The elegant and simple enzymatic method of assay has replaced older methods. The method employed in this research is based on the work of Allain et al. [1]. In this method, cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and fatty acids:

\[
\text{Cholesterol esters} \rightarrow \text{Cholesterol + fatty acids esterase}
\]

Cholesterol is oxidized by cholesterol oxidase to 
\(\Delta 4\)-Cholestene and the simultaneous production of hydrogen peroxide:

\[
\text{Cholesterol} + \text{O}_2 \rightarrow \Delta 4\text{-Cholestene} + 4\text{H}_2\text{O}_2
\]

In the presence of peroxidase, hydrogen peroxide oxidizes phenol and 4-aminoantipyrine to give a quinoneimine dye colored in red:

\[
\text{Peroxidase} \rightarrow \text{Quinoneimine dye + 4H}_2\text{O}
\]

The intensity of the color produced is proportional to the concentration of cholesterol in the sample.

**REAGENT DESCRIPTION**

**Cholesterol Rapid Liquid Reagent**

Reactives:
- 4-Aminoantipyrine 0.6 mmol/L
- Phenol 10 mmol/L
- Cholesterol Oxidase ≥ 300 U/L
- Cholesterol Esterase ≥ 120 U/L
- Peroxidase 25000 U/L

Non-reactives:
- Buffers, stabilizers and fillers

**PRECAUTIONS**

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety: Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

**PREPARATION**

Ready to use liquid reagent, no preparation required.

**STORAGE**

Stable until the expiration date on the label when stored at 2–8 °C. Protect from light and microbial contamination.

**INDICATIONS OF INSTABILITY**

If the absorbance of the reagent without sample added is over 0.600 at 500 nm, discard the reagent. If the reagent fails to recover stated values for control sera, discard the reagent. Discard the reagent after the expiration date on the label.

**SPECIMEN**

**Collection and preparation** [2]

**SERUM:** Use serum collected by standard venipuncture technique.

**PLASMA:** Use Heparin or EDTA plasma collected by standard venipuncture technique.

The patient should sit quietly for about 5 minutes before the sample is drawn. A minimum of 10 µL of serum or plasma is required for this assay. The optimum quantity of serum or plasma is 0.5 mL.

**Interfering substances**

- Bilirubin: No significant interference observed at 20 mg/dL.
- Hemolysis: No significant interference observed at 500 mg/dL.
- Lipemia: No significant interference observed at 1000 mg/dL.
- Young [3,4] has published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including that of cholesterol.

**Storage**

Cholesterol levels in the sample have been reported to be stable for 5-7 days at 4°C or room temperature, 3 months at -20°C, and many years at -70°C. [5]

**MANUAL PROCEDURE**

**Material Provided**

Cholesterol Rapid Liquid Reagent

**Cholesterol Standard (300 mg/dL)**

**Materials required but not provided**

1. Spectrophotometer or colorimeter capable of measuring absorbance at 500 nm.
3. Constant temperature incubator set at 30 °C, or 37 °C. Use the same temperature for assay of standard, controls and samples.
4. Pipettes to measure reagent and samples.

**INSTRUCTIONS**

**Wavelength:** 500 nm

**Test:** 1 mL reagent + 10 µL sample

**Standard:** 1 mL reagent + 10 µL standard

**Blank:** 1 mL reagent

**Incubate:** 5 minutes at 37 °C or 10 minutes at 30 °C

**Reading:** Set instrument to 0 absorbance with the blank. Read absorbance (A) of the test and standard within 3 minutes from the end of the incubation period recommended above.

**CALIBRATION**

Use a reliable, commercially available standard or Cliniqua Multi-Analyte Serum Calibrator (R60010) with established values for cholesterol concentrations.

It is recommended that each laboratory establish its own procedures for corrective action if calibration is not acceptable.

**QUALITY CONTROL**

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Cliniqua Assayed Control Serum Level 1 (R83082) and Level 2 (R83083) or a commercially available control material with established values for cholesterol concentrations may be used.

**RESULTS**

\[
C_a = \frac{C_s \times V_a}{V_s} = \text{Cholesterol in sample in mg/dL}
\]

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A standard
Sample Calculation:
If the absorbance of the sample is 0.235 and that of the standard is 0.470 and the value of the standard is 300 mg/dL:
\[
\frac{0.235}{0.470} \times 300 = 150 \text{ mg/dL Cholesterol}
\]

LIMITATIONS OF THE PROCEDURE
1. Samples with cholesterol concentrations exceeding the linearity of this assay (700 mg/dL) should be diluted with an equal volume of physiological saline (150 mmol/L sodium chloride in water) and assayed again; multiply results by 2.
2. Plasma levels may be approximately 3% lower than serum values. It is recommended that if plasma is used, the plasma value should be converted to a serum value according to the following factor:
   Serum cholesterol = Plasma cholesterol \times 1.03 \[2\].

PERFORMANCE
1. Linearity: The assay is linear to 700 mg/dL.
2. Correlation: Results obtained with this reagent in 107 serum samples, ranging in cholesterol value from 55 to 545 mg/dL, were compared with those obtained using Raichem Cholesterol Reagent. The correlation coefficient was 0.998 and the regression equation was \[y = 1.006x - 1.422\].
3. Precision: Precision studies conducted in accordance with NCCLS EP5-T2, using this reagent and control serum, at 37 °C, yielded the following:
<table>
<thead>
<tr>
<th></th>
<th>Within Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/dL)</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>SD</td>
<td>2.2</td>
<td>3.0</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.7</td>
<td>2.4</td>
</tr>
<tr>
<td>N</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>239</td>
<td>239</td>
</tr>
<tr>
<td>SD</td>
<td>1.7</td>
<td>4.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>N</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

4. Sensitivity: 1mg/dL = 0.0016 A

REFERENCE RANGE
The use of risk groups to classify accepted concentrations of total cholesterol in serum have been suggested by recent studies \[7,8\]. The risk groups have been identified as follows:

<table>
<thead>
<tr>
<th>Risk Classification</th>
<th>Total Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desirable</td>
<td>&lt; 200 mg/dL</td>
</tr>
<tr>
<td>Borderline High</td>
<td>200-239 mg/dL</td>
</tr>
<tr>
<td>High</td>
<td>≥ 240 mg/dL</td>
</tr>
</tbody>
</table>

At least two measurements of cholesterol on separate occasions should be made before a medical decision is made. A single point total cholesterol measurement may not represent a patient’s usual cholesterol concentration. Cholesterol results that are at the decision points should be followed with a repeat measurement.

It is recommended that each laboratory establish its own reference range.

BIBLIOGRAPHY

For in vitro diagnostic use
See package insert for proper use

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RE-ORDER INFORMATION
Cholesterol Rapid Liquid Reagent

Catalog No.    Catalog No.    Catalog No.
               R85464          R85465          R85545
Made in the USA