Alinity c Hemoglobin A1c (HbA1c)-16

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SUPERSEDES: Procedure titled _____

WARNING: The Alinity c Hemoglobin A1c assay has significant interference with the fetal hemoglobin (HbF). Hemoglobin A1c results are invalid for patients with abnormal amounts of HbF, including those with known Hereditary Persistence of Fetal Hemoglobin. For more information regarding the specific concentrations of HbF that were found to interfere with the Alinity c Hemoglobin A1c assay, refer to the SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity section of this package insert.

INTENDED USE

The Alinity c Hemoglobin A1c assay is used in clinical laboratories for the quantitative *in vitro* measurement of percent hemoglobin A1c (NGSP) or HbA1c fraction mmol/mol (IFCC)

in human whole blood and hemolysate on the Alinity c analyzer.

Hemoglobin A1c measurements are used as an aid in the diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

SUMMARY AND EXPLANATION OF THE TEST

HbA1c is the fraction of hemoglobin A that is first reversibly, then irreversibly glycated at one or both *N*-terminal valines of the *β*-chain.[1] The longer red blood cells are in circulation and the higher the ambient glucose levels, the higher the concentration of HbA1c. HbA1c reflects the average blood glucose level during the preceding 2 to 3 months. The HbA1c assay is useful as an aid in the:

- diagnosis of diabetes mellitus,
- identification of patients at risk for developing diabetes, and
- monitoring of patients with diabetes mellitus.[2, 3, 4, 5, 6]

For monitoring diabetic patients, it is recommended that glycemic goals are individualized following current professional society recommendations.[7] As recommended by the American Diabetes Association (ADA), patients in the range of 5.7 to 6.4 %HbA1c (39 to 46 mmol/mol) would be in the category of increased risk for diabetes and results ≥ 6.5% (48 mmol/mol) may aid in the diagnosis of diabetes.[7]

Several studies, including the Diabetes Control and Complications Trial (DCCT), have shown that long-term control of diabetes can prevent complications such as cardiovascular disease, retinopathy, nephropathy, and neuropathy. Measurement of HbA1c can be invaluable in the monitoring of glycemic control of diabetic patients.[8, 9, 10]

This method is certified to the National Glycohemoglobin Standardization Program (NGSP), standardized to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and traceable to DCCT.

PRINCIPLES OF THE PROCEDURE

The Alinity c Hemoglobin A1c assay consists of two separate concentration measurements: glycated hemoglobin (HbA1c) and total hemoglobin (THb). The two concentrations are used to determine the percent HbA1c (NGSP units) or the hemoglobin fraction in mmol/mol (IFCC units).

The individual concentration values of HbA1c and THb generated by the Alinity c Hemoglobin A1c assay are used only for calculating the percent hemoglobin A1c or HbA1c fraction, and must not be used individually for diagnostic purposes.

The anticoagulated whole blood specimen is lysed automatically on the system for the Whole Blood application or may be lysed manually using the Hemoglobin A1c Diluent (A1cDIL) for the Hemolysate application.

Glycated Hemoglobin (HbA1c)

The Alinity c Hemoglobin A1c assay utilizes an enzymatic method that specifically measures *N*-terminal fructosyl dipeptides of the *β*-chain of HbA1c.

- In the pretreatment process, the erythrocytes are lysed and the hemoglobin is transformed to methemoglobin by reaction with sodium nitrite.
- With the addition of Reagent 1 (R1) to the sample, the glycosylated *N*-terminal dipeptide (fructosyl-VH) of the *β*-chain of hemoglobin is cleaved by the action of protease. The hemoglobin is transformed to stable methemoglobin azide by the action of sodium azide and the concentration of the hemoglobin is determined by measuring absorbance.
- Addition of Reagent 2 (R2) starts a reaction and fructosyl peptide oxidase (FPOX) is allowed to react with fructosyl-VH. The HbA1c concentration is measured by determining the resultant hydrogen peroxide.

