| **HDL Cholesterol** |
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| **Purpose** | This procedure provides instructions for performing HDL CHOLESTEROL ON ABBOTT INSTRUMENTATION. The Alinity c Ultra HDL assay is used for the quantitation of high-density lipoprotein (HDL) cholesterol in human serum or plasma on the Alinity c analyzer.  |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Alinity c at Children’s Minnesota Laboratory. |
| **Principle** | The Ultra HDL assay is a homogeneous method for directly measuring HDL cholesterol concentrations in serum or plasma without the need for off‑line pretreatment or centrifugation steps. The method uses a two-reagent format and depends on the properties of a unique detergent. This method is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL cholesterol selectively using a specific detergent. In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction with DSBmT yielding a colorless product. The second reagent consists of a detergent (capable of solubilizing HDL cholesterol), cholesterol esterase (CE), and chromagenic coupler to develop color for the quantitative determination of HDL cholesterol.**Methodology:** Accelerator Selective Detergent *For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.* |
| **Clinical Significance** | Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids, and proteins. Phospholipids, free cholesterol, and proteins constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglyceride. These particles serve to solubilize and transport cholesterol and triglyceride in the bloodstream. The relative proportions of protein and lipid determine the density of these lipoproteins and provide a basis on which to begin their classification. The classes are: chylomicron, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and varied effects on coronary heart disease risk. The principle role of HDL cholesterol in lipid metabolism is the uptake and transport of cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport (a proposed cardioprotective mechanism). Low HDL cholesterol levels are strongly associated with an increased risk of coronary heart disease. Hence, the determination of serum HDL cholesterol is a useful tool in identifying high-risk patients. The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that in all adults 20 years of age and over, a fasting lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride) should be obtained once every five years to screen for coronary heart disease risk. |
| **Analyzer** | **Minneapolis: Abbott Alinity ci (Sunquest method code: MACC)****St. Paul: Abbott Alinity c (Sunquest method code: SALIC)** |
| **Sunquest Test Codes** | **HDLC** |
| **Specimen** | Sample: Plasma or Serum (with or without gel barrier) **Preferred: Lithium Heparin** **Alternative:** SST, Sodium heparinThe National Cholesterol Education Program (NCEP) recommends using fasting specimens for a lipoprotein profile. **Minimum sample volume:** 0.6 mL blood, 0.2 mL serum/plasma**Stability when separated from cells/gel:** **20 to 25°C** 2 days**2 to 8°C** 7 days **-20°C** 3 months**Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma**Preparation:** 1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Specimens should be free of particulate matter.
4. Transfer serum or plasma to a properly labeled sendout tube. Short samples should be pipetted into an Abbott sample cup and nested on a sendout tube; any amount remaining after sampling should be pipetted into the sendout tube and tightly capped.
5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Reagent Handling** Upon receipt, place reagent cartridges in an upright position for 8 hour 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. Use a pipette to remove all bubbles prior to loading on the Alinity or Architect system.* Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Alinity c Ultra HDL Reagent Kit | 07P7520 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**On-board**: 28 days |
| Abbott Alinity c Lipid Multiconstituent Calibrator | 09P1403 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**Opened expiration:** 7days |
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| **Risk and Safety** | 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.**WARNING** Contains methylisothiazolones.May cause an allergic skin reaction. The following warnings and precautions apply to: Lyophilized Calibrator: Contains methylisothiazolone and gentamicin sulfate.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** |

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| Assay Range: | 7 to 180 mg/dL |
| Reference Material: | Lipid Multiconstituent Calibrator |
| Suggested Calibration Levels: | 1 level |
| Calibration Scheme: | Linear data reduction method |
| Calibration Frequency: | 28 days |
| AMR | AMR is verified twice annually using the Maine Standards LP Product # 501ab by running all applicable levels in triplicate. Assay results are submitted to Maine Standards for compilation and comparison to peers. Results are reviewed and approved by the Technical Specialist. Questionable results are investigated and corrective actions documented. |

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| **Quality Control** | **QC Material:** Bio-Rad Liquichek Multiqual Unassayed Chemistry Control Levels 1 and 3**Frequency:** Two levels (Level 2 from each control) each day of use **Stability:** Once thawed, opened, and stored tightly capped at 2 to 8°C, product will be labeled with an expiration date equal to the shortest stability of the included analytes, which is **7 days.** **Preparation**: This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used. * To thaw the product, allow it to stand at room temperature (18° to 25°C) until completely thawed but no longer than one (1) hour
* After thawing, the products **MUST** be gently swirled and inverted several times to ensure homogeneity.
* For optimal analyte stability in the thawed state, promptly return to 2 to 8°C storage after each use and minimize the time at room temperature to no more than 20 minutes daily.
* **Before each use**, gently swirl the contents until homogeneous with no visible signs of precipitate.

**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Interferences** | **Hemolysis, Icterus & Lipemia (HIL) Index Values:**

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At HIL levels at or above the specified cutoff value, append the appropriate comment AFTER visually confirming presence of interferent: -HP for “Hemolysis present, may affect results.” -BIN for “Bilirubin Interference”-LINT for “Lipid Interference”Greater than 10% differences are seen at the following levels:* Hemoglobin: n/a
* Intralipid: 2000 mg/dL
* Bilirubin: 63.3 mg/dL(conjugated)
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| **Reference Intervals** | Normal: >=40 Abnormal <40 |
| **Critical Values** | Not applicable |
| **Limitations** | * N-acetyl-L-cysteine at elevated concentrations may lead to falsely low results.
* Interferences from medication or endogenous substances may affect results.
* Do not perform HDL testing on specimens clarified by ultracentrifugation.
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| **Dilutions** | Do not dilute |
| **Result Reporting** | * Results between 7 and 180 mg/dL without error messages are released
* Results below 7 without error messages are reported as < 7 mg/dL.
* Results > 180 should be reported as > 180 mg/dL
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity c Ultra HDL Instructions for Use, Abbott Diagnostics Division, Abbott Park IL, USA. Revised February 2018.
2. Bio-Rad Laboratories Liquichek Multiqual 1,2,3 Unassayed QC Product Insert, Bio-Rad Laboratories, Irvine CA, USA.
3. 2018 Guideline on the Management of Blood Cholesterol, GUIDELINES MADE SIMPLE A Selection of Tables and Figures. American College of Cardiology, acc.org/GMSCholesterol. Updated June 2019.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Elauteria Earnhardt | April 21, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Added AMR, no dilutions, interferences, reference intervals, references, correct Alinity instrument in Mpls, changed title and gave procedure a number. |