

What is HDL cholesterol?

HDL cholesterol is cholesterol that is associated with high density glycoprotein particles. HDL particles are a heterogeneous group of particles which are comprised of cholesterol and Apolipoproteins (Apo A1 and Apo A2). It is the smallest and densest of a wider group of lipoprotein particles which include chylomicrons, VLDL, IDL and LDL). All of which contain cholesterol. Therefore the principal challenge for the measurement of HDL-cholesterol methods is to only measure the HDL associated cholesterol and not to be subject to interference from other lipoprotein associated cholesterol.

Methods have therefore been developed that rely on the separation of the protein particles and then the determination of cholesterol using chemical, enzymatic or other methods.

Ultracentrifugation

Ultracentrifugation of lipoprotein particles and the subsequent measurement of cholesterol is the currently recognized reference method for the determination of HDL associated cholesterol. In ultracentrifugation methods, serum is applied to a tube containing solutions of different densities usually created by using solutions of sodium chloride or Sodium/Potassium bromide. During ultracentrifugation at speeds over 30000 - 70000 rpm (depending on the ultracentrifuge and rotor configuration) a density gradient is formed, the lipoprotein particles sink or float to a point where they have the equivalent density. The density layers are then differentially removed and cholesterol is determined by chemical, enzymatic or other methods.

The advantages of this methodology include:

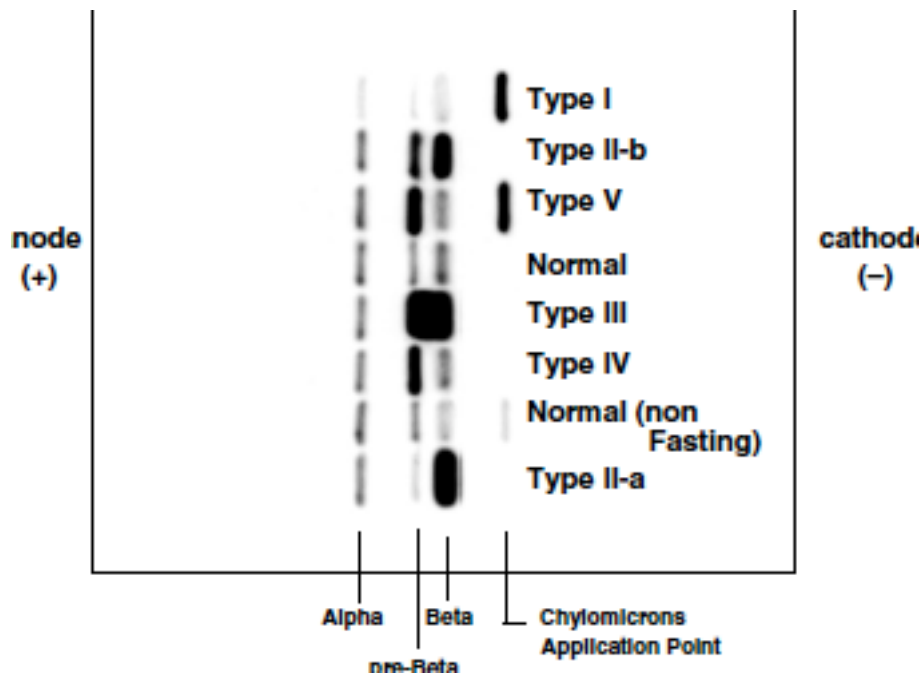
- Good resolution of lipoprotein fractions can be obtained

The disadvantages of this methodology include:

- Time consuming - centrifugation runs can take up to 48 hours
- Dilution of sample - need sensitive cholesterol methods
- In some methods large volumes of sample are required
- Decreased sample throughput
- Ultracentrifugation equipment is not widely available
- Labile lipoproteins can be substantially altered by the high salt concentrations and centrifugal forces used
- Difficult to reproduce due to the wide range of equipment used.
- Can get cross contamination of lipoprotein fractions.

Electrophoresis

Fredrickson and Lees proposed a system for phenotyping hyperlipoproteinemia in 1965. The basis of this classification system was the separation of lipoprotein fractions using cellulose acetate or agarose electrophoresis. In these systems lipoproteins are separated on the basis of mass to charge ratio and are visualized using lipophilic stains such as Fat Red 7B. Quantitation of fractions can be achieved by scanning densitometry.



The advantages of this methodology are:

- Relatively easy to do.
- Inexpensive

The disadvantages of this methodology are:

- No quantitative determination of HDL. As the Fredrickson classification is no longer used this has limited its usefulness
- Can be difficult to interpret

Precipitation Methods

Chemical precipitation uses polyanions, sometimes combined with divalent cations, to selectively aggregate and render insoluble the lower density lipoproteins, leaving HDL in solution. The insoluble lipoproteins can then be sedimented by low-speed centrifugation. The resulting supernatant solution can be analysed for cholesterol analysis as a measure of HDL cholesterol. Heparin with $MnCl_2$ was a popular early combination,

Advantages of this methodology are:

- Simple to do
- Cheap and cost effective
- Traceable to ultracentrifugation methods

Disadvantages of this methodology are:

- Manual step needed and not suited to large numbers of samples
- Problems with specificity of methods in some cases.
- Interference from triglycerides

Direct Methods (Homogenous Methods)

Homogeneous assays do not require off-line pretreatment and separation, eliminating the need for manual pipetting, mixing, and centrifugation steps. They rely on the sequential addition of reagents for example sulfated α -cyclodextrins together with Mg^{2+} have been found to selectively block but not precipitate chylomicrons and VLDL, providing selectivity

without the need for a clearing reagent. HDL-Cholesterol can then be measured enzymatically.

Advantages

Homogeneous assays have been shown to be reasonably well suited for use in routine clinical laboratories, generally meeting the NCEP criteria for precision, accuracy, and total error.

Small volumes

Full automation

Disadvantages

Discrepant results compared with the reference methods have been observed with some of the assays, and the sources of discrepancies are not well characterized.

NMR

This technique simultaneously quantifies the number and size of HDL, LDL and VLDL

Advantages can measure particle numbers and particle size.

Disadvantages

- No directed HDL cholesterol concentration
- Validation of particle size and number with respect to risk equations still needs to be performed.
- Instrumentation not widely available.

Immunologic Methods

Have been reported but not in commercial use.

Various HPLC methods have been used to fractionate lipoproteins, including HDL, but have been hindered by poor stability of the columns. An improved HPLC technique separates lipoproteins, including HDL and its subclasses, on the basis of size and quantifies the cholesterol with enzymatic reagent detection.