Total Hemoglobin (THb)

The hemoglobin is oxidized to stable methemoglobin azide by the action of sodium nitrite and sodium azide and the concentration of the hemoglobin is determined by measuring absorbance (sample + R1).

Hemoglobin A1c Calculations[11]

The final result is expressed as %HbA1c (NGSP) or mmol/mol HbA1c (IFCC) and is automatically calculated by the system from the HbA1c/THb ratio as follows:

mmol/mol HbA1c IFCC:

HbA1c (mmol/mol) = (HbA1c/THb) × 1000

%HbA1c DCCT/NGSP:

HbA1c (%) = IFCC x 0.09148 + 2.152

Methodology: Enzymatic

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

REAGENTS

Kit Contents

Alinity c Hemoglobin A1c Reagent Kit 08P43

NOTE: This product is composed of 3 components, which are packaged as a 2 cartridge reagent set. Both cartridges are required to perform the assay.

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

REF	**08P4320**
Tests per cartridge set	260
Number of cartridge sets per kit	5
Tests per kit	1300
R1	46.0 mL

REF	**08P4320**
R2	18.3 mL
A1cDIL	67.6 mL

R1 Active ingredients: 10-(carboxymethylaminocarbonyl)- 3,7-bis(dimethylamino) phenothiazine sodium salt (0.000817%), protease (bacterial) (< 1 MU/dL). Inactive ingredient: sodium azide (< 0.1%) as a stabilizer. Preservatives: sodium azide (< 0.1%), ProClin 300 (0.05%).

R2 Active ingredients: peroxidase (horseradish) (5 to 15 kU/dL), fructosyl-peptide-oxidase (*E. coli*, recombinant) (300 to 900 U/dL). Preservative: ofloxacin (0.001%).

A1cDIL Active ingredient: sodium nitrite (> 0.05 to < 0.3%). Preservative: ProClin 300 (0.01%).

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.[12], [13], [14], [15]

The following warnings and precautions apply to: R1

DANGER	Contains morpholinoethanesulfonic acid, monohydrate*, N,N-dimethylformamide, methylisothiazolones and sodium azide.
H317	May cause an allergic skin reaction.
H360	May damage fertility or the unborn child.
H316*	Causes mild skin irritation.
EUH032	Contact with acids liberates very toxic gas.
Prevention	

P201	Obtain special instructions before use.
P261	Avoid breathing mist / vapors / spray.
P280	Wear protective gloves / protective clothing / eye protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
P308+P313	IF exposed or concerned: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

The following warnings and precautions apply to: R2	
H316*	Causes mild skin irritation.
P332+P313*	If skin irritation occurs: Get medical advice / attention.

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

The following warnings and precautions apply to: A1cDIL	
	
WARNING	Contains sodium nitrite*, maleic acid and methylisothiazolones.
H317	May cause an allergic skin reaction.
H402*	Harmful to aquatic life.
Prevention	
P261	Avoid breathing mist / vapors / spray.

P272	Contaminated work clothing should not be allowed out of the workplace.
P273*	Avoid release to the environment.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

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

- Reagents are shipped refrigerated or on cold packs.
- 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.

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

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	50 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.**

A1cDIL can be replaced independently of R1 and R2.

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 Hemoglobin A1c assay files must be installed on the Alinity c analyzer prior to performing the assay.

For the Whole Blood application, the following assay files are required:

- THbWB
- HbA1cWB
- A1cWB (for results in mmol/mol (IFCC) units) or %A1cWB (for results in %HbA1c (NGSP) units)

For the Hemolysate application, the following assay files are required:

- THbH
- HbA1cH
- A1cH (for results in mmol/mol (IFCC) units) or %A1cH (for results in %HbA1c (NGSP) units)

The Alinity ci-series software version 2.0 or higher must be installed 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.**

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen type used for this assay is whole blood only.

The specimen type listed below was verified for use with this assay.

