Pacific Biomarkers
The Value of Expertise

HDL-C Subclasses by Precipitation (HDL2/HDL3)

Analyte: HDL subfractions HDL2/HDL3

Specimen Type: Serum

Optimum Volume: 1 mL

Stability:

2-8 Degrees C	-20 Degrees C	-70 Degrees C
5 days	2 months	2 years

Reporting Units: mg/dL

Method: Precipitation & Enzymatic

Biological or Clinical Significance:

HDL subclasses are separated by selective precipitation using dextran sulfate (50 kDa) and Mg2+ followed by quantitation of cholesterol in the total-HDL and HDL-3 fractions; the HDL-2 cholesterol content is obtained by calculation. The total HDL particle-contaning fraction is prepared by removing Apo B-containing lipoproteins (VLDL and LDL) by dextran sulfat/Mg2+ precipitation of serum or EDTA plasma. The HDL-3 particle-containing fraction is prepared from a second aliquot of sample by adding a higher concentration of dextran sulfate and Mg2+ to remove VLDL, LDL and HDL-2 by precipitation. Cholesterol is measured in the HDL-3 supernate and the total HDL supernate. HDL-2 cholesterol compositions are calculated by subtracting HDL-3 components from total HDL components. Selective chemical precipitations have been shown to agree well wtih preparative and analytical ultracentrifugation.

Principle of Test Method:

Separation of the high density lipoprotein fraction from plasma or serum in the clinical laboratory is routinely performed by chemical precipitation. Subsequent analysis of lipids (particularly cholesterol) in the fraction assists in the determination of risk for heart disease.

This method involves a sulfated polysaccharide, which together with the divalent cation Mg2+, selectively precipitates LDL and VLDL leaving HDL in the supernate. The dextran sulfate method is especially appropriate when analysis is to be performed using enzymatic procedures, as it does not interfere with most reagents. Separation of HDL subclasses is achieved by performing a routine total HDL determination (The total HDL method employed is study specific.), followed by a method modification employing higher precipitation reagent concentrations. This modified reagent produces a supernate that is analyzed for the HDL3 subclass. HDL2 is obtained by the following equation.

$$HDL2 = Total\ HDL - HDL3$$

The HDL rich supernates produced from the subclasses procedure can be analyzed for other HDL components such as Apo E, Free Cholesterol, Triglycerides, and Phospholipids.