

TBIL – TOTAL BILIRUBIN

Intended Use The Alinity c Total Bilirubin assay is used for the quantitation of total bilirubin in human serum or plasma of adults and neonates on the Alinity c analyzer.

Clinical Significance Total bilirubin is the sum of the unconjugated and conjugated fractions. Total bilirubin is elevated in hepatitis, cirrhosis, hemolytic disorders, several inherited enzyme deficiencies, and conditions causing hepatic obstruction. Neonatal bilirubin quantitation is used to monitor diseases causing jaundice in the newborn, chiefly erythroblastosis fetalis (also called hemolytic disease of the newborn or HDN). HDN is caused by maternal alloimmunization to RhD, antibodies involving additional blood groups, and ABO incompatibility.

Methodology Traditional methods of measuring bilirubin are based on the reaction of bilirubin with a diazo reagent to form the colored compound azobilirubin. The diazo reaction can be accelerated by the addition of various chemicals. For example, Malloy-Evelyn³ used methanol, Jendrassik-Gróf⁴ used caffeine, and Walters-Gerarde⁵ used dimethyl sulfoxide (DMSO). Modifications of these methods included the addition of surfactants as solubilizing agents.⁶ 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. Methodology: Diazonium Salt.

Specimen

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Type of Specimen

Specimen Type	Collection Vessel	Special Conditions
Serum	Serum tubes (with or without gel barrier)	Protect from light.
Plasma	Collection tubes Acceptable anticoagulants are: Lithium heparin (with or without gel barrier) Sodium heparin EDTA	Protect from light. The use of tubes containing sodium fluoride/ potassium oxalate is not recommended due to the potential for hemolysis with this anticoagulant.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.
2. Specimens should be protected from bright light as bilirubin is photolabile. 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. For accurate results, plasma specimens should be free of platelets and other particulate matter. Ensure centrifugation is adequate to remove platelets.
3. Use serum or plasma specimens without visible hemolysis or lipemia. Specimen should be free of bubbles.
4. To ensure consistency in results, recentrifuge specimens prior to testing if they contain fibrin, red blood cells, or other particulate matter. NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

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Specimen Type	Temperature	Maximum Storage Time
Serum/ Plasma	20 to 25°C	1 day
	2 to 8°C	7 days
	-20°C	6 months
	-80°C	6 months

Sample Dilution Procedures

Serum/Plasma

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 either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:5 or a 1:10 dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedure

Dilute the sample with saline (0.85% to 0.90% NaCl). The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

Reagents

Reagent Handling

- Reagents are shipped on wet ice.
- 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.
- Do not invert reagent cartridges prior to use.
- 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

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Reagent Storage and Stability

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position
Onboard	System Temperature	21 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Calibration

Calibration Required

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5. Calibration is stable for approximately 14 days (336 hours), but is required with each change in reagent lot. Verify calibration with at least 2 levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary. This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

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Calibration Preparation

Calibration material is the Alinity c Bilirubin Calibrator Kit. Allow temperature before use. Prior to each use, mix by gentle inversion.

Calibration Storage and Stability

	Calibrator Storage	Stability Once OPEN
Bili Cal (CAL 1 and CAL 2)	Unopened: 2 to 8 (until expiration date) Protect from light.	2 to 8 °C: 7 days

Calibration Information

1. Calibrator values may be configured using e-files accessed and imported from www.corelaboratory.abbott, or from Abbott Mail.
2. Verify that the correct calibrator values have been entered into the calibration file.
3. Calibration is performed by running Alinity c Bilirubin Calibrator Kit.
4. For information on configuring calibrator data, refer to the Alinity ci-series Operations Manual, Section 2.

Quality Control

See Policy [Chemistry Quality Control Policy](#)

Sample Processing

See Policy [RIV-PPP-1199](#)

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Reference Range

Test unit= mg/dL for all ages and both sexes.

Test	Age Day Low	Age Day High	Age Year Low	Age Year High	Ref Low	Ref High
TBILI	31			250	0	1.2
TBILI	0	1			0	6
TBILI	1	2			0	7
TBILI	2	3			0	7
TBILI	3	4			0	12
TBILI	4	5			0	12
TBILI	5	6			0	12
TBILI	6	31			0	1.2
NTBILI	0	1			0	6
NTBILI	1	2			0	7
NTBILI	6	31			0	1.2
NTBILI	2	3			0	7
NTBILI	3	4			0	12
NTBILI	4	5			0	12
NTBILI	5	6			0	12
NTBILI	31			250	0	1.2

Analytic Range

AMR Low	AMR High	CRR Low	CRR High
0.1	25.0	0.1	250.0

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