

NHANES 1999–2000 Public Data Release

Laboratory 13 – Total Cholesterol and HDL-Cholesterol (September 2003)

Description

Total Cholesterol and HDL-Cholesterol

The goals of this component are: 1) to monitor the prevalence and trends in major cardiovascular conditions and risk factors in the U.S.; and 2) to evaluate prevention and treatment programs targeting cardiovascular disease in the U.S.

The main element of the cardiovascular disease laboratory component in NHANES is blood lipid levels. Cardiovascular disease is the leading cause of death in the United States. The data will be used to monitor the status of hyperlipidemia and the success of the National Cholesterol Education Program.

Eligible Sample

Participants aged 3 years and older who do not meet any of the exclusion criteria were sampled.

Data Collection Methods

In the mobile examination center (MEC) laboratory, blood specimens are processed, stored, and shipped to the Johns Hopkins University Lipoprotein Analytical Laboratory for analysis.

Examination Protocol

Detailed specimen collection and processing instructions are described in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Vials were stored under appropriate temperature conditions (stored at -20 degrees Centigrade) until they were shipped to Johns Hopkins University Lipoprotein Analytical Laboratory for testing. The analytical methods are described in the Analytic Methodology section of this document.

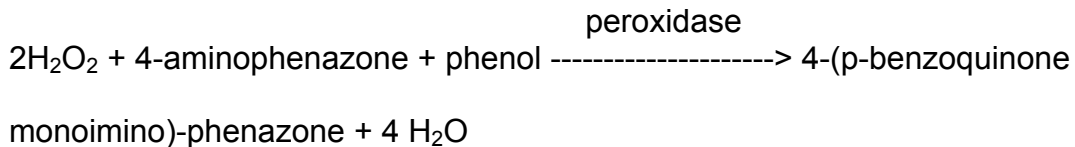
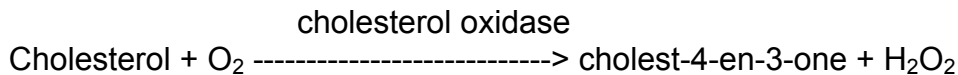
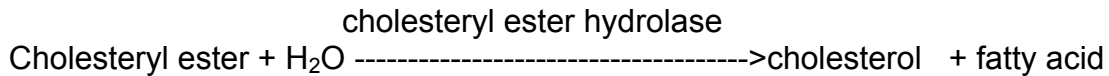
Data Collection

Detailed specimen collection and processing instructions are discussed in the LPM. Each chapter in the LPM specifies the procedure to be used for preparation, labeling, processing, preservation, and transport of the specimens.

Analytic 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, H₂O₂ 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:



HDL-Cholesterol

In NHANES 1999-2000, HDL-cholesterol was measured using two methods. A heparin-manganese (Mn) precipitation method and a direct immunoassay technique were used. The heparin-Mn method was used for participants ages 6 years and above. The direct method is used for participants ages 3-5 and for participants with no heparin-manganese HDL-cholesterol values, usually as a result of limited sample volume. HDL-cholesterol values less than 40 mg/dL are associated with increased coronary heart disease risk in adults.

Heparin-Mn Precipitation Method

Apolipoprotein B containing lipoproteins are removed by precipitation with heparin sulfate and MnCl₂ 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 apolipoprotein B 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 are purchased from Roche/Boehringer-Mannheim Diagnostics. The method uses sulfated alpha-cyclodextrin in the presence of Mg⁺², which forms complexes with apoB containing lipoproteins, and 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 $\xrightarrow{\text{PEG-cholesteryl esterase}}$ HDL-unesterified cholesterol + fatty acid

Unesterified cholesterol + $O_2 \xrightarrow{\text{PEG-cholesterol oxidase}}$ 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.

Correction of HDL Method

Initial observations of the participants' HDL-cholesterol values showed approximately a 6% decrease in mean values when compared to NHANES III HDL-cholesterol values. The precipitation and direct immunoassay methods were investigated for analytical bias. The heparin-manganese precipitation method and direct immunoassay method for 1999-2000 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-cholesterol and the Abell-Kendall method to measure cholesterol.

The NHANES 1999-2000 HDL-cholesterol values for both the precipitated and direct methods were corrected as follows:

Corrected HDL = $\frac{(\text{Solomon Park assigned HDL value}) \times (\text{Participant HDL})}{(\text{Quality Control HDL value associated with Participant sample})}$

A batch of participants' HDL-cholesterol values was run with Solomon Park quality controls during NHANES 1999-2000. 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 +5.2% for 1999-2000. The participants' direct HDL-cholesterol values were corrected by an average of +0.4%. The variance, skewness, and kurtosis of the uncorrected and corrected HDL-cholesterol distributions of 1999-2000 participants compared well. The corrected precipitated HDL-cholesterol method was compared to the corrected direct HDL-cholesterol method for 872 participants. The linear regression revealed:

Precipitated HDL = 1.02 x (direct HDL), with non-significant intercept and a r-square of 0.932.

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

1. Use corrected direct HDL values for ages 3-5 and compute corrected precipitated HDL from linear regression (above).
2. Use corrected direct HDL for all ages when precipitated HDL was unavailable and compute corrected precipitated HDL from linear regression (above).
3. Use corrected precipitated HDL when available for ages greater than 5 years.

Analytic Notes

LBXTC:

The Laboratory 13 data file contains laboratory test results for total cholesterol (LBXTC), which uses the reference analytic method. However, the NHANES Laboratory 18 biochemistry profiles also include measurements of total cholesterol (Laboratory 18 variable name: LBXSCH). In general, for most analyses, the appropriate variable to use is LBXTC. The value from the biochemistry profile (LBXSCH) should not be used routinely.

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 analytic methodology section above.

LBDHDLSI:

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

Sample Weights

Use the full sample weight (WTSMEC2YR) and the jackknife replicate sample weights (WTMREP01-WTMREP52) for serum total cholesterol and high density lipoprotein (HDL) cholesterol analyses.

The full sample weights are used to estimate means, percentages, medians and other percentiles, and regression coefficients.

The 52 jackknife replicate weights are used to estimate standard errors of these statistics.

Special Notes for this Dataset

The analysis of NHANES 1999-2000 laboratory data must be conducted with the key survey design and basic demographic variables. The NHANES 1999-2000 Household Questionnaire data files contain demographic data, health indicators, and other related information collected during the household components. The Household Questionnaire data files include all of the survey design variables and sample weights required to analyze these data. The Phlebotomy Examination file includes auxiliary information on duration of fasting, the time of day of the venipuncture, and the conditions precluding venipuncture. The Household Questionnaire and Phlebotomy Exam files may be linked to the laboratory data file using the unique survey participant identifier SEQN.