**Intended Use**
The Q-Line™ LDL-Cholesterol reagent is intended for the in vitro quantitative determination of Low Density Lipoprotein Cholesterol in human serum or plasma. The reagent can assist in the diagnosis and treatment of patients at risk for developing coronary heart disease.

**Clinical Significance**
Low Density Lipoproteins (LDL) are synthesized in the liver by the action of various lipolytic enzymes on triglyceride-rich Very Low Density Lipoproteins (VLDLs). Specific LDL receptors exist to facilitate the elimination of LDL from plasma by liver parenchymal cells. It has been shown that most of the cholesterol stored in atherosclerotic plaques originates from LDL. For this reason the LDL-Cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis. 2-8

Accurate measurement of LDL-Cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture.

**METHOD**

**Direct Enzymatic (PVS/PEGME)**

**Assay Principle**
The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. 9 LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of R2 containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H2O2 which is quantified by the Trinder reaction.

**Reagents-“Working Solutions”**

**REAGENT R1:**

<table>
<thead>
<tr>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES buffer (pH 6.5)</td>
</tr>
<tr>
<td>Polyvinyl sulfonic acid</td>
</tr>
<tr>
<td>Polyethylene glycol methyl ester</td>
</tr>
<tr>
<td>MgCl2</td>
</tr>
<tr>
<td>EDTA</td>
</tr>
<tr>
<td>4-aminobipyrine</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
</tr>
<tr>
<td>Cholesterol oxidase</td>
</tr>
</tbody>
</table>

**REAGENT R2:**

<table>
<thead>
<tr>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES buffer (pH 6.5)</td>
</tr>
<tr>
<td>EDTA</td>
</tr>
<tr>
<td>TOOB N, N-Bis (4-sulfobutyl)-3-methylaniline</td>
</tr>
</tbody>
</table>

**Precautions:**
1. For in vitro diagnostic use only.
2. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8935).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid ingestion and contact with skin or mucous membranes. See Material Safety Data Sheet.
5. Reagents are light-sensitive. Do not let bottles remain open. Keep containers tightly closed.
6. Do not use the reagents after the expiration date labeled on the outer box.

**Reagent Handling**
The Q-Line™ LDL-Cholesterol reagents (R1, R2) are liquid stable, ready-to-use REAGENTS.

**Reagent Stability and Storage**
Unopened REAGENTS are stable until the expiration date printed on the outer box when stored at 2-8°C. The REAGENT should be clear. If turbid, REAGENT may have deteriorated.

**Specimen Collection and Preparation**
Use fresh patient serum and plasma samples (EDTA, Citrate). Fasting and non-fasting samples can be used. 9 If samples contain LDL cholesterol greater than 250 mg/dL, they should be diluted with saline.

**Materials Provided**
See “Reagents-Working Solution” section.

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**Materials Required But Not Provided**
Any instrument with temperature control of 37 ± 0.5°C that is capable of reading absorbance accurately at 578 nm (578-600 nm) may be used.

The Q-Line™ HDL-LDL-Calibrator (REF: HDL-LDL-CAL)*
The Q-Line™ Multi CON-N & Q-Line™ Multi CON-H for validating the performance of the Q-Line™ LDL-Cholesterol Reagent.*
Saline (NaCl 9 g/L) for diluting serum samples.*

**Reference Range**
Each laboratory must establish its own range of expected values.

**Commercial**

- LDL-Cholesterol
- HDL-Cholesterol
- VLDL-Cholesterol
- LDLCR

**Application**

- Direct Enzymatic (PVS/PEGME)
- Manual Method
- Semi-automatic Analyzers
- Fully automatic Analyzers

**Reference**

The expected values for serum LDL Cholesterol are as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-Cholesterol</td>
<td>3.0 - 6.0 mmol/L</td>
</tr>
</tbody>
</table>

**Wavelength:**
578 nm

**Temperature:**
37°C

**Absorbance:**
Wavelength: 578 nm

**CALCULATION**

LDL-Chol (mg/dl) = \( \frac{OD_{578} - OD_{578,\text{blank}}}{x \text{ Calibrator Conc. (mg/dL)}} \)

**Conversion factor:**
mg/dL x 0.02586 = mmol/L LDL-Cholesterol

**Calibration**
The Q-Line™ HDL-LDL-Calibrator (REF: HDL-LDL-CAL) should be used to calibrate the Q-Line™ LDL-Cholesterol Reagent. 0.9% Saline should be used as CALIBRATOR 0 (If 2-point calibration is used)

The Q-Line™ HDL-LDL-Calibrator is provided in lyophilized form and is stable until the expiration date printed on the outer box when stored at 2-8°C. Reconstitute contents with distilled water as per instructions on vials and mix gently. Let vials equilibrate to room temperature for 30 minutes before use. Reconstituted calibrator is stable for 7 days when capped tightly and stored at 2-8°C.

