Principle of the Method
Polyethylene glycol, average MW 6000, in aqueous solution, is used to precipitate lipoproteins VLDL and LDL. After centrifugation, the clear supernatant containing HDL fraction is suitable for enzymatic determination of cholesterol.

Kit Components
For in vitro diagnostic use only. The components of the kit are stable until expiration date on the label. Keep away from direct light sources.

Reagent A: 4 x 100 ml (liquid) blue cap
Composition: polyethylene glycol 16%, non reactive additives and stabilizers.
Store all components at 2-8°C.

Materials Required but Not Supplied
The kit CT F400 CH or CT 150F CH CHOLESTEROL FL and your STANDARD are needed to perform the colorimetric assay run.

Reagent Preparation
Use reagent ready to use.
Stability: up to expiration date on labels at 2-8°C. Stability since first opening of vials: preferably within 60 days at 2-8°C.

Precautions
Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

Specimen
Serum, plasma EDTA.
Sample is stable 3 days at 2-8°C and 1 month at -20°C.

Test Procedure - Precipitation Step
Pipet in centrifuge tubes:

<table>
<thead>
<tr>
<th>µl</th>
<th>Sample</th>
<th>Reagent</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix by inversion, incubate 5 minutes, centrifuge at 3000 r.p.m. for 10 minutes. Separate supernatant and use it as sample into following procedure.

Test Procedure - Quantitative Step

<table>
<thead>
<tr>
<th>µl</th>
<th>Sample</th>
<th>Reagent</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml</td>
<td>1 ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dispense: blank standard sample
Temperature: 37°C
Lightpath: 1 cm

Results Calculation

HDL cholesterol mg/dl = Ax/Ax as stand. value x 2 (standard value + dilution factor)

Expected Values

<table>
<thead>
<tr>
<th>Men</th>
<th>Average</th>
<th>Low Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 40 mg/dl</td>
<td>40-50 mg/dl</td>
<td></td>
</tr>
<tr>
<td>&gt; 50 mg/dl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Women</th>
<th>Average</th>
<th>Low Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 45 mg/dl</td>
<td>45-60 mg/dl</td>
<td></td>
</tr>
<tr>
<td>&gt; 60 mg/dl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each laboratory should establish appropriate reference intervals related to its population.

Quality Control and Calibration
It is suggested to perform an internal quality control. For this purpose a suitable human based control sera has to be used. Please contact Customer Care for further information.

SUMMARY OF TEST

Although the metabolism of chylomicrons, VLDL, IDL, and LDL is fairly well understood, our knowledge regarding HDL is relatively new and still growing. HDL consists of a number of polydisperse and heterogeneous particles that vary with respect to size and content of lipid and apolipoprotein. HDL can be separated not only by ultra centrifugation and electrophoresis but also by polyacryanic precipitation. About 50% of HDL mass is protein, 30% is phospholipid, and 20% is cholesterol. The major apolipoproteins found in HDL are A-I and A-II and constitute about 90% of total HDL protein. The ratio of apo A-I to apo A-II is approximately 3:1 by weight.

Both the liver and the intestine are involved in the production of HDL, although the exact roles and relative importance of each are not fully understood. The half-life of HDL in plasma in normal subjects is approximately 4 days. Little is known about the sites of HDL catabolism; however, the liver and kidneys are probably involved. HDL appears to play an important part in cholesterol efflux from tissues, thereby reducing the amount of cholesterol stored there; this is apparently mediated by a particular lipoprotein particle containing only apo A-I, known as Lp-AI. HDL also has a role in returning cholesterol from the periphery to the liver for removal as bile acids, a process known as reverse cholesterol transport. Evidence tends to support the suggestion that HDL serves as a scavenger of lipid and apoprotein during the normal catabolism of chylomicrons and VLDL. HDL acquires free cholesterol released from these particles, and plasma LAQT converts this free cholesterol to its esters (with fatty acids derived from lecithin). These esters are later transferred back to VLDL and IDL by way of apo D or a protein known as lipid transfer protein. HDL is also known to function as a plasma reservoir for apo C-II. The relationship of HDL and VLDL with chylomicrons is exemplified by the fact that defects in triglyceride-rich lipoprotein catabolism are commonly associated with marked reduction in HDL levels. Epidemiological studies have suggested that HDL protects against cardiovascular disease, and a significant amount of research has been devoted to HDL in order to demonstrate its role in reverse cholesterol transport.

The importance of HDL-C as an independent risk factor for developing CAD is now recognized by the scientific community. An outcome from an NCEP consensus conference on the relation of HDL-C to CAD recommended that HDL levels, in addition to total cholesterol, be routinely measured in the serum or plasma sample of all patients at risk for CAD. This recommendation clearly reflects the new epidemiological data that suggest that low HDL level is a common and strong risk factor for CAD in both men and women, that it can and should be modified, and that it still can be a factor even when total cholesterol levels are desirable. Because it currently is extremely difficult and impractical to quantitate HDL directly, most methods depend on the measurement of the HDL in serum or plasma and measurement of the cholesterol concentration in the supernatant containing the HDL.

Numerous studies have compared the different methods for measuring HDL-C. Isolation procedures involving preparative ultracentrifugation are considered reference methods. Various precipitation techniques are now recommended. Techniques for preparative isolation of the lipoprotein classes by precipitation have now been adopted as routine clinical chemistry for the separation of HDL in small volumes of plasma. The lipoprotein classes can be selectively precipitated by appropriately chosen polyanions. For clinical purposes, a reagent-precipitating system must form an insoluble complex with all the plasma lipoproteins except HDL so that HDL remaining in the supernatant after centrifugation can be quantitated by its cholesterol content. The presentation reagent uses a solution of polyethylene glycol with an average MW of 6000 (PEG 6000). A determined concentration of this polyion could get precipitation of various macromolecules, including lipoproteins. After precipitation and centrifugation, the HDL fraction of cholesterol is measured in the supernatant.