Serum High Density Lipoprotein Cholesterol

The Different types of Lipoproteins which are called high density lipoprotein (HDL), low density Lipoprotein (LDL), very low density Lipoprotein (VLDL) and chylomicrons have a relatively low density compared with other plasma proteins. Lipoproteins also differ in their electric charge and can be separated by electrophoresis. The variation in the densities of the Lipoprotein fractions is due differences in the proportions of lipids and proteins. Chylomicrons and VLDL are mostly lipid, whereas HDL is only about 50% lipid. Conversely, the protein content is lowest in chylomicrons and highest in HDL. The nature of the lipids also varies from one fraction to another. Chylomicrons and VLDL contain predominantly triglyceride, while the lipid in LDL and HDL is mainly cholesterol and phospholipids.

HDL have a density between 1.063-1.21. they are the smallest of lipoprotein particles. The dry weight of HDL is approximately 50% protein, 28% phospholipids, and 20% cholesterol. Most of the cholesterol is esterified. Only a small amount of triglyceride is present.

HDL have been further divided into two subgroups, HDL₂ and HDL₃, based on centrifugal analysis, HDL₂ has a density of 1.063-1.12 and Wt. of about 375,000 HDL₃ has a density of 1.12-1.21 and M. Wt. Of 200,000 and the two subtypes have different biologic functions.

Most Lipoproteins transport lipids, including cholesterol, from one body [Cell to another where are stored or metabolized. In contrast, there is evidence that HDL functions in the removal of cholesterol from body cells and its rotation to the liver. In the liver cholesterol is catabolized and excreted in the bile. Of particular importance is the removal of cholesterol from the cells of
blood vessel walls, including those of the heart (coronary arteries) and the brain (cerebral arteries). When lipid deposits, especially cholesterol, accumulate in vessel walls, a condition called atherosclerosis is produced. Atherosclerosis is the basis of many important diseases such as myocardial infarction (the usual type of heart attack) and cerebrovascular accidents (strokes). The mechanism by which HDL removes cholesterol from cells involves activation of the enzyme lecithincholesterol acyltransferase (LCAT) by one or more of the apoproteins of HDL. LCAT catalyzes the esterification of free cholesterol in the cell, an essential step in the removal process.

HDL in usually measured by first separating it from other Lipoproteins and then determining its cholesterol content. Ordinary when the term HDL is used, what is meat is HDL-Cholesterol. Under normal fasting conditions about 17% of plasma cholesterol is carried by HDL.

**Principle:**

Numerous techniques are available for HDL cholesterol quantitation. They all, however, are based on two steps:

1. isolation of the HDL and
2. quantitative of the cholesterol in the isolated HDL.

The various method differ primarily on how the HDL fraction is isolated. The cholesterol analyses are usually done by one of the many acceptable cholesterol methods available. One of the most important methods which are used for the isolation of HDL is the "precipitation with polyanion solutions", in which the differential precipitation of lipoproteins with various polyanion solutions is common practice and is more suited for the clinical laboratory because of its simplicity, elimination of expensive instrumentation, speed, and low cost. These techniques are a based on the ability of various agents to precipitate
selectively the major Lipoprotein fractions, except HDL, which is left in the supernatant solution to be quantitative. The agents most frequently employed include:

1. Heparin - manganese chloride.
2. Dextran sulfate - magnesium chloride.
3. Sodium phosphotungstate.
4. Polyethylene glycol.

Of these, the heparin - manganese chloride and phosphotungstate are two of the most frequently used precipitating reagents. According to the phosphotungstate procedure (Method of Lopes - Virella et. al. 1977), the chylomicron, VLDL and LDL are precipitated by phosphotungstate in the presence of magnesium ions and the cholesterol is then estimated in the supernatant.

Note: If the supernatant is not clear completely, dilute the serum (1:1) with Tris - buffer and repeat the whole analysis.

**Clinical Significance**

Total serum Cholesterol is known to useful in predicting the risk of developing atherosclerosis, especially coronary artery disease (CAD), coronary atherosclerosis may lead to myocardial infarction, which is the leading cause of death in the United States and most other countries. Increases in total cholesterol are associated with increased risk of atherosclerosis.

The total cholesterol is a better indicator in persons under age 50 than it is in older age groups. In person over 50 years of age, HDL is a much better indicator of CAD than is total serum Cholesterol. High HDL levels tend to protect against atherosclerosis. The best predictive power is obtained by calculating the ratio of HDL to total Cholesterol.
Concentration of HDL vary from one person to another and from
time to time in a given individual. Inheritance seems to be important.
It is known that persons with familial hyperalphalipoproteinemia,
who have had coronary artery disease (and presumably low HDL
levels) have lower than normal HDL values.

Sex is also a determinant. After puberty, males have
substantially lower HDL levels than females. HDL decreases
with advancing age. Physical activity raises HDL, while
inactivity lowers it. Obese persons tend to have lower than
normal HDL. Smoking decreases HDL and alcohol increases it.

Those factors known to increase the likelihood of
atherosclerosis and coronary artery decrease serum HDL levels.
Low HDL levels predispose development of atherosclerosis and
diseases which result from it. As more is learned about the
functions of HDL in the metabolism of cholesterol, it is likely that it
will become more and more useful in clinical medicine.