**Intended Use**
For the direct quantitative determination of low density lipoprotein cholesterol (LDL-C) in human serum or plasma. For in vitro diagnostic use only.

**Summary**
Plasma lipoproteins are spherical particles that contain varying amounts of cholesterol, triglycerides, phospholipids, and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, the inner core contains mostly esterified cholesterol and triglycerides. These particles serve to solubilize and transport cholesterol and triglycerides in the bloodstream. 

The relative proportions of protein and lipid determine the density of these plasma lipoproteins and provide a basis for their classification. The classes are: very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have varied effects. 

Plasma lipoproteins are spherical particles that contain varying amounts of cholesterol, triglycerides in the bloodstream. These particles serve to solubilize and transport cholesterol and triglycerides. These particles serve to solubilize and transport cholesterol and triglycerides in the bloodstream.

**Principle**
The autoLDL™ Cholesterol Reagent is a two-part, liquid stable method for directly measuring LDL-C levels in serum or plasma. The method depends on the properties of a unique detergent which eliminates the need for any offline pre-treatment or centrifugation steps. This detergent (Reagent 1) solubilizes only the non-LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

**autoLDL™ Cholesterol Reagent Set**
Reagent 1: Reagent 1 is ready to use.
Reagent 2: Reagent 2 is ready to use.

**Components**
- **Appearance**: Liquid
- **Ingredients**: MES Buffer (pH 6.3)
- **Detergent**: Detergent 1
- **Cholesterol esterase**: Cholesterol esterase
- **Cholesterol oxidase**: Cholesterol oxidase
- **Peroxidase**: Peroxidase
- **4-aminoantipyrine**: 4-aminoantipyrine
- **Ascorbic acid oxidase**: Ascorbic acid oxidase
- **Preservative**: Preservative

**Specimen Collection and Storage**
Serum, EDTA-treated or heparinized plasma are the recommended specimens. Patients are not required to fast prior to blood collection. Serum: Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection (within 3 hours). Plasma: Specimens may be collected in EDTA or heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).

**Interferences**
All interference studies were conducted according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry. Hemoglobin at levels up to 250 mg/dl, Bilirubin at levels up to 20 mg/dl and Triglycerides to 1000 mg/dl were found to exhibit negligible interference (<10%) on this method. Samples with levels of interfering substances higher than the upper limits should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor. For a comprehensive review of drug interference on serum LDL cholesterol levels see Young et al.

**Precautions**
1. Reagent is intended for in vitro diagnostic use only.
2. Do not pipette by mouth.
3. All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing.
4. Do not use the reagents beyond the expiration date printed on the kit label.

**Reagent Composition**
<table>
<thead>
<tr>
<th>Components</th>
<th>Appearance</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagent 1</strong></td>
<td>Liquid</td>
<td>MES Buffer (pH 6.3)</td>
</tr>
<tr>
<td><strong>Detergent 1</strong></td>
<td>Cholesterol esterase</td>
<td>Cholesterol oxidase</td>
</tr>
<tr>
<td><strong>Peroxidase</strong></td>
<td>4-aminoantipyrine</td>
<td>Ascorbic acid oxidase</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Reagent 2**
<table>
<thead>
<tr>
<th>Components</th>
<th>Appearance</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Detergent 2</strong></td>
<td>Liquid</td>
<td>MES Buffer (pH 6.3)</td>
</tr>
<tr>
<td><strong>N,N-bis (4-sulfobutyl)-m-Toluidine-disodium</strong> (DSBmT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cholesterol Oxidase from Nocardia sp., Cholesterol Esterase from Pseudomonas sp., Peroxidase from Horseradish, Ascorbic Acid Oxidase from Cucurbita sp.**

**Reagent Preparation**
Reagent 1: Reagent 1 is ready to use.
Reagent 2: Reagent 2 is ready to use.

**Reagent Storage and Stability**
All reagents are stable until the expiration date on the label when stored at 2 to 8°C.

