Bilirubin, Total, Serum or Plasma

Purpose

This procedure provides instructions for TOTAL BILIRUBIN on Abbott Instrumentation. The Alinity c TBILI2 assay is used for the quantification of Total Bilirubin in human serum or plasma on the Alinity c analyzer.

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. In newborn infants, the Total Bilirubin2 assay is intended to measure the levels of total bilirubin (conjugated and unconjugated) in serum or plasma to aid in the diagnosis and management of neonatal jaundice and hemolytic disease of the newborn.[1]

Policy Statements

This procedure applies to all personnel responsible for operating the Abbott Alinity c at Children's Minnesota Laboratory.

Principle

Methodology: Diazonium Salt

The Total Bilirubin2 assay is an automated clinical chemistry assay. Total (conjugated and unconjugated) bilirubin couples with a diazo reagent in the presence of a surfactant to form azobilirubin. The diazo reaction is accelerated by the addition of surfactant as a solubilizing agent. The increase in absorbance at 548 nm due to azobilirubin is directly proportional to the total bilirubin concentration.[1]

Clinical Significance

Bilirubin is a degradation product of hemoglobin. Bilirubin bound to albumin is insoluble in water and is known as unconjugated (indirect) bilirubin. In the liver, unconjugated bilirubin is coupled with glucuronide; this form is called conjugated (direct) bilirubin. It is water soluble and is mostly excreted in bile.

The sum of direct and indirect bilirubin is called total bilirubin and the indirect fraction of the total usually makes up to approximately 85%. In cases of hyperbilirubinemia, bile pigment is deposited in the skin, sclera, and mucous membranes and so the patient has yellowish color; this condition is called jaundice or icterus. In newborns or in people with familial hyperbilirubinemia the presence of jaundice and elevation of total bilirubin may indicate inherited metabolic disorders (e.g., Gilbert, Crigler-Najjar, Lucey-Driscoll, Dubin-Johnson, and Rotor syndromes).

Fractionation of total bilirubin into conjugated and unconjugated may help in the diagnosis of hyperbilirubinemia. For example, conjugated bilirubin is increased in cases of cholestasis caused by several liver diseases (e.g., hepatitis, hepatic obstruction, and cirrhosis), while a high level of unconjugated bilirubin may indicate a hemolytic disorder. Inherited metabolic disorders may also have differences in conjugated and unconjugated fractions: increased conjugated bilirubin suggests Dubin-Johnson or Rotor syndromes, while unconjugated bilirubin is prevalent in Gilbert, Crigler-Najjar, or Lucey-Driscoll syndromes. The total bilirubin test is used as an aid in the differential diagnosis and management of liver diseases, and neonatal jaundice, as well as hemolytic, and inherited metabolic diseases.[1]

Materials

Product Description	*Product Code*	*Stability*
Total Bilirubin2 Reagent (2500 tests per box; each box contains 10 reagent sets of 350 tests). Each reagent set consists of an R1 and R2 component. Alinity c **R1:** Active ingredient: Brij L23 (233.333 mL/L). **R2:** Active ingredients: 2,4-dichlorobenzenediazonium 1,5-naphthalenedisulfonate hydrate (1845.000 mg/L) and Brij L23 (100.000 mL/L). Abbott Diagnostics	**MFR#** 04U0520 **CHC#** 33275	**Store at:** 2-8 °C **Unopened:** Until Expiration **Opened:** Stable 30 days onboard the analyzer. Stable until expiration if returned to 2-8 °C storage with replacement caps tightly secured and in upright position.
Total Bilirubin2 Reagent (900 tests per box; each box contains 4 reagent sets of 225 tests). Each reagent set consists of an R1 and R2 component. Architect **R1:** Active ingredient: Brij L23 (233.333 mL/L). **R2:** Active ingredients: 2,4-dichlorobenzenediazonium 1,5-naphthalenedisulfonate hydrate (1845.000 mg/L) and Brij L23 (100.000 mL/L). Abbott Diagnostics	**MFR#** 04T0920 **CHC#** 32553	**Store at:** 2-8 °C **Unopened:** Until Expiration **Opened:** Stable 30 days onboard the analyzer. Stable until expiration if returned to 2-8 °C storage with replacement caps tightly secured and in upright position.
Consolidated Chemistry Calibrator (ConCC) Each calibrator set consists of 6 lyophilized calibrator vials, and 6 empty barcode labeled vials. Abbott Diagnostics	**Alinity** **MFR#** 04V6201 **CHC#** 37653 **Architect** **MFR#** 04V1501 **CHC#** TBD	**Store at:** 2-8 °C **Unopened:** Until Expiration **Reconstituted:** Stable 7 days if returned to 2-8 °C storage with replacement caps tightly secured and in upright position.
Biorad MultiQual Controls Levels 1 and 3	**Level 1 MFR#** 697 **Level 1 CHC#** 34574	**Store at:** -20°C or colder **Unopened:** Until Expiration

