| **Alkaline Phosphatase** |
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| **Purpose** | This procedure provides instructions for ALKALINE PHOSPHATASE (ALK or ALP) ON ABBOTT INSTRUMENTATION. The alkaline phosphatase method is an *in vitro* diagnostic test for the quantitative measurement of albumin in human serum and plasma on the Abbott Architect c4000 or Abbott Alinity c automated chemistry analyzers. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Architect c4000 or Abbott Alinity c at Children’s Minnesota Laboratory.  |
| **Principle** | Several substrates have been used to measure alkaline phosphatase activity such as glycerophosphate, phenyl phosphate, and *p-*nitrophenyl phosphate. Bowers and McComb improved the method of Bessey et al. to include a kinetic measurement. Tietz et al. optimized this method to include a chelated metal-ion buffer of zinc, magnesium, and HEDTA. This Alkaline Phosphatase procedure is a modification of this method. Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless *p*-nitrophenyl phosphate (*p*- NPP) to give *p*-nitrophenol and inorganic phosphate. At the pH of the assay (alkaline), the *p*-nitrophenol is in the yellow phenoxide form. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample. Methodology: Para-nitrophenyl Phosphate with AMP Buffer  |
| **Clinical Significance** | Human alkaline phosphatase consists of a group of at least five tissue-specific isoenzymes which catalyzes the hydrolysis of phosphate mono-esters at alkaline pH. A variety of disease processes can result in the release of increased quantities of alkaline phosphatase into the blood, primarily those of bone and liver. Normal processes, such as bone growth and the last trimester of pregnancy, can also cause increased alkaline phosphatase. The highest levels occur within a few weeks after birth. The levels fall during the first year of life to values that are 2 to 3 times the normal adult levels. A rise may occur at puberty, followed by a rapid fall to the adult level. |
| **Analyzer** | **Minneapolis: Abbott Alinity c (Sunquest method code: MALIC) or Abbott Alinity ci (Sunquest test code: MACC)****St. Paul: Abbott Architect c4000 (Sunquest method code: ARCH4), Abbott Alinity c (Sunquest method code: SALIC)** |
| **Sunquest Test Codes** | ALK: Alkaline Phosphatase in serum and plasma. |
| **Specimen** | Sample: Plasma (lithium heparin with or without gel) preferred. Sodium heparin plasma or serum (with or without gel) also acceptable. Refer to specimen collection procedures.**Minimum sample volume:** 200 µL preferred, 150 µL minimum**Stability when separated from cells/gel:** RT / 7 days, 2-8 °C / 7 days, < -20°C / 2 months**Rejection criteria:** Unlabeled tube, sample type other than serum or heparinized 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 pilot tube or aliquot cup. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
5. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Alinity c and Architect c4000:****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. 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.

**Alinity c:**Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.**Alinity c:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Alinity c Alkaline Phosphatase ReagentCHC# 32619 | 08P20-20 | **Store at:** 2 – 8 °C**Unopened:** Manufacturer’s printed expiration date (R2 reagent is light sensitive. Store protected from light.)**On-board: 8 days****Opened, off the analyzer (with clean caps):** Manufacturer’s printed expiration date. (Reagents may be stored on or off the system. The system tracks time onboard.) |

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|  | **Architect c4000:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Architect Alkaline Phosphatase ReagentCHC# 32523 | 07D55-22 | **Store at:** 2 – 8 °C**Unopened:** Manufacturer’s printed expiration date (R2 reagent is light sensitive. Store protected from light.)**On-board: 8 Days** |

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| **Risk and Safety** |

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| **CAUTION:** For in vitro diagnostic use. This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Appropriate Personal Protective Equipment (PPE) must be worn according to Children’s Minnesota Laboratory policies. Current SDSs are kept on the [Children’s StarNet](https://msdsmanagement.msdsonline.com/a07dc954-23d8-42a9-b591-ef5763cdfd33/ebinder/?nas=True) page. R1 and R2 reagents can cause skin irritation or allergic reactions.Reagents can be disposed of in regular trash if no tests remain in the reagent pack. If the reagent has not be used up, dispose in regulated medical waste (red trash.) |