**Procedure for ELTech Clinical Systems Selectra Analyzers**

**Applications:** Applications are available on request

**Wavelength:**
578 nm

**Temperature:**
37°C

**Reference**

- LDL-Cholesterol
- HDL-Cholesterol
- VLDL-Cholesterol

**Sample Handling**

- LDL-Cholesterol
- HDL-Cholesterol
- VLDL-Cholesterol

**Materials Provided**
See “Reagents-Working Solution” section.

**Reference**

- LDL-Cholesterol
- HDL-Cholesterol
- VLDL-Cholesterol

**Application**

- Direct Enzymatic (PVS/PEGME)
- Manual Method
- Semi-automatic Analyzers
- Fully automatic Analyzers

**Reference**

- LDL-Cholesterol
- HDL-Cholesterol
- VLDL-Cholesterol
- LDLCR

**Application**

- Direct Enzymatic (PVS/PEGME)
- Manual Method
- Semi-automatic Analyzers
- Fully automatic Analyzers
Within-Run Precision:
respectively were tested with 2 runs per day in duplicates over 5 working days. In the study, one levels of serum specimen containing about 70 mg/dL LDL conducted in accordance to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, three samples ranging from 4.0 to 232.0 mg/dL gave a correlation coefficient of 0.9804. The LOB = mean + 3SD = 1.64 mg/dL. The Limit of Blank (LOB) of the Q-Line™ LDL-Cholesterol Reagent was determined using a Hitachi 917 chemistry analyzer. Results may vary from individual laboratories. All performance characteristics were determined at Diazyme Laboratories, USA using the listed concentrations: Triglyceride at 1000 mg/dL, Ascorbic Acid at 10 mM, Bilirubin at 40 mg/dL, Bilirubin Conjugate at 30 mg/dL, Hemoglobin at 1000 mg/dL.

On-board Stability & Calibration frequency
REAGENT on-board stability is at least 60 days. Calibration curve is stable for at least 14 days. Recalibrate when reagent lots change, when quality control results fall outside the established range, and after a maintenance operation.

Quality Control
We recommend that each laboratory use the Q-Line™ Multi CON-N & Q-Line™ Multi CON-H to validate the performance of the Q-Line™LDL-Cholesterol Reagent. If the results from the controls fall outside the acceptable limits, as determined by their assigned values, the test should not be performed. We recommend that the quality control requirements should be established in accordance with local, state, and/or federal regulations.

Limitations
1. A sample with an LDL-Cholesterol level exceeding the linearity limit should be diluted with 0.9% saline and re-assayed incorporating the dilution factor in the calculation of the value.
2. Store the reagents at 2-8°C. Do not freeze REAGENTS.

WASTE MANAGEMENT
Disposal of all waste material should be in accordance with local and legal requirements.

Performance Characteristics
All performance characteristics were determined at Diazyme Laboratories, USA using a Hitachi 917 chemistry analyzer. Results may vary from individual laboratories.

Limit of Blank
The Limit of Blank (LOB) of the Q-Line™ LDL-Cholesterol Reagent was determined as follows: LDL zero calibrator was tested 12 replicates on Hitachi 917. The LOB = mean + 3SD = 1.64 mg/dL.

Accuracy
The performance of this Reagent was compared to a reagent of a legally marketed LDL-Cholesterol Reagent using serum samples. Seventy nine serum samples ranging from 4.0 to 232.0 mg/dL gave a correlation coefficient of 0.9804. Linear regression analysis gave the following equation: y = 1.0863x + 0.6078

Precision
The precision of the Q-Line™ LDL-Cholesterol Reagent was evaluated according to Clinical Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, three serum specimens containing about 95, 146 and 210 mg/dL LDL-Cholesterol were tested on Hitachi 917 with 2 runs per day in duplicates over 20 working days. This method has not been tested or certified by the Cholesterol Reference Method Laboratory Network (CRMLN).

