CHOLESTEROL

This package insert contains information to run the Cholesterol assay on the ARCHITECT c Systems™ and the AEROSET System.

NOTE: Changes Highlighted

NOTE: This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Support

United States: 1-877-4ABBOTT
Canada: 1-800-387-8378 (English speaking customers)
1-800-465-2675 (French speaking customers)
International: Call your local Abbott representative

Symbols in Product Labeling

- **CAL[1-2]**: Calibrators 1 and 2
- **CONC**: Concentration
- **EC[REP]**: Authorized Representative in the European Community
- **INGRED**: Ingredients
- **INVD**: In vitro diagnostic medical device
- **LOT**: Batch code/Lot number
- **RT**: Reagent 1
- **REF**: Catalog number/List number
- **SN**: Serial number
- **I**: Consult instructions for use
- **M**: Manufacturer
- **°C**: Temperature limitation
- **Use by/Expiration date**
INTENDED USE
The Cholesterol assay is used for the quantitation of cholesterol in human serum or plasma.

SUMMARY AND EXPLANATION OF TEST
Measurement of serum cholesterol levels can serve as an indicator of liver function, biliary function, intestinal absorption, propensity toward coronary artery disease, and thyroid function. Cholesterol levels are important in the diagnosis and classification of hyperlipoproteinemias. Stress, age, gender, hormonal balance, and pregnancy affect normal cholesterol levels.

The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that all adults 20 years of age and over should have a fasting lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride) once every five years to screen for coronary heart disease risk.2

PRINCIPLES OF PROCEDURE
The use of enzymes to assay cholesterol has been studied by many investigators.3,4 This reagent is based on the formulation of Allain, et al.5 and the modification of Roeschlau6 with further improvements to render the reagent stable in solution. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide that is originally present is then oxidized by cholesterol oxidase to cholesterol and free fatty acids. Hydrogen peroxide combines with hydroxybenzoic acid (HBA) and 4-aminopyridine to form a chromophore (quinoneimine dye) which is quantitated at 500 nm.

Methodology: Enzymatic

REAGENTS
Reagent Kit
REF 7D62 Cholesterol is supplied as a liquid, ready-to-use, single reagent kit which contains:

RT 10 x 84 mL

Estimated tests per kit: 3,032

Calculation is based on the minimum reagent fill volume per kit.

<table>
<thead>
<tr>
<th>Reactive Ingredients</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Oxidase (Microbial)</td>
<td>&gt; 200 U/L</td>
</tr>
<tr>
<td>Cholesterol Esterase (Microbial)</td>
<td>&gt; 500 U/L</td>
</tr>
<tr>
<td>Peroxidase (Horseradish)</td>
<td>&gt; 300 U/L</td>
</tr>
<tr>
<td>4-Aminopyridine</td>
<td>0.25 mmol/L</td>
</tr>
<tr>
<td>HBA</td>
<td>10 mmol/L</td>
</tr>
</tbody>
</table>

The Abbott Clinical Chemistry Cholesterol reagent is certified to be traceable to the National Reference System for Cholesterol, against the Abell-Kendall reference method in a CDC-Certified Cholesterol Reference Method Laboratory Network (CRMLN).

REAGENT HANDLING AND STORAGE
Reagent Handling
Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.

CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration which could impact results.

Reagent Storage
Unopened reagents are stable until the expiration date when stored at 2 to 8°C.

Reagent stability is 30 days if the reagent is uncapped and onboard.

WARNINGS AND PRECAUTIONS
Precautions for Users
1. For in vitro diagnostic use.
2. Do not use components beyond the expiration date.
3. Do not mix materials from different kit lot numbers.
5. CAUTION: This product requires the handling of human specimens.

It is recommended that all human sourced materials are considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.7 Biosafety Level 28 or other appropriate biosafety practices6,10 should be used for materials that contain or are suspected of containing infectious agents.

SPECIMEN COLLECTION AND HANDLING
Suitable Specimens
Serum and plasma are acceptable specimens. The National Cholesterol Education Program (NCEP) recommends using fasting specimens.2

- Serum: Use serum collected by standard venipuncture techniques into glass or plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. Separate serum from red blood cells or gel as soon after collection as possible.

- Plasma: Use plasma collected by standard venipuncture techniques into glass or plastic tubes. Acceptable anticoagulants are lithium heparin (with or without gel barrier) and sodium heparin. Ensure centrifugation is adequate to remove platelets. Separate plasma from red blood cells or gel as soon after collection as possible.

Refer to the specimen collection tube manufacturer’s instructions for processing and handling requirements.

For total sample volume requirements, refer to the instrument-specific ASSAY PARAMETERS section of this package insert and Section 5 of the instrument-specific operations manual.

