**Method**: Endpoint  
**Specimen Type**: Serum or Plasma  
**Linear Range**: Up to 20 mmol/L (774 mg/dL)  

**Stability**  
This reagent is intended for the in vitro quantitative determination of cholesterol in human serum or plasma.  

**Clinical Significance**  
Measurement of serum cholesterol levels can serve as an indicator of liver function, biliary function, intestinal absorption, propensity toward coronary artery disease, thyroid function and adrenal disease. Cholesterol levels are important in the diagnosis and classification of hyperlipoproteinaemias. Stress, age, gender, hormonal balance and pregnancy affect normal cholesterol levels.  

**Methodology**  
The use of enzymes to assay cholesterol has been studied by many investigators. This reagent is based on the formulation of Allain et al and the modification of Roeschlau with further improvements to render the reagent stable in solution.  

1. Cholesterol Esters \( \xrightarrow{\text{CE}} \) Cholesterol + Fatty Acids  
2. Cholesterol + \( \text{O}_2 \) \( \xrightarrow{\text{CO}} \) Cholest-4-en-3-one + \( \text{H}_2\text{O}_2 \)  
3. \( \text{2H}_2\text{O}_2 + \text{HBA} + \text{4-AAP} \) \( \xrightarrow{\text{POD}} \) Quinoneimine Dye + 4\( \text{H}_2\text{O} \)  

Where:  
CE = Cholesterol Esterase  
CO = Cholesterol Oxidase  
HBA = Hydroxybenzoic Acid  
4-AAP = 4-aminoantipyrine  
POD = Peroxidase  

1. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids.  
2. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.  
3. The hydrogen peroxide combines with HBA and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which may be quantitated at 500-550 nm. For bichromatic analyzers the blank wavelength should be set to 600 or 660 nm.  

**Application**  
Reagent is supplied ready to use.  

**Stability and Storage**  
When stored refrigerated at 2-8°C in sealed air-tight containers and protected from light the reagent is stable until the expiry date on the bottle.  

**Indications of Reagent Deterioration:**  
- Turbidity.  
- Reagent Absorbance >0.2 AU at 500 nm; and/or  
- Failure to recover control values within the assigned range.  

**Specimen Collection and Handling**  
Collection: No special preparation of the patient is necessary, however it is recommended that prior to collection, patients should be following their usual diet and be in their usual state of health. Patients who are acutely ill, losing weight, pregnant or have had a myocardial infarction in the previous 6 months should be rescheduled. Blood should be collected by venipuncture, after the patient has been in a seated position for at least 5 minutes. Tourniquet usage should be kept to a minimum and the specimen should be allowed to clot for 30 minutes at room temperature.  

Serum: The best specimen is non-haemolysed serum collected as per the above instructions.  

Plasma: Heparinized plasma is a suitable specimen.  

Storage: Specimens should be analysed on the day of collection. When stored at 4°C, specimens are stable for 3-4 days. Specimens are stable at -20°C for several months.  

**Additional Equipment Required but Not Provided**  
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance between 500 and 550 nm.  
- Analyzer specific consumables, e.g.: sample cups.  
- Normal and Abnormal control Material.  
- Calibrator traceable to NRS/CHOL material.  

**Assay Procedure**  
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.  

**System Parameters**  
- Temperature: 30/37°C  
- Primary Wavelength: 500 nm (500 - 550nm)  
- Secondary Wavelength: 660 nm (600 - 660nm)  
- Assay Type: End Point  
- Incubation Time: 300 seconds  
- Reagent Blank Limits: Low 0.0 AU  
- Linearity: 0 - 20 mmol/L (0 - 774 mg/dL)  
- Analytical Sensitivity: 82.1 mA per mmol/L  

**Calculations**  
Results are calculated, usually automatically by the instrument, as follows:  

\[
\text{Cholesterol} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}
\]

**Example:**  
Absorbance of calibrator = 0.42
Absorbance of unknown = 0.25
Value of calibrator = 7.0 mmol/L (271 mg/dL)

Cholesterol = \( \frac{0.25}{0.42} \times 7.0 = 4.2 \text{ mmol/L} \)

Cholesterol = \( \frac{0.25}{0.42} \times 271 = 161 \text{ mg/dL} \)

NOTES
1. Specimens with cholesterol values greater than 20 mmol/L (774 mg/dL) should be diluted and reassayed. Multiply the results by the dilution factor.
2. The assay can be performed at 30°C by increasing the incubation time to 10 minutes or at 25°C by incubating for 15 minutes.
3. The color development is stable for 30 minutes.
4. Unit conversion mmol/L x 38.7 = mg/dL.

