Kit for the determination of Low Density Lipoprotein Cholesterol on HITACHI Clinical Analyzer

2011-11 Rev.0

Intended Use
The S TEST LDL is intended for the quantitative determination of low density lipoprotein cholesterol concentration in serum or heparin plasma using the HITACHI Clinical Analyzer. The S TEST LDL is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

Method
Enzymatic direct method

Test Summary and Explanation
Low Density Lipoprotein (LDL)-Cholesterol is known to be a risk factor for arteriosclerotic disease, especially coronary disease. Determination of total cholesterol has been conventionally and widely used in the diagnosis of hyperlipidemia, which is considered a cause of arteriosclerotic disease. However, it has been reported that ischemic heart disease is more strongly correlated with LDL-Cholesterol than with total cholesterol. 1

LDL-Cholesterol level is generally calculated from the measurements of total cholesterol, HDL-Cholesterol, and triglycerides using Friedewald's formula, but this calculation method is not sufficient for an accurate determination of LDL-Cholesterol. The reference method for LDL-Cholesterol is the ultracentrifugation method, but the method requires specialized instrumentation and a long measurement time. This makes the reference method difficult to perform in routine laboratory tests, and direct calculation method is not sufficient for an accurate determination of LDL-Cholesterol. The method requires specialized instrumentation and a long measurement time. This makes the reference method difficult to perform in routine laboratory tests, and direct methods are widely used.

Principle of the Test
The method combines two surfactants as follows. The first reaction is a color reaction, and the second reaction is a colorless reaction. This makes the reference method difficult to perform in routine laboratory tests, and direct methods are widely used.

The first reaction
HDLC, VLDLC, CM Cholesterol esterase Cholesterol oxidase H2O2 + 4-Aminoantipyrine Colorless

The second reaction
LDLC Cholesterol esterase Cholesterol oxidase H2O2 + 4-Aminoantipyrine + DSbmT Purple-red pigment

Reagent Requirements- one cartridge per patient sample

Reagent Composition
The S TEST LDL reagent cartridge has the following composition:

LDL Reagent (1):
- 4-Aminoantipyrine 0.01% • Cholesterol esterase (Microbial) <0.5 U/mL
- Cholesterol oxidase (Microbial) 1.2 U/mL
- Peroxidase (Horseradish) <1.3 ppg U/mL
- Surfactant 1: Good’s Buffer (pH 6.3)

LDL Reagent (2):
- Surfactant 2
- N,N-bis-(4-sulfobutyl)-m-toluidine, disodium salt (DSbmT) 0.04%
- Good’s Buffer (pH 6.3)

Preparation and Labelling
LDL reagent is provided in a ready-to-use cartridge. The 2D code label on the front of each cartridge automatically identifies the reagent to the system.

Reagent Cartridge

Measurement

Presentation of Result
Each Patient Report includes the data and time, sample ID number (as programmed), and control results appear on the display. For detailed explanations on flags and error messages, refer to the User Manual.

Calculation
LDL-Cholesterol concentration is directly determined by multiplying the change in absorbance of the unknown samples by the calibrator factor on the 2D code. Patient and control results appear on the display.

Quality Control
Users should follow federal, state and local regulatory requirements regarding quality control practices. See instrument manual for procedures on how to run controls. Good laboratory practice includes the use of at least two levels of control material to ensure the test performance. The frequency and limits of QC testing should be determined according to individual laboratory standard QC procedures. Controls should be run at least once every 30 days and:
1. When test results do not match patient symptoms or clinical findings.
2. When using a new lot or shipment of reagents.
3. When laboratory environmental conditions have significantly changed.
4. When training or retraining of personnel occurs.

Reading and Reporting Results

Expected Value
- Reportable range: 8 – 400 mg/dL
- Reference range: 70 – 139 mg/dL
- Optimal: <100 mg/dL. Near optimal: 100 – 129 mg/dL. Borderline high: 130 – 159 mg/dL. High: 160 – 189 mg/dL. Very high: >189 mg/dL.
- It is recommended that each laboratory determine the expected values for its particular population.

Interpretation of Results
There may be reactions with non-target substances or interfering reactions. If measured results seem unreliable, repeat the measurement (if necessary after dilution) or try another analytical measurement.

Handling Critical Values
If the result of a sample exceeds the measurement range, dilute the sample with physiological saline solution, and repeat the measurement.

Performance Characteristics
Please note: this assay has not been certified by the Cholesterol Reference Method Laboratory Network (CRMLN), but is traceable to the CRMLN method.

