| **Direct Bilirubin** |
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| **Purpose** | This procedure provides instructions for DIRECT BILIRUBIN ON ABBOTT INSTRUMENTATION. The direct bilirubin (also known as “DBIL”) method is an *in vitro* diagnostic test for the quantitative measurement of direct bilirubin 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** | Bilirubin determination is generally based on the reaction of bilirubin with a diazotized sulfanilic acid, described by Ehrlich. In this method, direct (conjugated fractions) bilirubin couples with a diazonium salt in the presence of sulfamic acid to form the colored compound azobilirubin. The increase in absorbance at 548 nm due to azobilirubin is proportional to the direct bilirubin concentration.Methodology: Diazo Reaction |
| **Clinical Significance** | Red blood cells at the end of their circulating life are broken down in the reticuloendothelial system, mainly the spleen. The resulting heme, once the iron is removed, is then converted to bilirubin. This process accounts for about 80% of the 500 μmol (300 mg) of bilirubin formed daily. Other sources of bilirubin include the breakdown of myoglobin and cytochromes and the catabolism of immature red blood cells in the bone marrow.Once formed, bilirubin is transported to the liver bound to albumin. This fraction of bilirubin is referred to as indirect or unconjugated bilirubin. In the liver, bilirubin is conjugated to glucuronic acid (monoanddiglucuronides) to form conjugated bilirubin by the enzyme uridyl diphosphate glucuronyl transferase. Conjugated bilirubin or direct bilirubin is excreted via the biliary system into the intestine, where it is metabolized by bacteria to a group of products known collectively as stercobilinogen. Elimination is almost complete and serum levels are normally negligible.Direct bilirubin is the sum of the conjugated fractions. Direct bilirubin is elevated in conditions causing hepatic obstruction, hepatitis, cirrhosis, several inherited enzyme deficiencies, and inherited defects in canalicular excretion. |
| **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** | **DBIL:** direct bilirubin in serum or plasma |
| **Specimen** | Sample: Plasma (lithium heparin with or without gel) preferred. Sodium heparin plasma, EDTA plasma, or serum (with or without gel) are also acceptable. Refer to specimen collection procedures.**Minimum sample volume:** 200 µL preferred, 150 µL minimum**Stability when separated from cells/gel:** RT / 2 days, 2-8 °C / 1 week, < -20°C / 3 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 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 – 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 Direct Bilirubin ReagentCHC# 32626 | 07P97-20 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**On-board:** 28 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.) |
| Abbott Alinity c Bilirubin CalibratorCHC# 32635 | 08P61-01 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**Opened:** 7 dayswhen opened and stored off the system. Protect from light. |

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|  | **Architect c4000:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Architect Direct Bilirubin ReagentCHC# 32548 | 08G63-21 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date.**On-board:** 28 Days |
| Abbott Architect Bilirubin CalibratorCHC# 32562 | 01E66-04 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**Opened:** 7 Days. Protect from light. |

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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 sulfamic acid. Causes severe skin burns and eye damage. Recap and dispose of in Acid waste stream.R2 Reagents: **DANGER** Contains hydrochloric acid. Causes severe skin burns and eye damage. Harmful if inhaled. May be corrosive to metals. Recap and dispose of in Acid waste stream.Bilirubin Calibrators 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: | 0.1 – 15.0 mg/dL |
| Reference Material: | Abbott Alinity Bilirubin CalibratorAbbott Architect Bilirubin Calibrator |
| Suggested Calibration Levels: |  See lot-specific assay set point documentation |
| Calibration Scheme: | 2 levels, Linear data reduction method |
| Calibration Frequency: | 14 Days |
| AMR | AMR is verified twice annually using the Maine Standards GC4 Product # 1400ab 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 and 3
* Bio-Rad Liquichek **Pediatric Control**, Level 2

**Frequency:** Three levels each day of use**Stability:** Once thawed, opened, and stored tightly capped at 2 to 8°C, **Unassayed Multiqual Chemistry Control** product will be labeled with an expiration date equal to the shortest stability of the included analytes, which is **7 days.**Once thawed, opened, and stored tightly capped and protected from light at2 to 8°C, the **Pediatric Control** is stable for 14 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 for Unassayed Multiqual Control and 30 minutes for Pediatric Control.
* 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 (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:**

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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 Division and effects were assessed by Dose Response and Paired Difference methods at the medical decision levels of the analyte. Interference is less than 10% for:* Hemoglobin: up to 500 mg/dL (higher concentrations not tested)
* Triglycerides: up to 1034 mg/dL
* Intralipids: up to 500 mg/dL
* Indocyanine Green: up to 25.0 mg/L

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 | Direct Bilirubin |
| 0 – 14 days | 0.3 – 0.7 mg/dL |
| 15 - 364 days | 0.1 – 0.3 mg/dL |
| 1 – 8 years | 0.1 – 0.2 mg/dL |
| 9 - 12 years | 0.1 – 0.3 mg/dL |
| 13 – 18 years | 0.1 – 0.4 mg/dL |
| Adult | 0.0 – 0.5 mg/dL |
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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 direct bilirubin 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 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 of 0.1 mg/dL, 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 0.1 – 15.0 mg/dL without error messages are released
* Results < 0.1 mg/dL without error messages are reported as < 0.1 mg/dL
* Results > 15.0 mg/dL should by diluted using the 1:4 manual dilution protocol described above. Release results without error messages following dilution.
* Results > 60.0 mg/dL following manual dilution are reported as > 60.0 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. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc., Hudson, OH, 5th Edition, 2001
2. Architect Direct Bilirubin Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, February 2017.
3. Alinity Direct Bilirubin Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, March 2018.
4. Alinity c Bilirubin Calibrator Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, March 2018.
5. Architect Bilirubin Calibrator Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, July 2016.
6. Bio-Rad Liquichek Unassayed Multiqual Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618
7. Bio-Rad Liquichek Pediatric Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618
8. CALIPER pediatric reference range database. (2019). Retrieved October 3, 2019, from https://caliper.research.sickkids.ca/#/
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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 | Erin Bartos, Elauteria Earnhardt | October 28, 2020 | Added St Paul Alinity c as an analyzer; added alinity ci, multiqual qc, HIL |