Specimen Type	Collection Vessel	Special Conditions
Whole Blood	Collection tubes Acceptable anticoagulants are: Dipotassium EDTA Lithium heparin Sodium heparin Sodium fluoride/disodium EDTA Tripotassium EDTA	Do not overfill specimen collection tubes. Whole blood samples greater than 78 mm in height from the bottom of tube will result in an instrument error and results will not be generated.

- Use only whole blood specimens collected by standard venipuncture techniques into plastic tubes.

Specimen Conditions

- Analyze fresh specimens if possible.
- Do not overfill specimen collection tubes. Whole blood samples greater than 78 mm in height from the bottom of tube will result in an instrument error and results will not be generated. Refer to the Alinity ci-series Operations Manual, Section 10.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- **Do not centrifuge samples.**
- Follow the tube manufacturer's collection instructions for specimen collection tubes.
- For testing whole blood samples less than 600 µL, use 12 x 75 mm polypropylene conical bottom tubes.
- Visually inspect the specimens. If fibrin clots or particulate matter is observed, remove with a clean applicator stick.
- Mix all specimens thoroughly by low speed vortexing or gently inverting 10 times prior to loading onto the Alinity c analyzer.

Prepare frozen whole blood specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.

Do not use Alinity c Sample Cups for whole blood samples. Refer to the Assay Procedure section of this package insert for further information.

Specimen Storage

Analyze fresh specimens if possible.

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Whole Blood	Room temperature	8 hours	
	2 to 8°C	7 days	
Hemolysate	Room temperature	4 hours	Do not freeze hemolyzed specimens.
	2 to 8°C	24 hours	

Whole Blood: If testing will be delayed more than 7 days, **store at -70°C or colder**.

CAUTION: Whole blood specimens that require freezing must be stored at -70°C or colder.

Avoid more than one freeze/thaw cycle.

Stored specimens must be inspected for particulates. If present, mix with a low speed vortex or by inversion.

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

08P43 Alinity c Hemoglobin A1c Reagent Kit

Materials Required but not Provided

- Alinity c Hemoglobin A1c assay files
- 08P4301 Alinity c Hemoglobin A1c Calibrators

Whole Blood application:

- Commercially available whole blood controls
- 12 x 75 mm polypropylene conical bottom tubes

Hemolysate application:

- 08P4310 Alinity c Hemoglobin A1c Controls or other commercially available controls
- Calibrated adjustable pipette capable of measuring 222 µL
- Calibrated micropipette capable of measuring 10 µL
- Vortex (optional)

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.**

Refer to the following table.

NOTE: No more than one replicate can be sampled from a sample cup or tube.

For information on ordering patient samples and controls, **refer to the Alinity ci-series Operations Manual, Section 5.**

	Sample Type	
	Whole Blood	**Hemolysate**
1. Order the application:	Whole Blood	Hemolysate
2. Prepare samples according to the instructions from this section of the package insert:	Assay Procedure, Whole Blood Preparation	Assay Procedure, Hemolysate Preparation
3. Load, then run:	Whole Blood samples	Prepared, Hemolyzed samples

	Sample Type	
	Whole Blood	**Hemolysate**
4. Report results in **percent (NGSP)** or mmol/mol (IFCC) hemoglobin A1c.		

Whole Blood Preparation

- **WARNING: Do not use Alinity c sample cups.**
- **WARNING: Do not overfill specimen collection tubes. Whole blood samples greater than 78 mm in height from the bottom of tube will result in an instrument error and results will not be generated. Refer to Section 10 of the Alinity ci-series Operations Manual.**
- Select the appropriate sample vessel using the following table:

If sample is:	Then:
< 200 µL	Do not use the Whole Blood application. Follow the instructions in the Assay Procedure, Hemolysate Preparation section of this package insert.
200 µL to 600 µL	Use 12 x 75 mm polypropylene conical bottom tubes only.
> 600 µL	Use suitable tubes or polypropylene conical bottom tubes.
> 78 mm in height in a large tube	Pipette 600 µL of sample into a suitable tube or polypropylene conical bottom tube.

