Intended Use
For the quantitative determination of Total Cholesterol in serum using the Mindray BS-480 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 Roeschla⁶ began using cholesterol esterase and oxidase, in a single reagent to determine total cholesterol in serum.
Trinder’s⁷ 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

\[
\begin{align*}
\text{Cholesterol Esters} & \xrightarrow{\text{C. Esterase}} \text{Cholesterol + Fatty Acids} \\
\text{Cholesterol + O}_2 & \xrightarrow{\text{C. Oxidase}} \text{Cholesterol-3-one + H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + 4\text{-AAP} + \text{Phenol} & \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + 4 H}_2\text{O} \\
\end{align*}
\]

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 >150u/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.
3. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

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

Precautions and Hazards
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.

Hazard Classifications: Reproductive Toxicity (Category 2)
Hazard Statements: H361: Suspected of damaging fertility or the unborn child
Precautionary Statements: Prevention: P202 Do not handle until all safety precautions have been read and understood.


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-480 Analyzer
2. BS-480 Operation manual
3. Chemistry Calibrator, catalog number CHEC480
4. Chemistry Control, catalog number CHEQ480

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 test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

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:
Desirable Cholesterol: <200 mg/dL
Borderline-High Cholesterol: 200-239 mg/dL
High Cholesterol: >240 mg/dL

Performance

1. Assay Range: 0-500 mg/dL
2. Comparison: A study was performed between the Mindray BS-480 and a similar analyzer using this method, resulting in the following:

<table>
<thead>
<tr>
<th>Method</th>
<th>Cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>84</td>
</tr>
<tr>
<td>Mean Cholesterol</td>
<td>210.8</td>
</tr>
<tr>
<td>Range (mg/dL)</td>
<td>57-398</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>73.9</td>
</tr>
<tr>
<td>Regression Analysis</td>
<td>y = 0.974x – 2.1</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9968</td>
</tr>
</tbody>
</table>

3. Precision: Precision studies were performed using the Mindray BS-480 analyzer following a modification of the guidelines which are contained in NCCLS document EPS-T2. \(^{12}\)

<table>
<thead>
<tr>
<th>Within Day</th>
<th>Sample</th>
<th>LOW</th>
<th>MID</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>137.4</td>
<td>287.3</td>
<td>504.3</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.5</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td>1.1%</td>
<td>0.3%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td></td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>Sample</th>
<th>LOW</th>
<th>MID</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>137.3</td>
<td>290.0</td>
<td>510.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>3.1</td>
<td>7.6</td>
<td>10.6</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td>2.3%</td>
<td>2.6%</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

4. Sensitivity: 2SD limit of detection (95% Conf) = 0 mg/dL

5. 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).
# CHEMISTRY PARAMETERS

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Chem:</td>
<td>CHOL</td>
<td>No.:</td>
<td>210</td>
<td>Sample Type:</td>
<td>Serum</td>
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<tr>
<td>Chemistry:</td>
<td>Cholesterol</td>
<td></td>
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<td>Print Name:</td>
<td>CHOL</td>
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<td>Reaction Type:</td>
<td>End Point</td>
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<td>Reaction Direction:</td>
<td>Positive</td>
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<td>Pri Wave:</td>
<td>505</td>
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<td>Sec Wave:</td>
<td>660</td>
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<tr>
<td>Unit:</td>
<td>mg/dL</td>
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<td></td>
<td>Decimal:</td>
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<td>Blank Time:</td>
<td>10</td>
<td>12</td>
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<td>Reaction Time:</td>
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<td>Standard:</td>
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<td>--- ul</td>
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<td>150 ul</td>
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<td>Decreased:</td>
<td>--- ul</td>
<td>--- ul</td>
<td>--- ul</td>
<td>R2:</td>
<td>--- ul</td>
<td>--- ul</td>
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<tr>
<td>Increased:</td>
<td>--- ul</td>
<td>--- ul</td>
<td>--- ul</td>
<td>R3:</td>
<td>--- ul</td>
<td>--- ul</td>
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<tr>
<td>Sample Blank</td>
<td></td>
<td></td>
<td></td>
<td>R4:</td>
<td>--- ul</td>
<td>--- ul</td>
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<tr>
<td>Auto Rerun</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**Slope/Offset Adjustment**

Slope: 1  Offset: 0

<table>
<thead>
<tr>
<th>Linearity Range (Standard)</th>
<th>0</th>
<th>500</th>
<th>Linearity Limit:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range (Decreased)</td>
<td></td>
<td></td>
<td>Substrate Depletion:</td>
</tr>
<tr>
<td>Linearity Range (Increased)</td>
<td></td>
<td></td>
<td>Mixed Blank Abs:</td>
</tr>
<tr>
<td>R1 Blank Abs:</td>
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<td></td>
<td>Uncapping Time</td>
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<td>Blank Response:</td>
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<td></td>
<td>Reagent Alarm Limit:</td>
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<tr>
<td>Twin Chemistry:</td>
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<td></td>
<td>Enzyme Linear Extension</td>
</tr>
</tbody>
</table>

- [ ] Prozone Check
- [ ] Rate Check
- [ ] Antigen Addition

Q1: Q2: Q3: Q4: PC: ABS:
CALIBRATION PARAMETERS

Calibrator Definition

Calibrator: * Lot No.: *
Exp Date: *

Carousel Pos
Sample Carousel 1 *
Sample Carousel 2
Sample Carousel 3

Reagent/Calibration

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Pos</th>
<th>Lot No.</th>
<th>Exp Date</th>
<th>Chem</th>
<th>Conc</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>W</td>
<td>*</td>
<td>*</td>
<td>CHOL</td>
<td>0</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Chemistry Calibrator</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>CHOL</td>
<td>*</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

Calibration Setup

Chem: CHOL

Calibration Settings

Math Model: Two-Point Linear
Factor: Replicates: 2

Acceptance Limits

Cal Time: * Hour
Slope Diff: --- SD: ---
Sensitivity: --- Repeatability: ---
Deter Coeff: ---

Auto Calib.
☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF CHO480
Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

Symbol Key

☐ Use by (YYYY-MM-DD) ☐ Temperature limitation
☐ Lot and batch code ☐ Consult instructions for use
☐ Catalog number ☐ In vitro diagnostic medical device

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