Each box contains 12 5mL vials of control material. Biorad Laboratories	**Level 3 MFR#** 699 **Level 3 CHC#** 34575	**Opened:** Stable 7 days at 2-8 °C storage with caps tightly secured.
Biorad Pediatric Controls Level 2 Each box contains 6 4mL vials of control material. Biorad Laboratories	**Level 2 MFR#** 355 **Level 2 CHC#** 20357	**Store at:** -20°C or colder **Unopened:** Until Expiration **Opened:** Stable 7 days at 2-8 °C storage with caps tightly secured.

Special Safety Precautions

The following warnings and precautions apply to: R1 **DANGER** Contains sodium metaborate tetrahydrate, polyoxyethylene lauryl ether and phosphoric acid. H360 May damage fertility or the unborn child. H314 Causes severe skin burns and eye damage. H290 May be corrosive to metals. **Prevention** P201 Obtain special instructions before use. P260 Do not breathe mist / vapors / spray. P264 Wash hands thoroughly after handling. P280 Wear protective gloves / protective clothing / eye protection. P234 Keep only in original container. **Response** P308+P313 IF exposed or concerned: Get medical advice / attention. P301+P330+P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water / shower. P310 Immediately call a POISON CENTER or doctor / physician. P390 Absorb spillage to prevent material damage. **Disposal** P501 Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: R2 **DANGER** Contains hydrochloric acid. H314 Causes severe skin burns and eye damage. H290 May be corrosive to metals. **Prevention** P260 Do not breathe mist / vapors / spray. P264 Wash hands thoroughly after handling. P280 Wear protective gloves / protective clothing / eye protection. P234 Keep only in original container. **Response** P301+P330+P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water / shower. P310 Immediately call a POISON CENTER or doctor / physician. P390 Absorb spillage to prevent material damage. **Disposal** P501 Dispose of contents / container in accordance with local regulations.[1]

Calibration material contains human-sourced and/or potentially infectious components.[3]

Sample

Sample: Plasma collected with lithium heparin with or without gel barrier is preferred. Alternatively, serum collected with or without gel barrier is also accepted.

Stability: 7 days at room temperature, 7 days at 2-8 C, or up to 3 months frozen at –20 C or colder.

Test Code

LIS Test code: TBIL for Total Bilirubin in Serum or Plasma.

Analyzer

Primary Analyzer or Method: MACC or MALIC (Minneapolis), SALIC or ARCH4 (St. Paul).

Backup: Each analyzer may act as a backup for the other.

Calibration

Calibration Information[3]

Reference Material	ConCC; 04V6201 or 04V1501
Calibration Frequency	Calibration is due every 42 days and with each new reagent lot.
Calibration Scheme	3-Point Calibration; 3 replicates per level. The TBILI2 assay utilizes the Linear data reduction method to generate a calibration and results.

Quality Control

Material: Biorad Multiqual, Levels 1 and 3. Biorad Peds, Level 2.
Frequency: Run 3 levels, once per day.
Acceptable Ranges: Acceptable ranges are set in Unity Real Time software.

Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, section 4 to ensure sufficient specimen is present.
- Minimum sample cup volume is calculated by the system and printed on the Order List report.
- To minimize the effect of evaporation, verify that adequate sample cup volume is present prior to running the test.
- Maximum number of replicates sampled from the same sample cup: 10
    - Sample volume for the first test: 2.6 uL + 50 uL for over-aspiration and dead volume
    - Sample volume for each additional test from the same sample cup: 1.6 uL
    - Automated Dilutions require 20 uL + 50 uL for over-aspiration and dead volume

Dilutions

Samples with a Total Bilirubin value exceeding 25.0 mg/dL are flagged with the code “>25.0 mg/dL” and may be diluted with the Automated Dilution Protocol.

Available Auto Dilutions:	1:5
Maximum Auto Dilution:	1:5
Maximum Manual Dilution:	None
Diluent:	Normal Saline

Manual Dilution Instruction:	Not Applicable

Calculations

Not applicable.

Interpretation and Resulting

Results will be reported to the first decimal (xx.x).
Results between 0.1 and 25.0 mg/dL without error messages are released.
Results below 0.1 mg/dL without error messages are reported as <0.1 mg/dL.
Results greater than 25.0 mg/dL are diluted 1:5 by auto dilution and the reported value up to 125.0 mg/dL is released.
Results greater than 125.0 mg/dL without error messages are reported as >125.0 mg/dL.

Reference Intervals

Critical Values:

Age	*Critical Values*
<24 hr	>12.0 mg/dL
≥1 day	>15.0 mg/dL

Critical values must be called and documented according to the Critical Results or Critical Test Notification and Documentation policy.

Reference Ranges[2]

Age	*Reference Interval (mg/dL)*
0 to <15 days	0.2-12.0
15 days to <1 year	0.1-0.7
1 to <9years	0.1-0.4
9 to <12 years	0.1-0.6
12 to <15 years	0.1-0.7
15 to 18 years	0.1-0.8
Adult	0.2-1.2

Method Performance Specifications

Analytical Measuring Range: 0.1-25.0 mg/dL

AMR/Calibration Verification Frequency: Not Required, 3 point calibration every 30 days or less

AMR recommended verification product: Maine Standards GC4
AMR proximity budget: 0.2 mg/dL (low) or 20% (high)
AMR systematic error budget: 50%

Allowable Error: 0.4 mg/dL or 20% (CAP)

Precision: 0.1 mg/dL SD or 6.0% CV, whichever is greater

Limitations

HIL Indices Cutoff Values[1,4]				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"
Interpretation	H	I	L	
Quant (Index)	N/A	N/A	N/A	
Semi Quant	N/A	N/A	N/A	

Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.

Specimens with indican levels greater than 1 mg/dL may cause falsely elevated results with the Total Bilirubin2 assay. Refer to the SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity, Interference section of this package insert for further information.[1]

Specimens with indocyanine green levels greater than 5 mg/L may cause falsely elevated results with the Total Bilirubin2 assay. Refer to the SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity, Interference section of this package insert for further information.[1]

For patients undergoing evaluations involving the administration of indocyanine green, it is recommended that samples are drawn after indocyanine green has been eliminated.[1]

Additional interference information can be found in reagent package insert, pages 4-6.[1]

References

1. Total Bilirubin2 for Alinity c Reagent Package Insert, Abbott Laboratories Diagnostic Division, Abbott Park, IL 60064, Revised December 2023
2. CALIPER Reference Interval Studies, accessed October 27 2020
3. ConCC for Alinity c Calibrator Package Insert, Abbott Laboratories Diagnostic Division, Abbott Park, IL 60064, Revised December 2020
4. Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis. CLSI guideline C56-A, 1st Ed, Wayne, PA: CLSI, 2012
5. Total Bilirubin2 for Architect Reagent Package Insert, Abbott Laboratories Diagnostic Division, Abbott Park, IL 60064, Revised December 2023

Appendices

Not applicable.

Training Plan/Competency Assessment

CH 1.04.T1 Abbott Alinity Training
QP 2.30 Orientation and Training
QP 2.40 Competency Assessment

Historical Record

Version	Author	Effective Date	Summary
1	Matt Johnson	1/7/2026	Initial Version