Specimen Storage
Serum and plasma

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Maximum Storage</th>
<th>Bibliographic Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 to 25°C</td>
<td>7 days</td>
<td>11</td>
</tr>
<tr>
<td>2 to 8°C</td>
<td>7 days</td>
<td>11, 12</td>
</tr>
<tr>
<td>-20°C</td>
<td>3 months</td>
<td>11</td>
</tr>
</tbody>
</table>

Guder et al.11 suggest storage of frozen specimens at -20°C for no longer than the time interval cited above. However, limitations of laboratory equipment make it necessary in practice for clinical laboratories to establish a range around -20°C for specimen storage. This temperature range may be established from either the freezer manufacturer’s specifications or your laboratory standard operating procedure(s) for specimen storage.

NOTE: Stored specimens must be inspected for particulates. If present, mix and centrifugate the specimen to remove particulates prior to testing.

PROCEDURE
Materials Provided
REF 7D62 Cholesterol Reagent Kit

Materials Required but not Provided
- REF 1E65 Multiconstituent Calibrator, CAL 3 x 5 mL
- Control Material
- Saline (0.85% to 0.90% NaCl) for specimens that require dilution
PROCEDURE (Continued)

Assay Procedure
For a detailed description of how to run an assay, refer to Section 5 of the instrument-specific operations manual.

Specimen Dilution Procedures
The ARCHITECT c Systems and the AEROSET System have automatic dilution features; refer to Section 2 of the instrument-specific operations manual for additional information.

Serum and plasma: Specimens with cholesterol values exceeding 705 mg/dL (18.26 mmol/L) are flagged and may be diluted using the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol
If using the Automated Dilution Protocol, the system performs a 1:4 dilution of the specimen and automatically corrects the concentration by multiplying the result by the appropriate dilution factor.

Manual Dilution Procedure
Manual dilutions should be performed as follows:
• Use saline (0.85% to 0.90% NaCl) to dilute the sample
• The operator must enter the dilution factor in the patient or control order screen. The system uses this dilution factor to automatically correct the concentration by multiplying the result by the entered factor.
• If the operator does not enter the dilution factor, the result must be multiplied by the appropriate dilution factor before reporting the result.

NOTE: If a diluted sample result is flagged indicating it is less than the linear low limit, do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to Section 5 of the instrument-specific operations manual.

CALIBRATION
Calibration is stable for approximately 30 days (720 hours) and is required with each change in reagent lot number. Verify calibration curve with at least two levels of controls according to the established quality control requirements and potential corrective actions.
• Two levels of controls (normal and abnormal) are to be run every 24 hours.
• If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
• If quality control results do not meet the acceptance criteria defined by your laboratory, patient values may be suspect. Follow the established quality control procedures for your laboratory.
• Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

QUALITY CONTROL
The following is the recommendation of Abbott Laboratories for quality control. As appropriate, refer to your laboratory standard operating procedure(s) and/or quality assurance plan for additional quality control requirements and potential corrective actions.

RESULTS
Refer to the instrument-specific operations manual for information on results calculations.

• ARCHITECT System Operations Manual—Appendix C
• AEROSET System Operations Manual—Appendix A

Representative performance data are given in the EXPECTED VALUES and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert. Results obtained in individual laboratories may vary.

LIMITATIONS OF THE PROCEDURE
Refer to the SPECIMEN COLLECTION AND HANDLING and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

EXPECTED VALUES
Reference Range
Serum/Plasma

<table>
<thead>
<tr>
<th>Range (mg/dL)</th>
<th>Range (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child&lt;sup&gt;13&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Desirable</td>
<td>&lt; 170</td>
</tr>
<tr>
<td>Borderline</td>
<td>170 to 199</td>
</tr>
<tr>
<td>High</td>
<td>≥ 200</td>
</tr>
</tbody>
</table>

Adult<sup>2</sup>:

<table>
<thead>
<tr>
<th>Range (mg/dL)</th>
<th>Range (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desirable</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>Borderline</td>
<td>200 to 239</td>
</tr>
<tr>
<td>High</td>
<td>≥ 240</td>
</tr>
</tbody>
</table>

To convert results from mg/dL to mmol/L, multiply mg/dL by 0.0259.

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III Report<sup>2</sup> recommends the adult classification shown above. Laboratories should follow recommendations for lipid ranges effective in their locale if they differ from those of the NCEP.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity
Cholesterol is linear up to 705 mg/dL (18.26 mmol/L). Linearity was verified using Clinical and Laboratory Standards Institute (CLSI) protocol NCCLS EP6-P<sup>14</sup>.