CALIBRATION
Calibration is required. A suitable aqueous standard or serum based calibrator traceable to NRS/CHOL material is recommended. Appropriate calibrator levels range from 5.2 to 7.8 mmol/L (200 - 300 mg/dL).

For Calibration Frequency on automated instruments refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:
- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL
To ensure adequate quality control, two levels of control, one in the normal range (4.5 - 5.2 mmol/L, 175 - 200 mg/dL), and one at the high level (6.2 - 6.7 mmol/L or 240 - 260 mg/dL) should be run as unknown samples:
- At least every eight hours or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or the local distributor.

LIMITATIONS
1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:

Haemoglobin: No interference from haemoglobin up to 500 mmol/L

Free Bilirubin: No interference from bilirubin up to 182 mmol/L (10.6 mg/dL).

Conjugated Bilirubin: No interference from bilirubin up to 58 mmol/L (3.4 mg/dL).

Lipaemia: No interference from lipaemia, measured as absorbance at 630 nm up to 1.68 AU.

2. Ascorbic acid at high abnormal levels may cause negative interference.
3. Other 3-beta-hydroxysteroids cause positive interference but are not normally present in significant quantities in human serum.
4. For a more comprehensive review of factors affecting cholesterol assays refer to the publication by Young.

EXPECTED VALUES
The following values are those recommended by the US National Cholesterol Education Program Expert Panel.9

Desirable blood Cholesterol < 5.2 mmol/L (200 mg/dL)
Borderline high blood Cholesterol 5.2 - 6.1 mmol/L (200 - 239 mg/dL)
High Blood Cholesterol > 6.2 mmol/L (240 mg/dL)

PERFORMANCE DATA
The following data was obtained using the Infinity Cholesterol Liquid Stable reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION
Imprecision was evaluated using two levels of commercial controls and following the NCCLS EP5-T procedure.10

<table>
<thead>
<tr>
<th>Level I</th>
<th>Level II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>20</td>
</tr>
<tr>
<td>Mean (mmol/L / mg/dL)</td>
<td>3.79 / 147</td>
</tr>
<tr>
<td>Within run:</td>
<td></td>
</tr>
<tr>
<td>SD (mmol/L / mg/dL)</td>
<td>0.08 / 3.1</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>2.8</td>
</tr>
<tr>
<td>Total:</td>
<td></td>
</tr>
<tr>
<td>SD (mmol/L / mg/dL)</td>
<td>0.11 / 4.1</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>2.8</td>
</tr>
</tbody>
</table>

METHOD COMPARISON
Comparison studies were carried out using a similar commercially available Cholesterol reagent as a reference. Calibrations were carried out using material traceable to the WHO lipid standardisation laboratory at Centres for Disease Control. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Number of sample pairs: 60
Range of sample results: 0.1-12.0 mmol/L (4-464 mg/dL)
Mean of reference method results: 5.7 mmol/L (221 mg/dL)
Mean of Cholesterol Infinity results: 5.7 mmol/L (221 mg/dL)
Slope: 0.989
Intercept: -0.02 mmol/L (-0.8 mg/dL)
Correlation coefficient: 0.999

LINEARITY
When run as recommended the assay is linear between 0 and 20 mmol/L (0 - 774 mg/dL).

ANALYTICAL SENSITIVITY
When run as recommended the sensitivity of this assay is 62 µmAbs per mmol/L or 1.6 µmAbs per mg/dL (1cm light path, 500nm).

REFERENCES