Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

NAME
Alinity c Cholesterol Reagent Kit (also referred to as Chol)

INTENDED USE
The Alinity c Cholesterol assay is used for the quantitation of cholesterol in human serum or plasma on the Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE 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.1

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 THE 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 combines with hydroxybenzoic acid (HBA) and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which is quantitated at 500 nm.

Methodology: Enzymatic
For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.

REAGENTS
Kit Contents
Alinity c Cholesterol Reagent Kit 07P76
Volumes (mL) listed in the table below indicate the volume per cartridge.

<table>
<thead>
<tr>
<th>REF</th>
<th>07P7620</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests per cartridge</td>
<td>400</td>
</tr>
<tr>
<td>Number of cartridges per kit</td>
<td>10</td>
</tr>
<tr>
<td>Tests per kit</td>
<td>4000</td>
</tr>
<tr>
<td>R1</td>
<td>67.3 mL</td>
</tr>
</tbody>
</table>

R1 Active ingredients: Cholesterol Oxidase (Microbial) (> 200 U/L), Cholesterol Esterase (Microbial) (> 500 U/L), Peroxidase (Hors eradish) (> 300 U/L), 4-Aminoantipyrine (< 0.5 mmol/L), HBA (10 mmol/L). Preservative: sodium azide (0.01%).

Alinity c 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).

Warnings and Precautions

- For In Vitro Diagnostic Use
- Rx ONLY
- R1 contains nonsterile bovine serum albumin.

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.7-10

The following warnings and precautions apply to: R1
- Contains sodium azide.
- EUH032 Contact with acids liberates very toxic gas.
- P501 Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 8.

Reagent Handling

- Upon receipt, place reagent cartridges in an upright position for 8 hours before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.
Reagent Storage

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Collection Vessel</th>
<th>Special Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Collection tubes</td>
<td>Acceptable anticoagulants are: Lithium heparin (with or without gel barrier) Sodium heparin</td>
</tr>
</tbody>
</table>

The National Cholesterol Education Program (NCEP) recommends using fasting specimens.2

- The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

**Specimen Conditions**

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- For accurate results, plasma specimens should be free of platelets and other particulate matter. Ensure centrifugation is adequate to remove platelets.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

**Preparation for Analysis**

- Follow the tube manufacturer’s processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross-contamination.
- To ensure consistency in results, recentrifuge specimens prior to testing if they contain fibrin, red blood cells, or other particulate matter.

**Specimen Storage**

- Avoid multiple freeze/thaw cycles.
- Guder et al. suggest storage of frozen specimens at -20°C for no longer than the time intervals cited above.11

Each laboratory may establish a range around -20°C from either the freezer manufacturer’s specifications or your laboratory standard operating procedure(s) for specimen storage. Stored specimens must be inspected for particulates. If present, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

**Specimen Shipping**

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
**PROCEDURE**

**Materials Provided**
07P76 Alinity c Cholesterol Reagent Kit

**Materials Required but not Provided**
- Alinity c Cholesterol assay file
- 08P6001 Alinity c Multiconstituent Calibrator Kit
- Commercially available controls containing cholesterol
- Saline (0.85% to 0.90% NaCl) for specimen dilution

For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1. For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

**Assay Procedure**
For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Minimum sample volume requirements:
  - Sample volume for single test: 1.5 µL.
  - NOTE: This amount does not include the dead volume plus the additional over-aspiration volume. For total sample volume requirements, refer to the Alinity ci-series Operations Manual, Section 4.
- Refer to the Alinity c Multiconstituent Calibrator Kit package insert and commercially available control material package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

**Sample Dilution Procedures**
Samples with a cholesterol value exceeding 705 mg/dL (18.26 mmol/L) are flagged with the code “> 705 mg/dL” (> 18.26 mmol/L) and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

**Automated Dilution Protocol**
The system performs a 1:4 dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

**Manual Dilution Procedure**
Dilute the sample with saline (0.85% to 0.90% NaCl).

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than the lower value of the measuring interval of 7 mg/dL (0.18 mmol/L), do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

**Calibration**
For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration is stable for approximately 30 days (720 hours), but is required with each change in reagent lot. Verify calibration with at least 2 levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

**Quality Control Procedures**
As appropriate, refer to your laboratory standard operating procedure(s) and/or quality assurance plan for additional 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, sample results may be suspect. Follow the established quality control procedures for your laboratory. Recalibration may be necessary. For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.
- Review quality control results and acceptance criteria following a change in reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance. For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

**Quality Control Guidance**
Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.

**Verification of Assay Claims**
For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

**RESULTS**

**Calculation**
The Alinity c Cholesterol assay utilizes the Linear data reduction method to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

**Flags**
Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

**Measuring Interval**
Measuring interval is defined as the range of values in mg/dL (mmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity c Cholesterol assay is 7 to 705 mg/dL (0.18 to 18.26 mmol/L).
**LIMITATIONS OF THE PROCEDURE**