Limit of Detection (LOD)
The LOD for Cholesterol is 5.0 mg/dL (0.13 mmol/L). The LOD is the mean concentration of an analyte-free sample + 2 SD, where SD = the pooled, within-run standard deviation of the analyte-free sample. A study performed on an ARCHITECT c System and an AEROSET System produced an LOD for the Cholesterol assay of 0.80 mg/dL (0.021 mmol/L).

Limit of Quantitation (LOQ)
The LOQ for Cholesterol is 6.2 mg/dL (0.161 mmol/L). The LOQ is the analyte concentration at which the CV = 20%.

Interfering Substances<sup>15</sup>
Interference studies were conducted using CLSI protocol NCCLS EP7-P<sup>7</sup> and EP7-P<sup>8</sup>. Interference effects were assessed by Dose Response and Paired Difference methods, at the medical decision level of the analyte.

<table>
<thead>
<tr>
<th>Interfering Substance</th>
<th>Concentration (mg/dL)</th>
<th>Target Concentration (mg/dL)</th>
<th>Observed %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>7.5 mg/dL (128 µmol/L)</td>
<td>4 252.3</td>
<td>91.7</td>
</tr>
<tr>
<td></td>
<td>1.5 mg/dL (257 µmol/L)</td>
<td>4 252.3</td>
<td>86.8</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>750 mg/dL (7.5 g/L)</td>
<td>4 241.1</td>
<td>109.5</td>
</tr>
<tr>
<td></td>
<td>1,000 mg/dL (10.0 g/L)</td>
<td>4 241.1</td>
<td>111.9</td>
</tr>
<tr>
<td>Intralipid</td>
<td>1,000 mg/dL (10.0 g/L)</td>
<td>4 236.1</td>
<td>102.5</td>
</tr>
<tr>
<td></td>
<td>2,000 mg/dL (20.0 g/L)</td>
<td>4 236.1</td>
<td>101.9</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>1.5 mg/dL (85 µmol/L)</td>
<td>4 282.2</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>3 mg/dL (170 µmol/L)</td>
<td>4 282.2</td>
<td>97.6</td>
</tr>
</tbody>
</table>

Hemoglobin solutions at the above concentrations were prepared by addition of a bilirubin stock to human serum pools. Intralipid solutions at the above concentrations were prepared by addition of Intralipid to human serum pools. Ascorbate solutions at the above concentrations were prepared by addition of ascorbic acid to human serum pools.

Precision
The imprecision of the Cholesterol assay is ≤ 3% Total CV. Representative data from studies using CLSI protocol NCCLS EP5-P<sup>17</sup> are summarized below.

<table>
<thead>
<tr>
<th>Control</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>261.4</td>
<td>129.2</td>
</tr>
<tr>
<td>Within Run SD</td>
<td>1.99</td>
<td>0.78</td>
</tr>
<tr>
<td>%CV</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Between Run SD</td>
<td>1.01</td>
<td>1.03</td>
</tr>
<tr>
<td>%CV</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Between Day SD</td>
<td>3.36</td>
<td>1.64</td>
</tr>
<tr>
<td>%CV</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Total SD</td>
<td>4.03</td>
<td>2.09</td>
</tr>
<tr>
<td>%CV</td>
<td>1.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>
SPECIFIC PERFORMANCE CHARACTERISTICS (Continued)

Method Comparison

Correlation studies were performed using CLSI protocol NCCLS EP9-A.\textsuperscript{18}

Serum results from the Cholesterol assay on the AEROSET System were compared with those from a commercially available enzymatic methodology.

Serum results from the Cholesterol assay on the ARCHITECT \textsuperscript{c}System were compared with the Cholesterol assay on the AEROSET System.

\begin{tabular}{|c|c|c|}
\hline
 & AEROSET vs. Comparative Method & ARCHITECT vs. AEROSET \\
\hline
N & 79 & 101 \\
Y - Intercept & 0.933 & -0.840 \\
Correlation Coefficient & 0.993 & 0.993 \\
Slope & 1.016 & 0.979 \\
Range (mg/dL)* & 70.6 to 416.8 & 39.5 to 687.6 \\
\hline
\end{tabular}

*AEROSET Range

BIBLIOGRAPHY


TRADEMARKS

AEROSET and ARCHITECT are registered trademarks of Abbott Laboratories.

\textsuperscript{c}System is a trademark of Abbott Laboratories.

