Alinity c Direct Bilirubin (Bili D)-13

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity c Direct Bilirubin assay is used for the quantitative analysis of direct bilirubin in human serum or plasma on the Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE TEST

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 (mono- and diglucuronides) 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.

PRINCIPLES OF THE PROCEDURE

Bilirubin determination is generally based on the reaction of bilirubin with a diazotized sulfanilic acid, described by Ehrlich.*1* 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

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity c Direct Bilirubin Reagent Kit 07P97

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	**07P9720**
Tests per cartridge	360
Number of cartridges per kit	4
Tests per kit	1440
R1	68.1 mL
R2	21.0 mL
R1 Active ingredient: Sulfamic acid (9.7 g/L).	
R2 Active ingredients: 2, 4-dichloroaniline (< 0.1 g/L), Sodium nitrite (< 0.1 g/L), HCl (33.54 g/L).	

Warnings and Precautions

- IVD

- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[2], [3], [4], [5]

The following warnings and precautions apply to: R1	
	
DANGER	Contains sulfamic acid.
H314	Causes severe skin burns and eye damage.
Prevention	
P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
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.
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.
H332	Harmful if inhaled.
H290	May be corrosive to metals.
Prevention	
P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P271	Use only outdoors or in a well-ventilated area.
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.
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
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.

Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

For the most current hazard information, see the product Safety Data Sheet.

Safety Data Sheets are available at www.corelaboratory.abbott or/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

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.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	28 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.

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.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity c Direct Bilirubin assay file must be installed on the Alinity c analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations Manual.**

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
mg/dL	17.1	µmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

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

- Abbott Laboratories has not verified the assay performance characteristics with neonatal specimens.

  NOTE: Abbott Laboratories recommends the use of sample interference indices in the semi-quantitative mode to assist in the determination of sample integrity for all specimens. Refer to the Sample Interference Indices (HIL) application sheets.

Specimen Conditions

- 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.
- Use serum or plasma specimens without visible hemolysis or lipemia. Refer to the SPECIFIC PERFORMANCE CHARACTERISTICS section of this package insert.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

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.

Specimen Storage

Specimens **should be protected from bright light as bilirubin is photolabile**.*6* Direct bilirubin is stable in serum and plasma as follows:

Specimen Type	Temperature	Maximum Storage Time
Serum/Plasma	20 to 25°C	2 days*7*
	2 to 8°C	7 days*7*, *8*
	-20°C	3 months*9*
	-80°C	3 months*9*

Avoid multiple freeze/thaw cycles.

Guder et al. suggest storage of frozen specimens at -20°C for no longer than the time

intervals cited above.[7]

Stored specimens must be inspected for particulates. If present, mix with a low speed vortex or by inversion and centrifuge the specimen to remove particulates prior to testing.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

07P97 Alinity c Direct Bilirubin Reagent Kit

Materials Required but not Provided

- Alinity c Direct Bilirubin assay file
- 08P6101 Alinity c Bilirubin Calibrator Kit
- Commercially available controls containing direct bilirubin
- Saline (0.85% to 0.90% NaCl) for specimen dilution

For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

Assay 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.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Minimum sample volume requirements:
  - Sample volume for single test: 5.0 µL.

    NOTE: This amount does not include the dead volume plus the additional over-aspiration volume. For total sample volume requirements, **refer to the Alinity ci-series Operations Manual, Section 4.**
- Refer to the Alinity c Bilirubin Calibrator Kit package insert and/or commercially available control material package insert for preparation and usage.
- For general operating procedures, **refer to the Alinity ci-series Operations Manual, Section 5.**
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, **Section 9. Perform maintenance more frequently when required by laboratory procedures.**

Sample Dilution Procedures

Samples with a direct bilirubin value exceeding 15.0 mg/dL (256.5 µmol/L) are flagged with the code "> 15.0 mg/dL" (> 256.5 µmol/L) and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

If using an automated dilution protocol, the system performs a dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor. For details on configuring automated dilutions, refer to the Alinity ci-series Operations Manual, Section 2.

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.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than the lower value of the measuring interval of 0.1 mg/dL (1.7 µmol/L), do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration

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.

Quality Control Procedures

- Two levels of controls (normal and abnormal) are to be run every day testing performed.
- If quality control results do not meet the acceptance criteria defined by laboratory procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[10]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity c Direct Bilirubin assay utilizes the Linear data reduction method to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, **refer to the Alinity ci-series Operations Manual, Section 5.**

Measuring Interval

Measuring interval is defined as the range of values in mg/dL (µmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity c Direct Bilirubin assay is **0.1 to 15.0 mg/dL** (1.7 to 256.5 µmol/L).

LIMITATIONS OF THE PROCEDURE

Some specimens may give a direct bilirubin result slightly greater than the total bilirubin result. During internal testing at Abbott Laboratories, specimens with total bilirubin concentrations of 0.2 mg/dL (3.4 µmol/L) or less occasionally gave a direct bilirubin result that slightly exceeded their respective total bilirubin result. This may be observed when nearly all reacting bilirubin is direct bilirubin.

For patients undergoing evaluations involving the administration of **indocyanine green (ICG),** it is recommended that samples are drawn after ICG has been eliminated. See the Interference section for additional information.[11, 12]

Abbott Laboratories has not verified the assay performance characteristics with neonatal specimens.

Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

EXPECTED VALUES

Manufacturer provide reference range will be used, effort made to verify in house

Serum[13]

	Range (mg/dL)	Range (µmol/L)
Adult	0.0 to 0.5	0.0 to 8.6

A study was conducted using 136 serum samples from volunteers ranging in age from 25 to 66 years. Data were analyzed as described by NCCLS C28-A.[14] From this study, 95% of all specimens fell within 0.0 to 0.5 mg/dL, with samples ranging from 0.1 to 0.6 mg/dL.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity c analyzer and the ARCHITECT c System and AEROSET System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity c analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.[15] Testing was conducted using 1 lot of the Alinity c Direct Bilirubin Reagent Kit, 1 lot of the Alinity c Direct Bilirubin Calibrator Kit, and 1 lot of commercially available controls and 1 instrument. Three controls and 2 human serum panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

			Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	**n**	**Mean (mg/dL)**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	120	0.4	0.02	3.9	0.02	3.9
Control Level 2	120	1.2	0.02	1.4	0.06	4.8
Control Level 3	120	2.3	0.02	0.8	0.09	4.0
Panel 1	120	3.5	0.04	1.3	0.06	1.7
Panel 2	120	8.6	0.08	0.9	0.11	1.3

[a] Includes within-run, between-run, and between-day variability.

Sample	n	Mean (µmol/L)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Control Level 1	120	6.8	0.28	4.1	0.28	4.1
Control Level 2	120	21.4	0.29	1.4	1.03	4.8
Control Level 3	120	39.7	0.32	0.8	1.58	4.0
Panel 1	120	60.5	0.76	1.3	1.03	1.7
Panel 2	120	147.6	1.37	0.9	1.88	1.3

[a] Includes within-run, between-run, and between-day variability.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.*16* Testing was conducted using 3 lots of the Alinity c Direct Bilirubin Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.

	mg/dL	µmol/L
LoB[a]	0.0	0.0
LoD[b]	0.1	1.7
LoQ[c]	**0.1**	1.7

[a] The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b] The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met.

Linearity

A study was performed based on guidance from CLSI EP06-A.*17*

This assay is linear across the measuring interval of **0.1 to 15.0 mg/dL** (1.7 to 256.5 µmol/L).

Interference

Potentially Interfering Substances

Potential interference in the Direct Bilirubin assay from 62 mg/dL (0.62 g/L) hemoglobin, 125 mg/dL (1.25 g/L) Intralipid, or 0.50 mmol/L Indican (indoxyl sulfate) is ≤ 10% or ± 0.1 mg/dL, whichever is greater, at the medical decision level of the analyte.

This study was performed on the ARCHITECT c System and the AEROSET System.

Potential interference in the Direct Bilirubin assay from 6.3 mg/L (8.1 µmol/L) indocyanine green is ≤ 10% or ± 0.1 mg/dL, whichever is greater, at the medical decision level of the

analyte.

This study was performed on the ARCHITECT c System.

These studies were performed based on guidance from NCCLS EP7-P.[18]

Interference effects were assessed by Dose Response and Paired Difference methods at the medical decision level of the analyte.

Potentially Interfering Substance	Interferent Level		Direct Bilirubin	
	Default Units	Alternate Units	Target Level (mg/dL)	Difference from Target (mg/dL)
Hemoglobin	31 mg/dL	0.31 g/L	0.4	-0.1
	62 mg/dL	0.62 g/L	0.4	-0.1
	125 mg/dL	1.25 g/L	0.4	-0.2
	250 mg/dL	2.50 g/L	0.4	-0.2
	500 mg/dL	5.00 g/L	0.4	-0.2
Human triglyceride	519 mg/dL	5.86 mmol/L	0.4	-0.1
	1034 mg/dL	11.68 mmol/L	0.4	0.3
Intralipid	125 mg/dL	1.25 g/L	0.4	-0.1
	250 mg/dL	2.50 g/L	0.4	0.1
	500 mg/dL	5.00 g/L	0.4	0.4
Indocyanine Green	6.3 mg/L	8.1 µmol/L	0.3	+0.1
	12.5 mg/L	16.1µmol/L	0.3	+0.3
	18.8 mg/L	24.2 µmol/L	5.1	+0.4
	25.0 mg/L	32.3 µmol/L	5.1	+0.5

Taki et al. reported indoxyl sulfate concentrations up to 8.62 mg/dL (0.40 mmol/L), with an average of 3.52 mg/dL (0.17 mmol/L), in 224 hemodialysis (HD) patients.[19] Indoxyl sulfate does not cause significant interference with this direct bilirubin method. Testing at Abbott Laboratories demonstrated that addition of 12.57 mg/dL (0.50 mmol/L) 3-indoxyl sulfate potassium salt to specimens increased the direct bilirubin concentration by a maximum of 0.1 mg/dL.

Indocyanine green solutions at the above concentrations were prepared by the individual addition of indocyanine green to two pools of plasma, one with a high concentration of bilirubin and one with a low concentration of bilirubin.

Interferences from medication or endogenous substances may affect results.[20]

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[21]

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity c Direct Bilirubin vs ARCHITECT Direct Bilirubin	Serum	mg/dL	132	1.00	0.00	1.00	0.2 to 14.7
		µmol/L	132	1.00	0.00	1.00	3.4 to 250.6

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