NHANES 2001-2002 Data Release
September 2004
Documentation for Laboratory Results

Laboratory 13 – Total Cholesterol and HDL-Cholesterol

(1) Documentation File Date – September 28, 2004

(2) Documentation File Name – Laboratory 13 – Total Cholesterol and HDL-Cholesterol

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

(4) Component Description

The data will be used to monitor the status of hyperlipidemia and the success of the National Cholesterol Education Program.

(5) Sample Description:

5.1 Eligible Sample

Participants aged 3 years and older were tested.

(6) Description of the Laboratory Methodology

Total Cholesterol

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase-catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

cholesteryl ester + H_2O	cholesteryl ester hydrolase →	cholesterol + fatty acid
cholesterol + O_2	cholesterol oxidase →	cholest-4-en-3-one + H_2O_2
$2H_2O_2$ + 4-aminophenazone + phenol	peroxidase →	4-(*p*-benzoquinone-monoimino)-phenazone + 4 H_2O

HDL-Cholesterol

In NHANES 2001-2002, HDL-cholesterol was measured by two methods including a heparin-manganese (Mn) precipitation method and a direct immunoassay technique. The heparin-Mn method was used for participants ages 6 and above. The direct method is used for participants ages 3-5 and for participants with no heparin-Mn HDL-cholesterol values, usually as a result of limited sample volume.

Heparin-Mn Precipitation Method
Apolipoprotein B (apoB)-containing lipoproteins are removed by precipitation with heparin sulfate and $MnCl_2$, and cholesterol is measured in the HDL-containing supernatant. Cholesterol in the HDL-containing supernatant is measured as described above for total cholesterol.

HDL-Cholesterol Direct Immunoassay Method
HDL is measured directly in serum. The apoB-containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay.

The reagents were purchased from Roche/Boehringer-Mannheim Diagnostics. The method uses sulfated α-cyclodextrin in the presence of Mg^{+2}, which forms complexes with apoB-containing lipoproteins, polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase, for the HDL-cholesterol measurement. The reactions are as follows.

apoB-containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 →
soluble non-reactive complexes with apoB-containing lipoproteins

HDL-cholesteryl esters + PEG-cholesteryl esterase →
HDL-unesterified cholesterol + fatty acid

unesterified cholesterol + O_2 PEG-cholesterol oxidase → cholestenone + H_2O_2

H_2O_2 + 5-aminophenazone + *N*-ethyl-*N*-(3-methylphenyl)-*N*′-succinyl ethylene diamine + H_2O + H^+ peroxidase → quinoneimine dye + H_2O

Absorbance is measured at 600 nm.

(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 specimens were processed, stored and shipped to Johns Hopkins Hospital, Baltimore, MD for analysis. Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the Description of the Laboratory Methodology section.

(9) Data Access:

All data are publicly available.

(10) Analytic Notes for Data Users:

Correction of HDL Method

Initial observations of the participants' HDL-cholesterol values showed approximately a 6% decrease in mean values when compared to participant HDL-cholesterol values in NHANES III. The precipitation and direct immunoassay methods were investigated for analytical bias. The heparin-manganese precipitation method and direct immunoassay method for 2001-2002 showed a negative bias when compared to HDL-cholesterol quality controls (Solomon Park Research Laboratories, Kirkland, WA), with assigned values established by the Centers for Disease Control and Prevention. The CDC HDL-cholesterol reference method uses heparin-manganese to precipitate HDL and the Abell-Kendall method to measure cholesterol.

The participants' HDL-cholesterol values for both the precipitated and direct methods were corrected as follows:

$$\text{corrected HDL} = \frac{\text{(Solomon Park assigned HDL value) x (participant HDL)}}{\text{(quality control HDL value associated with participant sample)}}$$

A batch of participants' HDL-cholesterol values was run with Solomon Park quality controls during 2001-2002. Each participant's HDL-cholesterol was adjusted by comparing the associated Solomon Park quality control value to the assigned HDL-cholesterol value. The participants' precipitated HDL-cholesterol values were corrected by an average of +4.3% for 1999-2000. The participants' direct HDL-cholesterol values were corrected by an average of +2.1%. The variance, skewness, and kurtosis of the uncorrected and corrected HDL-cholesterol distributions of 2001-2002 participants compared well.

The derived HDL-cholesterol (LBDHDL) was generated as follows:

1. Use corrected direct HDL value if corrected precipitated HDL value is not available.
2. Use corrected precipitated HDL values if corrected direct HDL value is not available.
3. If both corrected precipitated and direct HDL values were analyzed:
    a. Use corrected direct HDL value for ages 3-5.
    b. Use corrected precipitated HDL values for ages greater than 5.

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

LBXTC:

The Lab 13 Total Cholesterol data file (at http://www.cdc.gov/nchs/about/major/nhanes/nhanes2001-2002/l13_b.xpt) contains laboratory test results for total cholesterol (LBXTC), which uses the reference analytic method. However, the NHANES Laboratory 40 biochemistry profiles also include measurements of total cholesterol (Laboratory 40 variable name is LBXSCH). The appropriate variable to use is LBXTC from Laboratory 13.

LBDTCSI:

The total cholesterol in mg/dL (LBXTC) was converted to mmol/L (LBDTCSI) by multiplying by 0.02586.

LBDHDL:

HDL-cholesterol was derived from two HDL-cholesterol methods. See this section (**Analytic Notes for Data Users**) above.

LBDHDLSI:

The HDL-cholesterol in mg/dL (LBDHDL) was converted to mmol/L (LBDHDLSI) by multiplying by 0.02586.

(11) References

See the references in the associated Laboratory Procedures Manual file.