N-Acetyl-L-Cysteine at therapeutically achieved concentrations may lead to falsely low results. Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

**EXPECTED VALUES**

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

**Reference Range**

<table>
<thead>
<tr>
<th>Serum/Plasma</th>
<th>Range (mg/dL)</th>
<th>Range (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child&lt;sup&gt;14&lt;/sup&gt;</td>
<td>Desirable &lt; 170</td>
<td>&lt; 4.40</td>
</tr>
<tr>
<td>Borderline 170 to 199</td>
<td>4.40 to 5.15</td>
<td></td>
</tr>
<tr>
<td>High ≥ 200</td>
<td>≥ 5.18</td>
<td></td>
</tr>
<tr>
<td>Adult&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Desirable &lt; 200</td>
<td>&lt; 5.18</td>
</tr>
<tr>
<td>Borderline 200 to 239</td>
<td>5.18 to 6.19</td>
<td></td>
</tr>
<tr>
<td>High ≥ 240</td>
<td>≥ 6.22</td>
<td></td>
</tr>
</tbody>
</table>

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**

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity c analyzer, and the ARCHITECT c System and AEROSET System utilize the same reagents and sample/reagent ratios. Unless otherwise specified, all studies were performed on the Alinity c analyzer.

**Precision**

**Within-Laboratory Precision**

A study was performed based on guidance from CLSI EP05-A2.<sup>15</sup> Testing was conducted using 1 lot of the Alinity c Cholesterol Reagent Kit, 1 lot of the Alinity c Multiconstituent Calibrator Kit, 1 lot of the commercially available controls and 1 instrument. Three control levels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (mg/dL)</th>
<th>SD</th>
<th>%CV</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Level 1</td>
<td>120</td>
<td>103</td>
<td>1.1</td>
<td>1.0</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Control Level 2</td>
<td>120</td>
<td>155</td>
<td>1.1</td>
<td>0.7</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Control Level 3</td>
<td>120</td>
<td>262</td>
<td>2.6</td>
<td>1.0</td>
<td>3.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (mmol/L)</th>
<th>SD</th>
<th>%CV</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Level 1</td>
<td>120</td>
<td>2.68</td>
<td>0.028</td>
<td>1.1</td>
<td>0.037</td>
<td>1.4</td>
</tr>
<tr>
<td>Control Level 2</td>
<td>120</td>
<td>4.01</td>
<td>0.028</td>
<td>0.7</td>
<td>0.039</td>
<td>1.0</td>
</tr>
<tr>
<td>Control Level 3</td>
<td>120</td>
<td>6.79</td>
<td>0.067</td>
<td>1.0</td>
<td>0.078</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Includes within-run, between-run, and between-day variability.

**Lower Limits of Measurement**

A study was performed based on guidance from CLSI EP17-A2.<sup>16</sup> Testing was conducted using 3 lots of the Alinity c Cholesterol Reagent Kit on each of 2 instruments over a minimum of 3 days. The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below. These representative data support the lower limit of the measuring interval.

<table>
<thead>
<tr>
<th>Substance</th>
<th>LoB&lt;sup&gt;a&lt;/sup&gt; mg/dL</th>
<th>LoQ&lt;sup&gt;b&lt;/sup&gt; mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>LoD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>LoQ&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.2</td>
<td>0.161</td>
</tr>
</tbody>
</table>

* The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.
* The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.
* The LoQ is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met.
* This value represents the observed LoQ on the ARCHITECT System. The LoQ observed on the Alinity c analyzer supports this LoQ.

**Linearity**

A study was performed based on guidance from CLSI EP06-A.<sup>17</sup> This assay is linear across the measuring interval of 7 to 705 mg/dL (0.18 to 18.26 mmol/L).

**Interference**

This study was performed on the AEROSET System. Potentially Interfering Substances

Interference studies were conducted using NCCLS EP7-P.<sup>18</sup> Interference effects were assessed by Dose Response and Paired Difference methods, at the medical decision level of the analyte.

**Lower Limits of Measurement**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Inferent Level</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>7.5 mg/dL</td>
<td>128 μmol/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>750 mg/dL</td>
<td>7.5 g/L</td>
</tr>
<tr>
<td>Intralipid</td>
<td>1000 mg/dL</td>
<td>10.0 g/L</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>1.5 mg/dL</td>
<td>85 μmol/L</td>
</tr>
<tr>
<td>3 mg/dL</td>
<td>170 μmol/L</td>
<td>282.2</td>
</tr>
</tbody>
</table>

The following drugs were tested on the ARCHITECT system for interference at the concentrations indicated using an acceptance criteria of ± 10% from the target value.

**Method Comparison**

A study was performed based on guidance from CLSI EP09-A3<sup>20</sup> using the Passing-Bablok regression method.
BIBLIOGRAPHY


Note for number formatting:

- A space is used as thousands separator (example: 10 000 specimens).
- A period is used to separate the integer part from the fractional part of a number written in decimal form (example: 3.12%).