**PRODUCT SUMMARY**

- **Stability**: 3 Months at 2-8°C
- **Linear Range**: Up to 20 mmol/L (0 - 774 mg/dL)
- **Specimen Type**: Serum
- **Method**: Endpoint
- **Reagent Preparation**: Add specified volume of distilled or deionised water.

**INTENDED USE**

This reagent is intended for the in vitro quantitative, diagnostic determination of cholesterol in human serum.

**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.1,2

**METHODOLOGY**

The use of enzymes to assay cholesterol has been studied by many investigators.1,2 This reagent is based on the formulation of Allain et al3 and the modification of Roeschlaub with further improvements to render the reagent stable in solution.

1. Cholesterol esters $\xrightarrow{CE}$ Cholesterol + Fatty Acids
2. Cholesterol + $O_2 \xrightarrow{CO}$ Cholest-4-en-3-one + $H_2O_2$
3. $2H_2O_2 + HBA + 4AAP \xrightarrow{POD}$ Quinoneimine Dye + $4H_2O$

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

1. Cholesterol esters are enzymatically hydrolysed 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.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>Active Ingredients</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol oxidase (microbial)</td>
<td>&gt; 100 U/L</td>
</tr>
<tr>
<td>Cholesterol Esterase (microbial)</td>
<td>&gt; 250 U/L</td>
</tr>
<tr>
<td>Peroxidase (Horseradish)</td>
<td>&gt; 150 U/L</td>
</tr>
<tr>
<td>4-aminoantipyrine</td>
<td>0.25 mmol/L</td>
</tr>
<tr>
<td>Buffer</td>
<td>10 mmol/L</td>
</tr>
<tr>
<td>HBA</td>
<td>50 mmol/L</td>
</tr>
<tr>
<td>Surfactants</td>
<td>pH 6.7 ± 0.1 at 20°C</td>
</tr>
</tbody>
</table>

**WARNING**: Do not ingest. Avoid contact with skin and eyes. If spill thoroughly wash affected area with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Cholesterol reagent Material Safety Data Sheet. The Packaging of This Product Contains Dry Natural Rubber. Exercise precaution when handling crimps and broken glass vials, as sharp edges can injure the user.

**STABILITY AND STORAGE**

**Prior to use**: When stored refrigerated at 2-8°C the reagent is stable until the expiry date stated on the bottle and kit box label.

**Reconstituted Reagent**: When stored capped at 2-8°C, the reagent is stable for at least 3 months.

**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 3 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.

**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**

- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance between 500 and 550 nm.
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal assayed controls.
- 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
- **Direction**: Increase
- **Sample/Reagent ratio**: 1:100
- **e.g. Sample vol**: 3 µL
- **Reagent vol**: 300 µL
- **Incubation Time**: 300 seconds
- **Reagent Blank Limits**: Low 0.0 AU; High 0.2 AU
- **Linearity**: 0 - 20 mmol/L (0 - 774 mg/dL)
- **Sensitivity**: 62 ± mA per mmol/L

**CALCULATIONS**

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

\[
\text{Cholesterol} = \frac{\Delta \text{Abs/min of Unknown}}{\Delta \text{Abs/min of Calibrator}} \times \text{Calibrator Value}
\]
If results are still out of control, contact Technical Services or the local distributor.

**QUALITY CONTROL**

To ensure adequate quality control, two levels of control, one in the normal range (4.5 - 5.2 mmol/L or 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.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

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 control reagent, then repeat the test.
- If results are still out of control, perform a calibration with freshly prepared 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 mg/dL.

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

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

   **Lipaemia:** No interference from lipaemia, measured as absorbance at 630nm 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.

- Desirable blood Cholesterol: < 5.2 mmol/L (200mg/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 Cholesterol reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

**IMPRECISION**

Imprecision was evaluated using two levels of commercial control.

<table>
<thead>
<tr>
<th>Level</th>
<th>Number of samples</th>
<th>Within Run: Mean (mmol/L / mg/dL)</th>
<th>SD (mmol/L / mg/dL)</th>
<th>C.V. (%)</th>
<th>SD (mmol/L)</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>40</td>
<td>3.13 / 121</td>
<td>0.13 / 4.9</td>
<td>0.70</td>
<td>0.05 / 1.9</td>
<td>10.5</td>
</tr>
<tr>
<td>II</td>
<td>40</td>
<td>6.94 / 269</td>
<td>0.27 / 10.5</td>
<td>3.89</td>
<td>0.24 / 9.3</td>
<td>36.69</td>
</tr>
</tbody>
</table>

**ACCURACY**

Comparison studies were carried out using a similar commercially available Cholesterol reagent as a reference. Calibrations were carried out using material with a cholesterol value 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: 48
- Range of sample results: 2.4 - 14.7 mmol/L (93 - 569 mg/dL)
- Mean of reference method results: 5.7 mmol/L (221 mg/dL)
- Mean of Cholesterol results: 5.9 mmol/L (228 mg/dL)
- Slope: 0.985
- Intercept: 0.24 mmol/L (9.3 mg/dL)
- Correlation coefficient: 0.985

**LINEARITY**

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

**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**