Hemolysate Preparation

The minimum sample volume requirement is 150 µL for Alinity c sample cups.

- **NOTE:** This volume supersedes the calculated minimum sample volume displayed on the User Interface.

Prepare the hemolysate samples as follows:

1. Using a calibrated pipette, dispense 222 µL A1cDIL into a tube or sample cup.
2. Using a calibrated micropipette, withdraw 10 µL of the well-mixed whole blood patient specimen.
3. Wipe excess blood from the exterior of the pipette to ensure accurate transfer of the sample.
4. Insert the pipette into the tube or sample cup containing the A1cDIL allowing the tip of the pipette to just make contact with the surface of the A1cDIL and dispense the 10 µL sample (1:23.2 dilution).
5. Withdraw and dispense twice to rinse the pipette, always keeping the tip of the pipette in contact with the fluid in the tube.

6. Mix hemolysate thoroughly by low speed vortexing or by gently inverting 10 times. Avoid foaming.
7. Allow the hemolysate to stand for a minimum of 1 minute at room temperature prior to testing.
8. If the hemolysate is prepared in a tube, transfer to a sample cup and place the cup on the instrument.

   NOTE: The number of tests per kit is based on the 222 µL of A1cDIL and 10 µL of specimen volumes stated in steps 1 and 2 above. However, alternate volumes may be used for the 1:23.2 dilution, such as 555 µL of A1cDIL and 25 µL of specimen.

- 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.
- Do not use Alinity c Sample Cups for whole blood samples.
- Minimum sample volume requirements:
  - Sample volume for single test: 200 µL (Whole Blood), 150 µL (Hemolysate).

    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 Hemoglobin A1c Calibrators package insert and the Alinity c Hemoglobin A1c Controls package insert 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 **cannot be diluted** for the Alinity c Hemoglobin A1c assay.

Calibration

For instructions on performing a calibration**, refer to the Alinity ci-series Operations Manual, Section 5.**

Calibration is stable for approximately **50 days (1200 hours),** but is required with each change in reagent lot. Verify calibration with 2 levels of controls according to the laboratory quality control procedure. 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.

Both the Whole Blood and Hemolysate applications use the Alinity c Hemoglobin A1c

Calibrators which are supplied separately.

Alinity c Hemoglobin A1c Calibrators are traceable **to NGSP and IFCC** reference methods.

Quality Control Procedures

- At least two levels of controls are to be run every day testing performed. The Alinity c Hemoglobin A1c assay uses:
- Commercially available whole blood controls for the Whole Blood application.
- Alinity c Hemoglobin A1c Controls or other commercially available controls for the Hemolysate application.

  Refer to the Alinity c Hemoglobin A1c Controls Value Sheet for NGSP and IFCC ranges of the Alinity c Hemoglobin A1c Controls.

- If quality control results do not meet the acceptance criteria defined by laboratory quality controls procedure, sample results may be suspect. Follow the laboratory 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.*16*

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

- The individual concentrations of the Whole Blood and Hemolysate applications are measured by the analyzer.

  NOTE: HbA1c or THb concentrations must not be used individually for clinical purposes.
- Refer to the LIMITATIONS OF THE PROCEDURE section of this package insert.

Conventional Units (NGSP)

The percent HbA1c (%HbA1c) is automatically calculated by the system per the calculation provided in the Principles of the Procedure section.

SI Units (IFCC)

The hemoglobin A1c fraction (mmol/mol HbA1c) is automatically calculated by the system

per the calculation provided in the Principles of the Procedure section.

Calculation

The Alinity c Hemoglobin A1c assay utilizes the Linear data reduction method to generate a calibration and results.

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 %HbA1c (mmol/mol) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity c Hemoglobin A1c assay is **4.0 to 14.0 %**HbA1c (20.22 to 129.51 mmol/mol HbA1c).

