| **Total Protein** |
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| **Purpose** | This procedure provides instructions for TOTAL PROTEIN ON ABBOTT INSTRUMENTATION. The total protein method is an *in vitro* diagnostic test for the quantitative measurement of total protein in human serum and plasma on the Abbott Architect c4000 or Abbott Alinity c automated chemistry analyzers, or in peritoneal, pericardial or chest fluid on the Minneapolis Abbott Alinity ci chemistry analyzer. |
| **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** | Polypeptides containing at least two peptide bonds react with biuret reagent. In alkaline solution, cupric ion forms a coordination complex with protein nitrogen with very little difference between albumin and globulin on a protein-nitrogen basis.Methodology: Biuret |
| **Clinical Significance** | Plasma proteins derive primarily from synthesis in the liver, plasma cells, lymph nodes, spleen, and bone marrow. In disease states both the total plasma protein level and the ratio of the individual fractions may be dramatically altered from their normal values. Hypoproteinemia may be caused by such conditions as nephrotic syndrome, extensive bleeding, sprue (deficient protein absorption), severe burns, salt retention syndromes, and Kwashiorkor (acute protein starvation). Hyperproteinemia may be observed in cases of severe dehydration and disease states such as multiple myeloma. Changes in the proportions of the plasma proteins may occur in one or several of the protein fractions and often without alterations in the quantity of the total protein. The A/G ratio has commonly been used as an index of the distribution between the albumin and globulin fractions. This ratio can be significantly altered in such conditions as cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, lupus erythematosis, and in some acute and chronic infections.No clinical claims are made in regards to body fluid testing. |
| **Analyzer** | **Minneapolis: Abbott Alinity c (Sunquest method code: MALIC) or Abbott Alinity ci (Sunquest method code: MACC)****St. Paul: Abbott Architect c4000 (Sunquest method code: ARCH4), Abbott Alinity c (Sunquest method code: SALIC)** |
| **Sunquest Test Codes** | TP: Total protein in serum and plasma**FTP**: Protein in body fluid |
| **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.Body Fluid: collected in a sterile container or red tube without gel. Ensure sample is not viscous or clotted. Centrifuge to remove particulate matter**Minimum sample volume:** 200 µL preferred, 150 µL minimum**Stability when separated from cells/gel:** RT / 7 days, 2-8 °C / 1 month, < -20°C / 2 months  Do not freeze body fluids; store in a refrigerated rack for 7 days.**Rejection criteria:** Unlabeled tube, sample type other than serum or heparinized plasma. See Interferences section for handling of hemolyzed samples.**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** | **Alinity c and Architect c4000:****Reagent Handling** Upon receipt, place reagent cartridges in an upright position for 1 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.

**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 Total Protein ReagentCHC# 32630 | 07P52-20 | **Store at:** 15 - 30°C**Unopened:** Manufacturer’s printed expiration date**On-board: 23 days****Opened, off the analyzer (with clean caps):** Manufacturer’s printed expiration date. (Reagents may be stored on or off the system at 2 – 8 °C. The system tracks time onboard.) |
| Abbott Alinity c Multiconstituent CalibratorCHC# 32633 | 08P60-01 | **Store at:** 2 – 8 °C**Unopened:** Manufacturer’s printed expiration date**On board expiration: 5 days when stored onboard.** The Alinity c tracks time on the system.**Opened expiration: 7 days** when opened and stored off the system. |

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|  | **Architect c4000:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Architect Total Protein ReagentCHC# 32554 | 07D73-21 | **Store at:** 15 - 30°C**Unopened:** Manufacturer’s printed expiration date.**On-board:** 23 Days |
| Abbott Architect Multiconstituent CalibratorCHC# 32557 | 01E65-05 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**Opened:** 7 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**Alinity c and Architect c4000:**R1 reagents: **DANGER**. Contains sodium hydroxide and copper sulfate. Causes serious eye damage. Causes skin irritation. Harmful to aquatic life with long lasting effects. May be corrosive to metals. Recap and dispose of in Alkaline Waste Stream. Multiconstituent Calibrator should be disposed of in Regulated Medical Waste (red trash). |

