample. The calculation of BAP concentration in the sample was based on concurrent testing of BAP calibrators and the Zero Diluent/calibrator.

For NHANES 2002, the Beckman Access Ostase assay was used to measure serum Bone Alkaline Phosphatase. The Access Ostase assay is a

one-step immunoenzymatic assay. A mouse monoclonal antibody specific to BAP is added to a reaction vessel with paramagnetic particles coated with goat anti-mouse polyclonal antibody. Calibrators, controls and samples containing BAP are added to the coated particles, and bind to the anti-BAP monoclonal antibody. Following the formation of a solid phase/capture antibody/BAP complex, separation in magnetic and washing remove materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos*530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of BAP in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

6.4 Urinary N-telopeptides

There were two methods used to measure Urinary N-telopeptides in NHANES 2001-2002.

In NHANES 2001, the Osteomark assay was used and is a competitive inhibition enzyme linked solid-phase immunosorbent assay for the quantitative measurement of the cross-linked N-telopeptides of type I bone collagen (Ntx) in human urine. The solid phase consists of microwells onto which cross-linked telopeptides (antigen) are adsorbed. Urine controls, test samples and calibrators were added to the antigen coated 96 well plates. Antibody to the N-telopeptide cross-links that are conjugated to horseradish peroxidase was then added to each well. During an initial incubation period, antigen in the sample competed with the solid phase antigen for binding to the antibody. The wells were then washed to remove unbound material.

Buffered substrate/chromogen reagent was then added to each well. During the final incubation, a blue color can develop when bound antibody-horseradish peroxidase conjugate was present in the well. The color intensity was a measure of the amount of conjugated antibody bound to the solid phase antigen, and was inversely proportional to the amount of antigen in the test sample. The reaction was stopped by the addition of stopping reagent (1N sulfuric acid) which results in a color change from blue to yellow. The absorbance values for the control, calibrators and test samples were determined spectrophotometrically at 450 nm with a 650 nm reference filter, by using a microtiter plate reader.

In NHANES 2002, N-telopeptides was measured using the Vitros Eci instrument. The Vitros NTx assay is a competitive immunoassay technique using a synthetic NTx peptide which has been coated on the reaction wells provided in the reagent pack. This assay depends on competition between this synthetic peptide and the NTx present in the specimen being tested.

These two sources of peptide compete for binding with a horse radish peroxidase (HRP) -labeled antibody conjugate (mouse monoclonal anti-NTx). The conjugate is captured by the peptide coated on the wells. Any unbound materials are removed through a washing step.

The bound HRP conjugate is then measured through the addition of a luminescent substrate. This signal reagent contains the luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative. This produces a light signal. The electron transfer agent (a substituted acetanilide) increases the level of the light and prolongs its emission. This light signal is read by the Vitros. The level of HRP conjugate bound is indirectly proportional to the concentration of NTx present.

(7) Laboratory Quality Control and Monitoring

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

(8) Data Processing and Editing

Blood and urine specimens are processed, stored and shipped to University of Washington, Seattle, Washington. Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the Analytic methodology section.

(9) Data Access:

All data are publicly available.

(10) Analytic Notes for Data Users:

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

files may be linked to the laboratory data file using the unique survey participant identifier SEQN.

10.2 Adjustment of Bone Alkaline Phosphatase was necessary for NHANES 2001 data because of a change of laboratory methods (see section 6.3) between 2001 (using the Hybritech method) and 2002 (using the Beckman Access method) The distributions of sample person results were compared between NHANES 2001 and NHANES 2002 and the BAP test had significantly ($p < 0.05$) different means. A cross-over study between the two methods was performed to establish regression equations to convert NHANES 2001 values to NHANES 2002 values. The regression equations were applied to the BAP test, and a t test was done after regression that showed no significant differences of BAP test means between the two methods after regression.

10.3 Adjustment of Urinary N-telopeptides was necessary for NHANES 2001-2002 data because of a change of laboratory methods (see section 6.4) from 2001 (Osteomark) to 2002 (Vitros). The distributions of sample person results were compared between NHANES 2001 and NHANES 2002 and the NTX was higher in 2001 when compared to 2002. A cross-over study between the two methods was performed to establish regression equations to convert NHANES 2002 values to NHANES 2001 equivalent values. The regressions were validated for NTX values up to 4000 nM. Many children (8-19 years) had values above 4000 nM, but only one adult (20+ years) had a value greater than 4000 nM. Data for adults (20+ years) was released and data for 8-19 years is available in Research Data Center at the National Center for Health Statistics.