LIMITATIONS OF THE PROCEDURE

- Do not use Alinity c Sample Cups for whole blood samples. Refer to the Assay Procedure section of this package insert for further information.
- This assay must be performed by qualified laboratory personnel, under appropriate laboratory conditions, solely for the intended use of the assay.
- Do not centrifuge samples.
- Do not freeze specimens that have been hemolyzed with the A1cDIL.
- Whole blood specimens that require freezing must be stored at -70°C or colder.
- Do not overfill specimen collection tubes. Whole blood samples greater than 78 mm in height from the bottom of tube will result in an instrument error and results will not be generated. Refer to Section 10 of the Alinity ci-series Operations Manual.
- Use specimen collection tubes, or for sample volumes < 600 µL use the 12 x 75 mm polypropylene conical bottom tubes as recommended in the PROCEDURE, Materials Required but not Provided section of this package insert.
- WARNING: The Alinity c Hemoglobin A1c assay should not be used to diagnose diabetes during pregnancy. Hemoglobin A1c reflects the average blood glucose levels over the preceding 3 months (i.e., the average life span of a red blood cell) and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red blood cell survival.[17], [18], [19], [20]
- Blood transfusions may impact the HbA1c concentration in the patient sample.
- The Alinity c Hemoglobin A1c assay should not be used to diagnose or monitor diabetes in patients with the following conditions:[17], [18], [19], [20]
  - hemoglobinopathies except as demonstrated to produce acceptable performance (e.g., sickle cell trait - refer to the SPECIFIC PERFORMANCE CHARACTERISTICS section of this package insert)
  - abnormal red blood cell turnover (e.g., anemias from hemolysis and iron deficiency)

- malignancies, and severe chronic hepatic and renal disease

- In cases of rapidly evolving Type 1 diabetes, the increase of HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentrations and/or the typical clinical symptoms.
- This test should not replace glucose testing for patients with Type 1 diabetes, pediatric patients, or pregnant women.
- The Alinity c Hemoglobin A1c assay is susceptible to interference effects from conjugated bilirubin at > 15.0 mg/dL (180 µmol/L) and unconjugated bilirubin at > 10.0 mg/dL
(171 µmol/L).
- The observed bias for samples containing HbC, HbD, HbE, HbS and HbA2 may be impacted by the method used to determine the reference Hemoglobin A1c concentration.
- The Alinity c Hemoglobin A1c assay is susceptible to interference effects from HbF at > 5%. Glycated HbF is not detected by the Alinity c Hemoglobin A1c assay as it does not contain the β-chain that characterizes HbA1c. However, HbF is measured in the total hemoglobin constituent assay and as a consequence, specimens containing high amounts of HbF (> 5%) may result in lower than expected mmol/mol HbA1c values (IFCC) and %HbA1c values (NGSP).
- Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert for specimen limitations.

EXPECTED VALUES

For monitoring diabetic patients, it is recommended that glycemic goals are individualized following current professional society recommendations.[7] The American Diabetes Association (ADA) recommendations[7] are summarized in the following table.

HbA1c Value	Glycemic Goal
< 8 %HbA1c (64 mmol/mol)	Less stringent
< 7 %HbA1c (53 mmol/mol)	General (non-pregnant adults)
< 6.5 %HbA1c (48 mmol/mol)	More stringent

HbA1c values above 6.5 %HbA1c (48 mmol/mol) are an indication of hyperglycemia during the preceding 2 to 3 months or longer. According to the recommendations of the ADA, HbA1c values above 6.5 %HbA1c (48 mmol/mol) are suitable for the diagnosis of diabetes mellitus. Patients with HbA1c values in the range of **5.7 to 6.4** %HbA1c (39 to 46 mmol/mol) may be at a risk of developing diabetes.[3, 21]

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 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.[22] Testing was conducted using 3 lots of the Alinity c Hemoglobin A1c Reagent Kit, 3 lots of the Alinity c Hemoglobin A1c Calibrators, and 1 lot of commercially available controls and 3 instruments. Three controls and 3 human whole blood panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