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| **Calibration** | **Alinity c and Architect c4000:**

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| Assay Range: | 9 – 4555 U/L |
| Reference Material: | Water and Alkaline Phosphatase reagent |
| Suggested Calibration Levels: |  The Alinity c and Architect c4000 Alkaline Phosphatase assays utilize the Factor data reduction method to generate a calibration and results. Calibration targets are determined by the Abbott assay configuration. |
| Calibration Scheme: | 2 levels, Factor data reduction method |
| Calibration Frequency: | 8 Days |
| AMR | AMR is verified twice annually using the Maine Standards CHEM Product # 104ab 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. Any questionable results are investigated and corrective actions documented.  |

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| **Quality Control** | **Alinity c and Architect c4000:** Bio-Rad Liquichek™ Unassayed Multiqual Chemistry Control (Human) Levels 1 & 3**Frequency:** Two levels each day of use**Stability:** Once thawed, opened, and stored tightly capped at 2 to 8°C, this 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 product **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 (insert hyperlink) 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** | **Alinity c and Architect c4000:** **All analyzers:** **Hemolysis, Icterus & Lipemia (HIL) Index Values:**

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Interference studies were conducted by Abbott Diagnostics Division using NCCLS EP07-A2. Interference is less than 10% at Alkaline Phosphatase concentration level of 150 U/L for:* Hemoglobin: up to 1000 mg/dL
* Bilirubin: up to 60 mg/dL
* Lipemia (Intralipid®): up to 1000 mg/dL

Interferences from medication or endogenous substances may affect results.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.  |
|  | **Alinity c and Architect c4000:**  |
| **Reference Intervals** |

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| Age | Alkaline Phosphatase  |
| 0 - 14 days | 90 – 273 U/L |
| 15 days - 1 year | 134 – 518 U/L |
| 1 - 9 years | 156 – 369 U/L |
| 10 - 12 years | 141 – 460 U/L |
| 13 - 14 years female | 62 – 280 U/L |
| 13 - 14 years male | 127 – 517 U/L |
| 15 - 16 years female | 54 – 128 U/L |
| 15 - 16 years male | 89 – 365 U/L |
| 17 - 18 years female | 48 – 95 U/L |
| 17 - 18 years male | 59 – 164 U/L |
| Adult | 40 - 150 U/L |
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| **Critical Values** | None specified. |
| **Limitations** | The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in albumin results. Refer to the [Abbott Architect](https://starnet.childrenshc.org/References/labsop/chem/operator/abbott-architect-operations-manual.pdf) or [Alinity](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) Operator’s Guides for the meaning of report flags and comments, and instructions for addressing them. Do not report results until a report containing flags and/or comments is resolved.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.  |
| **Dilutions** |

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| **Alinity c and Architect c4000:**  |
| Auto Dilution: | None |
| Maximum Manual Dilution: | 1:4 |
| Diluent: | Saline |
| Manual Dilution: | Follow Abbott [Architect Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/abbott-architect-operations-manual.pdf) or [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming manual dilutions. The operator must enter the dilution factor when ordering the manual dilution. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 9 U/L, do not report the result. Rerun using an appropriate (lower) dilution or investigate for other possible causes. |

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| **Result Reporting** | **Alinity c and Architect c4000:** * Results between 9 – 4555 U/L without error messages are released.
* Results below 9 U/L: report as < 9 U/L instead of the numerical value.
* Results >4555 should be manually diluted with a 1:4 dilution.
* Results >18220 following 1:4 manual dilution without error messages are reported as > 18220 g/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. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc., Hudson, OH, 5th Edition, 2001
2. Architect Alkaline Phosphatase Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, March 2017.
3. Alinity Alkaline Phosphatase Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, December 2017.
4. CALIPER pediatric reference range database. (2019). Retrieved October 3, 2019, from https://caliper.research.sickkids.ca/#/
5. Bio-Rad Liquichek Unassayed Multiqual Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Erin Bartos | 10/15/2019 | New Procedure for Abbott analyzers |
| 2 | Elauteria Earnhardt, Erin Bartos | 10/28/2020 | Added St Paul Alinity c as analyzer, added Alinity ci Mpls, Changed QC  |
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