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

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| Assay Range: | 1.0 – 13.0 g/dL (blood)1.0 - 10.0 g/dL (body fluid) |
| Reference Material: | Abbott Alinity Multiconstituent Calibrator |
| Suggested Calibration Levels: |  See lot-specific assay set point documentation |
| Calibration Scheme: | 2 levels, Linear data reduction method |
| Calibration Frequency: | 23 Days |
| AMR | AMR is verified twice annually using the Maine Standards GC1 Product # 1100ab and Body Fluid product 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™ Multiqual 1,2,3 Unassayed Chemistry Control 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:** **Hemolysis, Icterus & Lipemia (HIL) Index Values apply to serum/plasma only:**

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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” Interference studies were conducted by Abbott Diagnostics using NCCLS EP7‑P. Interference effects were assessed by Dose Response and Paired Difference methods, at the medical decision level of the analyte. Interference is less than 10% at total protein levels at the medical decision levels of the analyte for:* Hemoglobin: up to 125 mg/dL
* Bilirubin: up to 60 mg/dL
* Lipemia (Intralipid®): up to 1000 mg/dL

Hemolysis greater than 125 mg/dL caused increased recovery of total protein greater than 10%. Samples that contain visible hemolysis should not be used, whenever possible. Otherwise, append -HP to the appropriate tests. 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 | Total Protein |
| 0 - 14 days | 5.3 – 8.3 g/dL |
| 15 days - 1 year | 4.4 – 7.1 g/dL |
| 1 - 5 years | 6.1 – 7.5 g/dL |
| 6 - 8 years | 6.4 – 7.7 g/dL |
| 9 – 18 years | 6.5 – 8.1 g/dL |
| Adult | 6.0 – 8.3 g/dL |
| Body Fluids do not have an applicable reference range. |

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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 total protein 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. Hemolysis greater than 125 mg/dL caused increased recovery of total protein greater than 10%. Samples that contain visible hemolysis should not be used, whenever possible. Otherwise, append –HP to the appropriate tests. |
| **Dilutions** |

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| **Alinity c and Architect c4000: (Blood specimens only)** |
| Automated Dilution: | None |
| Maximum Manual Dilution: | 1:2 |
| 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, including the manual dilution factor. The system will 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, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | **Alinity c and Architect c4000:** * Results between 1.0 – 13.0 g/dL without error messages are released (1 to 10.0 for body fluids)
* Results below 1.0 g/dL: report as < 1.0 g/dL instead of the numerical value, for blood or body fluid.
* Results >13.0 g/dL without error messages are diluted 1:2 manually with saline and reported the numerical value or as > 26.0 g/dL for blood specimens. Results >10.0 g/dL for body fluids are reported as such.

Hemolysis greater than 125 mg/dL caused increased recovery of total protein greater than 10%. Samples that contain visible hemolysis should not be used, whenever possible. Otherwise, append –HP to the appropriate tests.  |
| **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. Do not freeze body fluids; instead, store in a refrigerated rack. |
| **References** | 1. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc., Hudson, OH, 5th Edition, 2001
2. Architect Total Protein Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, December 2016.
3. Alinity Total Protein 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. Alinity c Multiconstituent Calibrator Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, December 2017.
6. Architect Multiconstiuent Calibrator Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, August 2017.
7. Bio-Rad Liquichek Multiqual Unassayed 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 |
|  | Elauteria Earnhardt | April 23, 2020 | Added St Paul Alinity c as an analyzer |
| 2 | Erin Bartos | 10/28/2020 | Added Alinity ci, TP on body fluids, new QC, HIL box, changed AMR, added body fluid cal ver material. |
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