

Graduate School of Public Health - Nutrition Laboratory

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Research Associates: 6

Students: 3

Facilities: The Nutrition Laboratory is located in five rooms which occupy 2,000 square feet in the Department of Epidemiology at the University of Pittsburgh's Graduate School of Public Health. The laboratory is equipped with two Sterilgard Biological Safety cabinets; an Abbott VP Supersystem Bichromatic Analyzer; Biorad model 550 microplate reader, Bio-Tek Synergy 2 absorbance, fluorescence luminescence microplate reader, Bio-Tek ELx405 microplate washer, an Elan Atac 8000 Automatic Analyzer; an Olympus AU400 Chemistry Analyzer; a Perkin-Elmer 8410 capillary gas chromatograph with FID detector and Shimadzu C-R3A integrator; a Perkin Elmer Clarus 500 gas chromatograph with an FID detector, an auto sampler, a HP Pentium 4 with a data handling system and a HP Laserjet 1300; a Waters HPLC System, (two 6000A pumps, WISP 712 automatic injector, 2996 Photodiode array detector, 490 Programmable detector, a Pentium 2 Gateway computer, Millennia software, a HP Laserjet 2100); a Packard Cobra II Gamma Counter; a LS-S fluorescence spectrophotometer; a Carey 50 uv/visible spectrophotometer; 2 Centra GP8R refrigerated centrifuges; an Optima TLX ultracentrifuge; three -20°C freezers; twenty -70°C freezers; a Mettler H542 analytical balance; an APX-100 analytical balance; a Buchler Vortex-Evaporator.

Quality Control: Outlines of the analytical procedures employed in the laboratory are given below. Initially the accuracy and precision of each technique is verified. Briefly, blanks, controls, samples and standards are ran on multiple occasions. Accuracy is estimated by comparing values obtained for the reference controls with their stated values. Precision is evaluated as the coefficient of variation both intra-assay and inter-assay (reported with procedure outline).

Ongoing quality control is monitored by plotting the mean values and the difference between the duplicates for the control samples versus time. The 95% and 99% confidence limits are indicated on the graphs.

The laboratory has maintained the required levels of proficiency to be included in the CDC-NHLBI Lipid Standardization Program since 1982.

At quarterly intervals, the CDC sends 36 samples to be analyzed for total cholesterol (high:100 to 400 mg/dl), total cholesterol (low:<100 mg/dl), HDL cholesterol and triglyceride. Nine samples are analyzed weekly for four weeks. The results are returned to CDC and the mean and standard deviation compared to the accepted mean and standard deviation.

In February 2004, the laboratory obtained CLIA certification. The laboratory was found to be in complete compliance with both the state's clinical laboratory regulation and those of the Centers for Medicare and Medicaid Services. Accordingly, the laboratory became enrolled in a proficiency testing program organized by the College of American Pathologists (CAP). Three times a year CAP sends samples for analysis of glucose, insulin, total cholesterol, HDLc and LDLc (both calculated and direct) and triglycerides. Results are evaluated for accuracy and precision.

Sample Collection. Blood is collected at the antecubital vein from subjects in a reclined position into red-top tubes (serum) and lavender tubes (EDTA, plasma) (Vacutainer, Becton-Dickinson). The samples are kept on ice and centrifuged (1500g for 15 min at 4°C) within 90 min of collection. The samples are then aliquoted (1 ml) and stored at -70°C until analysis.

Sample Shipment. Samples are shipped to Pittsburgh (533 Parran Hall, GSPH, University of Pittsburgh, 130 DeSoto Street, Pittsburgh, PA 15261, Attention: Ms. B. Hauth) in accordance with Department of Transport regulations for biological hazards, overnight and on dry ice. Delivery on weekends should be avoided.

Procedures: (Volumes refer to plasma/serum)

Total Cholesterol (100 µl of sample). Total cholesterol is determined using the enzymatic method of Allain et al (1974). This procedure involves the hydrolysis of cholesterol esters by cholesterol esterase, oxidation of cholesterol by cholesterol oxidase with formation of hydrogen peroxide and finally, a peroxidase catalyzed reaction of hydrogen peroxide with 4-aminoantipyrine and phenol to form Quinoneimine dye. The intensity of the color produced is directly proportional to the total cholesterol concentration in the sample. Duplicate samples with standards, control sera and serum calibrators are included in each run. The coefficient of variation between runs is 1.3%.

Triglycerides (100 µl of sample). Triglycerides are determined enzymatically using reagents from Clinical Diagnostics based on the procedure of Bucolo et al (1973). Briefly, the triglycerides are hydrolyzed by lipase; the glycerol phosphorylated by ATP and glycerol kinase to yield glycerol-1-phosphate. The Glycerol phosphate is oxidized in the presence of glycerol phosphate oxidase to produce hydrogen peroxide. The hydrogen peroxide reacts with the p-chlorophenol and 4-aminoantipyrine in the reagent to produce a Quinoneimine dye which is read at 510/630 nm on the ATAC 8000 Chemistry analyzer. The intensity of the color produced is directly proportional to the total triglyceride concentration in the sample. Duplicate samples, standards and control sera are included in each run. Coefficient of variation between runs is 1.7%.

HDL cholesterol Direct (3µl of plasma or serum). This is a two reagent method using materials obtained from Vital Diagnostics. In the first step non HDL-esterified and free cholesterol are selectively consumed by cholesterol oxidase, peroxidase and DSBmt (5 min at 37°C) to form colorless products. A second reagent is added containing cholesterol esterase, a chromogenic coupler, and a detergent capable of selectively solubilizing the HDL cholesterol permitting the reaction of HDL cholesterol with cholesterol esterase and cholesterol oxidase. The H₂O₂ formed reacts with a peroxidase, DSBmT and 4-AAP (5 min at 37°C) to produce a colored product measured at 578 nm. The change in absorbance is proportional to the concentration of HDL cholesterol in the sample. Duplicate samples, calibrators and controls are run with each set. The reportable range is 2.5 to 200 mg/dl.

LDL. LDL is calculated indirectly using Friedewald (1972) equation:
$$LDL_c = \text{Total Cholesterol} - HDL_c - 0.2 (\text{total TG})$$

(Not applicable if total TG > 400 mg/dL). Note: if Lp(a) is measured then this calculation should be modified to include subtracting: 0.3[Lp(a)].

LDL Direct (3 μ l of sample [serum or plasma]). LDL is measured directly using an automated spectrophotometric assay, LDL Direct Liquid Select, from Equal Diagnostics. The principle of the assay involves solubilizing non LDL particles and removing their cholesterol and then solubilizing the LDL and measuring their cholesterol content. In brief, 3 μ l of sample is incubated with 300 μ l of buffer containing detergent, cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine and ascorbic acid oxidase for 5 min. at room temperature. Then, 100 μ l of buffer containing detergent and N, N-bis (4-sulphobutyl) -m- toluidine-disodium is added and the mixture incubated at room temperature for 10 min. The absorbance is then read at 550/650 nm. Standards (100-200 mg/dL), blanks and control pools are run with each assay. The coefficient of variation is $2.0 \pm 0.3\%$ (22). The analysis works with non-fasting samples (TG<1,300 mg/dL).

Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-475.

Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19:476-482

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.