Interference (per CLSI EP7-A2)
The data demonstrated that the LDL test system was not affected by high levels of the following substances at the levels noted:
- Hemoglobin: no interference up to 1000 mg/dL
- Unconjugated bilirubin: no interference up to 25 mg/dL
- Lipemia: no interference up to 614 mg/dL
- Ascorbic acid: no interference up to 50 mg/dL
- Lack of interference was defined as recoveries between 90% and 110% of the neat value, and assay performance claims were established on the HITACHI Clinical Analyzer by testing two serum pools containing approximately 30 mg/dL and 60 mg/dL LDL-Cholesterol.

Precision (per CLSI EPS-A2)
Four levels of serum samples were assayed 2 times per run, 2 runs per day, for total of 20 days. The precision was found to be:

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean (mg/dL)</th>
<th>SD (mg/dL)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>2.0</td>
<td>5.2</td>
</tr>
<tr>
<td>2</td>
<td>104</td>
<td>3.6</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>176</td>
<td>6.0</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>299</td>
<td>7.9</td>
<td>2.7</td>
</tr>
</tbody>
</table>

n = 80 per level

Precision (POL sites)
Three levels of samples (A, B, and C) were tested by three POL sites, six times a day for five days. The precision estimates are described below.

<table>
<thead>
<tr>
<th>Site #</th>
<th>Sample</th>
<th>Mean (mg/dL)</th>
<th>Regression Equation</th>
<th>Total Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>y = 0.94x + 3.8</td>
<td>CI Slope CI Intercept</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>41</td>
<td>2.0</td>
<td>0.90 to 0.99 -1.7 to 9.4</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>42</td>
<td>1.9</td>
<td>0.8 to 1.8 1.8</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>41</td>
<td>2.0</td>
<td>0.8 to 2.0 2.0</td>
</tr>
<tr>
<td>1</td>
<td>B</td>
<td>109</td>
<td>1.3</td>
<td>1.4 1.3</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>112</td>
<td>1.4</td>
<td>1.5 1.5</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>109</td>
<td>2.1</td>
<td>2.2 2.0</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>183</td>
<td>1.4</td>
<td>3.1 1.7</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>191</td>
<td>1.2</td>
<td>3.5 1.8</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>181</td>
<td>1.4</td>
<td>3.1 1.7</td>
</tr>
</tbody>
</table>

n = 30 replicates per sample per site

Patient Correlation (POL sites)
A series of 50 or more serum specimens with LDL-Cholesterol values ranging from 25 mg/dL to 283 mg/dL were assayed on the HITACHI Clinical Analyzer at three sites using S TEST LDL (y) and a comparative method as the reference method (x). Linear regression analysis (least squares) yielded the following results.

<table>
<thead>
<tr>
<th>Site #</th>
<th>n</th>
<th>Range (mg/dL)</th>
<th>Regression Equation</th>
<th>Total Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>26 to 274</td>
<td>y = 0.94x + 3.8</td>
<td>CI Slope CI Intercept</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>26 to 283</td>
<td>y = 0.93x + 3.6</td>
<td>0.89 to 1.01 -3.5 to 10.8</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>25 to 276</td>
<td>y = 0.93x + 6.4</td>
<td>0.89 to 0.98 0.6 to 12.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 to 370</td>
<td>y = 0.94x + 7.6</td>
<td>CI Slope CI Intercept</td>
</tr>
<tr>
<td>122</td>
<td></td>
<td>8 to 370</td>
<td>0.98</td>
<td>12.5 3.0 to 12.1</td>
</tr>
</tbody>
</table>

Cl = 95% confidence interval

Serum/Plasma Comparison Study
A study was performed to validate the use of heparinized plasma as well as serum for the HITACHI Clinical Analyzer with S TEST LDL. Approximately 40 matched serum/plasma samples that spanned the dynamic range were assayed in singleton and the results were compared using least squares linear regression (plasma = y-axis). The performance characteristics were as follows.

y = 1.01x - 4.4
Correlation coefficient (r) = 0.99
95% confidence interval of the slope = 0.99 to 1.02
95% confidence interval of the y-intercept = 6.4 to -2.3

Detection limit (per CLSI EP17-A)
The detection limit was determined to be 0.8 mg/dL.

Linearity (per CLSI EP6-A)
The assay linearity was determined to be 3 mg/dL to 430 mg/dL.

Reportable Range
8 mg/dL to 400 mg/dL

Routine Maintenance and Troubleshooting
For complete information on operation, see the User Manual for the HITACHI Clinical Analyzer.

Technical Support/Instrument Service
1. First contact to your local distributor.
2. Hitachi Chemical Co., Ltd. (Japan)

Reference

80-7868-00-00 05/12

Hitachi Chemical Co., Ltd.
2-1-1, Nishi-Shinjuku, Shinjuku-ku, Tokyo
163-0449, Japan