| **Total Bilirubin** |
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| **Purpose** | This procedure provides instructions for TOTAL BILIRUBIN ON ABBOTT INSTRUMENTATION. The total bilirubin (also known as “TBIL”) method is an *in vitro* diagnostic test for the quantitative measurement of total 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** | Total (conjugated and unconjugated) bilirubin couples with a diazo reagent in the presence of a surfactant which acts as a solubilizing agent to form azobilirubin. The increase in absorbance at 548 nm due to azobilirubin is directly proportional to the total bilirubin concentration.Methodology: Diazonium Salt |
| **Clinical Significance** | Measurement of total bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological and metabolic disorders, including hepatitis and disorders of the biliary tract.Total bilirubin in neonates is also known as neonatal bilirubin. The Total Bilirubin assay is intended to measure the levels of bilirubin in the serum or plasma of newborn infants for use in the diagnosis and management of neonatal jaundice and hemolytic disease of the newborn.Bilirubin is a degradation product of hemoglobin and other hemecontaining compounds. There are four principle forms of bilirubin in the serum: unconjugated, mono-, and di-glucuronide conjugated, and δ-bilirubin. The unconjugated form, which is mostly insoluble in water, is transported to the liver by albumin. Once in the liver, unconjugated bilirubin is made water soluble by conjugation with glucuronic acid, forming the mono- and di-glucuronide conjugated species. These conjugated species are mostly excreted with bile. However, conjugated bilirubin can also react with albumin forming δ-bilirubin. Conjugated bilirubin is often called direct bilirubin. Unconjugated bilirubin, which is the difference between total and direct bilirubin, is often referred to as indirect bilirubin. Direct bilirubin assays measure conjugated bilirubin fractions (mono- and di-glucuronide, and δ-bilirubin). However, when the concentration of unconjugated bilirubin is high the direct assay may overestimate the conjugated bilirubin concentration due to cross-reactions within the assay. Neonatal bilirubin quantitation is used to monitor diseases causing jaundice in the newborn. Physiologic jaundice is seen at serum bilirubin concentrations from 7 to 17 mg/dL (119.7 to 290.7 μmol/L), and serum bilirubin concentrations greater than 17 mg/dL (290.7 μmol/L) may be pathologic for an average full-term newborn infant. In addition, there is concern for bilirubin encephalopathy or kernicterus (the yellow staining of the basal ganglia observed in infants), and erythroblastosis fetalis (also called hemolytic disease of the newborn or HDN) which is caused by maternal alloimmunization to RhD, antibodies involving additional blood groups, and ABO incompatibility.Additional causes of neonatal jaundice are hematoma/hemorrhage, hypothyroidism, Crigler-Najjar syndrome, obstructive jaundice, galactosemia, sepsis, syphilis, toxoplasmosis, cytomegalovirus, rubella, glucose-6‑phosphate dehydrogenase (G-6-PDH) deficiency, pyruvate kinase deficiency, and spherocytosis. |
| **Analyzer** | **Minneapolis: Abbott Alinity c (Sunquest method code: MALIC) or 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** | **TBIL:** total 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. See Interferences section on handling of lipemic 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 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 8 hours 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 Total Bilirubin ReagentCHC# 33275 | 04V51-21 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date**On-board:** 21 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 Total Bilirubin ReagentCHC# 32553 | 06L4521 | **Store at:** 2 – 8°C**Unopened:** Manufacturer’s printed expiration date.**On-board:** 21 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 Reagent: **DANGER** Contains hydrochloric acid and sodium metaborate. May damage fertility or the unborn child. Causes severe skin burns and eye damage. May be corrosive to metals. Recap and dispose of in Acid waste stream.R2 Reagent: **DANGER** Contains hydrochloric acid. Causes severe skin burns and eye damage. 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: | 0.3 – 22.0 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: | **Alinity c**: 21 Days**Architect c4000**: 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 MultiqualChemistry 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 2000 mg/dL

At low levels of total bilirubin, Indican and Indocyanine Green cause moderately increased results. Wait until Indocyanine Green (contrast dye) has cleared before attempting total bilirubin quantification to avoid spurious results.Lipemia may cause moderate interference for low levels of total bilirubin. Samples should be cleared by ultracentrifugation prior to analysis. Program the sample manually, inserting LP into the barcode ID, so that results do not autofile.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 Bilirubin |
| 0 – 14 days | 0.2 – 16.6 mg/dL |
| 15 - 364 days | 0.1 – 0.7 mg/dL |
| 1 – 8 years | 0.1 – 0.4 mg/dL |
| 9 - 11 years | 0.1 – 0.6 mg/dL |
| 12 – 14 years | 0.1 – 0.7 mg/dL |
| 15 - 18 years | 0.1 – 0.8 mg/dL |
| Adult | 0.2 – 1.2 mg/dL |
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| **Critical Values** | Less than 1 day old: >12.0 mg/dLGreater than 1 day old: > 15.0 mg/dL.Critical values must be called and documented according to the Critical Limit Test Value Policy. |
| **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 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.Lipemia may cause moderate interference for low levels of bilirubin. Samples should be cleared by ultracentrifugation prior to analysis. Program the sample manually, inserting LP into the barcode ID, so that results do not autofile. |
| **Dilutions** |

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| **Alinity c and Architect c4000:**  |
| Auto Dilution: | 1:5 and 1:10 (maximum) |
| Maximum Manual Dilution: | Do not manually dilute |
| Diluent: | Onboard 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 automated instrument 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 0.3 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 0.3 – 12.0 mg/dL without error messages are released
* Results of patients less than 1 day old > 12.0 mg/dL and of patients greater than 1 day old > 15.0 mg/dL must be called and documented as critical according to the critical values policy.
* Results < 0.3 mg/dL without error messages are reported as < 0.3 mg/dL
* Results > 22.0 mg/dL should by diluted using the 1:5 automated dilution protocol described above. If necessary, the 1:10 (maximum) automated dilution may be used. Release results without error messages following dilution.
* Results > 220.0 mg/dL following automated dilution are reported as > 220.0 mg/dL.

Lipemia may cause moderate interference for low levels of bilirubin. Samples should be cleared by ultracentrifugation prior to analysis. Program the sample manually, inserting LP into the barcode ID, so that results do not autofile. |
| **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 Total Bilirubin Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, February 2017.
3. Alinity Total Bilirubin Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, February 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 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 Mpls Alinity ci, changed AMR, added HIL, changed QC material |