**Intended Use**
For the quantitative determination of Total Cholesterol in serum using the Mindray BS-200 analyzer.

**Method History**
A Cholesterol method developed in the late 1800’s by Lieberman1 and Burchard2 is still in use today despite its corrosive nature and its susceptibility to many interfering substances. Work on an enzymatic procedure was begun by Flegg3 and Richmond4 in the early 70’s. Allain5 and Roeschlau6 began using cholesterol esterase and oxidase, in a single reagent to determine total cholesterol in serum. Trinder’s color system of peroxidase/phenol/4-aminoantipyrine has been used successfully for some time now. With appropriate calibrator value assignment, this method has been shown to provide excellent accuracy in relation to the reference methodology.

**Principle**

\[
\text{C. Esterase} \\
\text{Cholesterol Esters} \rightarrow \text{Cholesterol + Fatty Acids}
\]

\[
\text{C. Oxidase} \\
\text{Cholesterol + O}_2 \rightarrow \text{Cholesterol-3-one + H}_2\text{O}_2
\]

\[
\text{Peroxidase} \\
\text{2H}_2\text{O}_2 + 4\text{-AAP} + \text{Phenol} \rightarrow \text{Quinoneimine + 4 H}_2\text{O (red dye)}
\]

The intensity of the red color produced is directly proportional to the total cholesterol in the sample when read at 500nm.

**Reagents**
4-Aminoantipyrine 0.25mM, Cholesterol Esterase >150u/L, Cholesterol Oxidase >1500u/L, Peroxidase >1500u/L, Phenol >15mM, Phosphate Buffer, pH 6.8, non-reactive stabilizers and preservatives.

**Reagent Preparation**
The reagent is ready to use.

**Reagent Storage**
1. Store reagent at 2-8°C.
2. The reagent is stable until the expiration date when stored at 2-8°C.

**Reagent Deterioration**
Do not use if:
1. The reagent is turbid.
2. The reagent does not meet stated performance parameters.

**Precautions**
1. This reagent is for in vitro diagnostic use only.
2. Not to be used internally in humans or animals. Normal precautions for handling laboratory reagents should be followed.
3. Additional safety information concerning storage and handling of this product is in the Material Safety Data Sheet for this product.

**Specimen Collection and Storage**
Nonhemolyzed serum is recommended. Cholesterol in serum is reported stable for seven days at room temperature (18-25°C) and six months when frozen and properly protected against evaporation.8,9

**Interferences**
A number of drugs and substances affect concentrations of cholesterol. See Young, et al.10

**Materials Provided**
Cholesterol Reagent

**Materials Required but not Provided**
1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry Control, catalog number 12-C7592-50

**Mindray BS-200 Test Parameters**

<table>
<thead>
<tr>
<th>Test :</th>
<th>CHOL</th>
<th>R1 :</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. :</td>
<td>012</td>
<td>R2 :</td>
<td>0</td>
</tr>
<tr>
<td>Full Name :</td>
<td>Cholesterol</td>
<td>Sample Volume:</td>
<td>3</td>
</tr>
<tr>
<td>Standard No. :</td>
<td>R1 Blank</td>
<td></td>
<td></td>
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<tr>
<td>Reac.Type :</td>
<td>Endpoint</td>
<td>Mixed Rgt. Blank:</td>
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</tr>
<tr>
<td>Pri. Wave :</td>
<td>510nm</td>
<td>Linearity Range:</td>
<td>0 - 500</td>
</tr>
<tr>
<td>Sec. Wave :</td>
<td>670nm</td>
<td>Linearity Limit:</td>
<td></td>
</tr>
<tr>
<td>Direction:</td>
<td>Increase</td>
<td>Substrate Limit:</td>
<td></td>
</tr>
<tr>
<td>Reac. Time:</td>
<td>0 / 20</td>
<td>Factor:</td>
<td></td>
</tr>
<tr>
<td>Incuba.Time:</td>
<td>□ Prozone check</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unit: mg/dl</td>
<td>q1:</td>
<td>q2:</td>
<td>q3:</td>
</tr>
<tr>
<td>Precision: Integer</td>
<td>PC:</td>
<td>Abs:</td>
<td></td>
</tr>
</tbody>
</table>

**Calibration Parameters**

<table>
<thead>
<tr>
<th>Rule: Two-point linear</th>
<th>Calibrator 1: Deionized Water</th>
<th>Calibrator 2: Chem Cal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicates: 2</td>
<td>Calibrator 3:</td>
<td></td>
</tr>
<tr>
<td>Interval (day):</td>
<td>Calibrator 4:</td>
<td></td>
</tr>
<tr>
<td>Difference Limit:</td>
<td>Calibrator 5:</td>
<td></td>
</tr>
<tr>
<td>SD:</td>
<td>Calibrator 6:</td>
<td></td>
</tr>
<tr>
<td>Blank Response:</td>
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<td></td>
</tr>
<tr>
<td>Error Limit:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation Coefficient:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Limitations**
Samples with values exceeding 500 mg/dl should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.
Calibration
Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer’s instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Calculation (Example)
Abs. = Absorbance

\[
\text{Abs. (Patient)} \times \text{Concentration of Std.} = \text{Cholesterol (mg/dl)}
\]

\[
\text{Abs. (Standard)} \quad \text{(mg/dl)}
\]

Example: Abs. (Patient) = 0.40, Abs. (Standard) = 0.32, Concentration of Standard = 200 mg/dl

\[
0.40 \times 200 = 250 \text{ mg/dl}
\]

0.32

Quality Control
Serum controls with known normal and elevated values should be run routinely to monitor the validity of the reaction. These controls should be run at least with every working shift in which Cholesterol assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values
Recommended Range:

<table>
<thead>
<tr>
<th>Category</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desirable Cholesterol</td>
<td>&lt;200 mg/dl</td>
</tr>
<tr>
<td>Borderline-High Cholesterol</td>
<td>200-239 mg/dl</td>
</tr>
<tr>
<td>High Cholesterol</td>
<td>&gt;240 mg/dl</td>
</tr>
</tbody>
</table>

Performance
1. Linearity: 500 mg/dl
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer using this method, resulting in a correlation coefficient of \( y = 1.068x - 2.5 \) with a correlation coefficient of 0.968.
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.12

<table>
<thead>
<tr>
<th>Within Run</th>
<th>Day to Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>140.4</td>
<td>3.1</td>
</tr>
<tr>
<td>269.1</td>
<td>4.0</td>
</tr>
</tbody>
</table>

4. Specificity: Cholesterol oxidase is not totally specific for cholesterol. Other analogs of cholesterol (dihydrocholesterol, 7-dehydrocholesterol, 20-hydroxycholesterol, etc.) are also oxidized. These analogs do not normally occur in any appreciable amounts in serum.

References
1. Lieberman, C., Ber. 18:1803 (1885).