Within-Run Precision:

<table>
<thead>
<tr>
<th>Level 1</th>
<th>95 mg/dL LDL</th>
<th>Level 2</th>
<th>146 mg/dL LDL</th>
<th>Level 3</th>
<th>210 mg/dL LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>97.14</td>
<td>147.37</td>
<td>211.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>1.00</td>
<td>1.19</td>
<td>1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV%</td>
<td>1.0%</td>
<td>0.8%</td>
<td>0.7%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Within-Laboratory Precision:

<table>
<thead>
<tr>
<th>Level 1</th>
<th>95 mg/dL LDL</th>
<th>Level 2</th>
<th>146 mg/dL LDL</th>
<th>Level 3</th>
<th>210 mg/dL LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>97.14</td>
<td>147.37</td>
<td>211.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>1.55</td>
<td>2.23</td>
<td>2.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV%</td>
<td>1.6%</td>
<td>1.5%</td>
<td>1.4%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

An additional precision study of the Q-Line™ LDL-Cholesterol Reagent was conducted in accordance to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, one levels of serum specimen containing about 70 mg/dL LDL were tested with 2 runs per day in duplicates over 9 working days. An additional precision study of the Q-Line™ LDL-Cholesterol Reagent was conducted in accordance to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, one levels of serum specimen containing about 70 mg/dL LDL were tested with 2 runs per day in duplicates over 9 working days.

Within-Run Precision:

<table>
<thead>
<tr>
<th>Level 1</th>
<th>70 mg/dL LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td>71.19</td>
</tr>
<tr>
<td>SD</td>
<td>0.5</td>
</tr>
<tr>
<td>CV%</td>
<td>0.70%</td>
</tr>
</tbody>
</table>

Within-Laboratory Precision:

<table>
<thead>
<tr>
<th>Level 1</th>
<th>70 mg/dL LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td>71.19</td>
</tr>
<tr>
<td>SD</td>
<td>1.15</td>
</tr>
<tr>
<td>CV%</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

Linearity
Sixteen levels of linearity set were prepared by diluting a serum sample containing 250 mg/dL of LDL with saline according to Clinical and Laboratory Standards Institute (formerly NCCLS) EP6-A. The linearity range is up to 250 mg/dL in serum. Results that exceed 250 mg/dL should be diluted with 0.9% saline and retested.

Interference
Interference for the LDL-Cholesterol reagent was evaluated on Hitachi 917. The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglyceride at 1000 mg/dL, Ascorbic Acid at 10 mM, Bilirubin at 40 mg/dL, Bilirubin Conjugate at 30 mg/dL, Hemoglobin at 1000 mg/dL.

References

General Technical Parameters

<table>
<thead>
<tr>
<th>Mode</th>
<th>End Point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>578 nm</td>
</tr>
<tr>
<td></td>
<td>None / No</td>
</tr>
<tr>
<td></td>
<td>Increasing / Positive</td>
</tr>
<tr>
<td></td>
<td>6 µL</td>
</tr>
<tr>
<td></td>
<td>600 µL</td>
</tr>
<tr>
<td></td>
<td>NA / NA</td>
</tr>
<tr>
<td></td>
<td>2 Seconds</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.300 Abs</td>
</tr>
<tr>
<td></td>
<td>One Point</td>
</tr>
<tr>
<td></td>
<td>NA (Calculated by System)</td>
</tr>
<tr>
<td></td>
<td>See the HDL-LDL-Calibrator Vial</td>
</tr>
<tr>
<td></td>
<td>250 mg/dL</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>37 °C</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&lt; 100 mg/dL</td>
</tr>
<tr>
<td></td>
<td>&gt; 130 mg/dL</td>
</tr>
</tbody>
</table>

Symbols:-

- In vitro diagnostic medical device use
- Use by
- Consult instruction for use
- REF Catalogue number
- Manufacturer
- R/R1/R2 Reagent
- Date of Manufacturing
- Std Standard
- LOT Batch code
- Temperature Limitation