**Vacuum Package**
- Detergent/Rgt1
- Detergent/Rgt2

**Color Development**
( Measured Bichromatically at 546 & 660nm)

**H₂O₂ + DSBmT + 4-AA → Color Development**

**Side Reactions**
- Non-solubilized
- Solubilized
- Consumed

**Substances**
- H₂O₂:
- Plasma:
- Patients:
- Specimens:
- Samples:
- Interferences:
- Hemoglobin:
- Bilirubin:
- Triglycerides:
- Drug:
- Comprehensive:
- Review:
- Drug:
- Interference:
- Serum:
- LDL cholesterol:
- Levels:
- Young et al.

**Specimen Handling**
All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing. Do not use the reagents beyond the expiration date printed on the kit label.
**autoLDL™ Cholesterol Reagent Set**

**Materials Provided**
autoLDL™ Cholesterol Reagent Set

**Materials Required but not Provided**
1. Pointe Scientific autoHDL/LDL™ Calibrator, Cat. No. H7545-CAL.
2. LDL cholesterol controls.
3. Beckman Coulter AU™ analyzer

**Procedure**
Below is a general example of the autoLDL™ test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations. For assistance with applications on automated analyzers, please contact Pointe Scientific’s Technical Service Department at (800)445-9853.

![Procedure Diagram](Image)

**Limitations**
1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Samples with values greater than 700 mg/dl must be diluted 1:1 with saline and re-assayed. Multiply the result by two.

**Calibration**
The autoHDL/LDL™ Cholesterol Calibrator is required for calibration. The values of the calibrator were assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Refer to autoHDL/LDL™ Cholesterol Calibrator package insert for instructions. If control results are found to be out of range, the procedure should be re-calibrated.

**Quality Control**
Reliability of test results should be routinely monitored with control materials that reasonably emulate the performance of patient specimens. Quality control materials are intended for use only as monitors of accuracy and precision. The recovery of control values within the appropriate range should be the criteria used in evaluation of future assay performance. Controls should be run with every working shift in which LDL-C assays are performed. It is recommended that each laboratory establish their own frequency of control determination. Quality control requirements should be determined in conformance with local, state, and/or Federal regulations or accreditation requirements.

**Results**
To convert from conventional units to S.I. units, multiply the conventional units by 0.02586.

**Expected Values**
The following NCEP recommendations for patient classifications are suggested for the prevention and management of coronary heart disease.:

<table>
<thead>
<tr>
<th>LDL Cholesterol</th>
<th>Classifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;130mg/dl (3.36mmol/L)</td>
<td>Desirable</td>
</tr>
<tr>
<td>130-159mg/dl (3.36-4.11mmol/L)</td>
<td>Borderline High Risk</td>
</tr>
<tr>
<td>160mg/dl (4.14mmol/L)</td>
<td>High Risk</td>
</tr>
</tbody>
</table>
It is highly recommended that each laboratory establish its own range of expected values.

Specific Performance Characteristics
1. Linearity Range: 0-700 mg/dl
2. Comparison: A comparison study performed between the Beckman Coulter AU™400 and Roche Hitachi 717 using this method resulted in correlation coefficient of $r = 0.993$ with a regression equation of $y = 1.1x + 5.10$. ($n = 30$, range 38 – 133 mg/dl)
3. Precision:
   Within - day precision study was performed using three levels of material. Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

<table>
<thead>
<tr>
<th>Within Day (N=20)</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>1.1</td>
<td>2.2</td>
<td>4.35%</td>
</tr>
<tr>
<td>163</td>
<td>3.0</td>
<td>1.8</td>
<td>1.13%</td>
</tr>
<tr>
<td>389</td>
<td>5.1</td>
<td>1.3</td>
<td>3.38%</td>
</tr>
<tr>
<td>Day to Day</td>
<td>Mean</td>
<td>S.D.</td>
<td>C.V.%</td>
</tr>
<tr>
<td>50</td>
<td>1.8</td>
<td>3.6</td>
<td>7.25%</td>
</tr>
<tr>
<td>168</td>
<td>3.3</td>
<td>2.0</td>
<td>1.18%</td>
</tr>
</tbody>
</table>

Precision and Linearity studies were performed following modifications of CLSI Protocols EP5 and EP6 using a Beckman Coulter AU™400 analyzer
4. Sensitivity: The analytical sensitivity for autoLDL™ Cholesterol was determined to be 0.0027 absorbance units per 1 mg/dl of LDL cholesterol.

References