All other trademarks, brands, product names, and trade names are the property of their respective companies.
### ARCHITECT® SYSTEMS ASSAY PARAMETERS

#### Cholesterol Serum/Plasma—Conventional and SI Units

#### Configure assay parameters — General

<table>
<thead>
<tr>
<th>General</th>
<th>Calibration</th>
<th>SmartWash</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay: Chol</td>
<td>Type: Photometric</td>
<td>Version: 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Configure assay parameters — Calibration

<table>
<thead>
<tr>
<th>General</th>
<th>Calibration</th>
<th>SmartWash</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay: Chol</td>
<td>Calibration method: Linear</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Calibrators

- **Calibrator set:**
  - MCC
  - Replicates: 3 [Range 1 – 3]

#### Volumes

- Diluent: Saline
  - Water volume: ___
- Diluent dispense mode: Type 0
  - Dispense mode: Type 0

#### Intervals

- Reaction definition:
  - Reagent: CHOL0
  - Reagent volume: 240
  - Diluent: Saline
  - Water volume: ___
  - Diluted Default
  - Dilution name: Sample/Diluent/Water
  - STANDARD: 2.4
  - Dilution: Sample/Diluent/Water
  - Default dilution factor: 1:1.00
  - Dilution name: Sample/Diluent/Water
  - Dilution: 1:4
  - Water volume: 75

#### Reaction definition

- Reaction check: None

#### Configure assay parameters — SmartWash

<table>
<thead>
<tr>
<th>General</th>
<th>Calibration</th>
<th>SmartWash</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay: Chol</td>
<td>COMPONENT</td>
<td>ASSAY</td>
<td>WASH</td>
<td>Volume</td>
</tr>
<tr>
<td>R1 ALBP0</td>
<td>Water</td>
<td>10% Detergent B</td>
<td>345</td>
<td></td>
</tr>
</tbody>
</table>

#### Configure assay parameters — Results — Conventional Units

<table>
<thead>
<tr>
<th>General</th>
<th>Calibration</th>
<th>SmartWash</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay: Chol</td>
<td>Result units: mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Low-Linearity:

- 7

#### High-Linearity:

- 705

#### Gender and age specific ranges:

- Either: 0 – 130 (Y)
- 0 – 199

#### Configure result units — Conventional Units

<table>
<thead>
<tr>
<th>General</th>
<th>Calibration</th>
<th>SmartWash</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay: Chol</td>
<td>Result units: mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Low-Linearity:

- 0.17

#### High-Linearity:

- 18.26

#### Gender and age specific ranges:

- Either: 0 – 130 (Y)
- 0.00 – 5.17

#### Configure result units — SI Units

<table>
<thead>
<tr>
<th>General</th>
<th>Calibration</th>
<th>SmartWash</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay: Chol</td>
<td>Result units: mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Low-Linearity:

- 4

#### High-Linearity:

- 5

#### Gender and age specific ranges:

- Either: 0 – 130 (Y)
- 0.00 – 5.17

#### Configure result units — SI Units

<table>
<thead>
<tr>
<th>General</th>
<th>Calibration</th>
<th>SmartWash</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay: Chol</td>
<td>Result units: mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Low-Linearity:

- 4

#### High-Linearity:

- 5

#### Gender and age specific ranges:

- Either: 0 – 130 (Y)
- 0.00 – 5.17

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† The linear low value (Low-Linearity) is LOQ rounded up to the number of decimal places defined in the decimal places parameter field.

‡ Refer to concentration specified on calibrator labeling or value sheet.

†† Displays the number of decimal places defined in the decimal places parameter field.
AEROSET SYSTEM ASSAY PARAMETERS

Cholesterol Serum/Plasma—Conventional Units

<table>
<thead>
<tr>
<th>Assay Configuration: Outline Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay Name</strong></td>
</tr>
<tr>
<td>Chol</td>
</tr>
<tr>
<td><strong>Quantitative Ranges</strong></td>
</tr>
<tr>
<td><strong>Min Text</strong></td>
</tr>
<tr>
<td>*</td>
</tr>
<tr>
<td><strong>Reference Ranges</strong></td>
</tr>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>0 Year</td>
</tr>
<tr>
<td><strong>Qualitative Ranges</strong></td>
</tr>
</tbody>
</table>

Cholesterol Serum/Plasma—SI Units

<table>
<thead>
<tr>
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<tr>
<td><strong>Assay Name</strong></td>
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</tr>
<tr>
<td><strong>Quantitative Ranges</strong></td>
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<tr>
<td><strong>Min Text</strong></td>
</tr>
<tr>
<td>*</td>
</tr>
<tr>
<td><strong>Reference Ranges</strong></td>
</tr>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>0 Year</td>
</tr>
<tr>
<td><strong>Qualitative Ranges</strong></td>
</tr>
</tbody>
</table>

Refer to **Assay Configuration** in Section 2 of the AEROSET System Operations Manual for information regarding assay parameters.

* User defined or instrument defined.

** The linear low value (L-Linear Range) is LOQ rounded up to the number of decimal places defined in the decimal places parameter field.