Whole Blood (%HbA1c)

Sample	Instrument	n	Mean (%HbA1c (NGSP))	Within-Run (Repeatability)		Within-Laboratory (Total)[a]		Precision with Additional Component of Between-Lot[b]	
				SD	%CV	SD	%CV	SD	%CV
Control 1	1	240	5.1	0.01	0.1	0.05	1.0	0.10	1.9
	2	240	5.1	0.04	0.7	0.06	1.2	0.10	1.9
	3	240	5.1	0.02	0.5	0.06	1.1	0.11	2.1
Control 2	1	240	9.2	0.03	0.3	0.07	0.7	0.12	1.3
	2	240	9.3	0.03	0.4	0.07	0.7	0.13	1.5
	3	240	9.3	0.03	0.4	0.06	0.7	0.11	1.2
Control 3	1	240	13.5	0.03	0.2	0.06	0.5	0.15	1.1
	2	240	13.6	0.04	0.3	0.08	0.6	0.16	1.2
	3	240	13.6	0.04	0.3	0.06	0.5	0.12	0.9
Panel near 4.0 %HbA1c	1	240	4.4	0.03	0.7	0.06	1.3	0.07	1.7
	2	240	4.4	0.03	0.7	0.05	1.2	0.07	1.5
	3	240	4.4	0.03	0.7	0.06	1.3	0.08	1.8
Panel near 6.5 %HbA1c	1	240	6.2	0.02	0.3	0.03	0.5	0.07	1.2
	2	240	6.2	0.02	0.4	0.04	0.6	0.07	1.2
	3	240	6.2	0.03	0.4	0.04	0.6	0.08	1.3

Sample	Instrument	n	Mean (%HbA1c (NGSP))	Within-Run (Repeatability)		Within-Laboratory (Total)[a]		Precision with Additional Component of Between-Lot[b]	
				SD	%CV	SD	%CV	SD	%CV
Panel near 8.0 %HbA1c	1	240	7.9	0.03	0.3	0.05	0.6	0.09	1.2
	2	240	7.9	0.04	0.5	0.06	0.7	0.09	1.2
	3	240	7.9	0.04	0.5	0.05	0.7	0.11	1.3

[a] Within-Laboratory variability contains within-run, between-run, and between-day variance components.

[b] Contains within-run, within-day, between-day, and between-lot variance components.

Whole Blood (mmol/mol)

Sample	Instrument	n	Mean (mmol/mol (IFCC))	Within-Run (Repeatability)		Within-Laboratory (Total)[a]		Precision with Additional Component of Between-Lot[b]	
				SD	%CV	SD	%CV	SD	%CV
Control 1	1	240	32.15	0.127	0.4	0.534	1.7	1.009	3.1
	2	240	32.40	0.195	0.6	0.546	1.7	0.959	3.0
	3	240	32.38	0.169	0.5	0.537	1.7	1.089	3.4
Control 2	1	240	77.17	0.153	0.2	0.627	0.8	1.332	1.7
	2	240	77.64	0.241	0.3	0.639	0.8	1.344	1.7
	3	240	77.80	0.182	0.2	0.582	0.7	1.204	1.5
Control 3	1	240	124.49	0.197	0.2	0.640	0.5	1.641	1.3
	2	240	125.22	0.373	0.3	0.734	0.6	1.693	1.4
	3	240	125.52	0.297	0.2	0.605	0.5	1.220	1.0
Panel near 20.0 mmol/mol	1	240	24.81	0.195	0.8	0.457	1.8	0.745	3.0
	2	240	24.90	0.146	0.6	0.428	1.7	0.682	2.7
	3	240	24.93	0.107	0.4	0.364	1.5	0.781	3.1

Sample	Instrument	n	Mean (mmol/mol (IFCC))	Within-Run (Repeatability)		Within-Laboratory (Total)[a]		Precision with Additional Component of Between-Lot[b]	
				SD	%CV	SD	%CV	SD	%CV
Panel near 47.5 mmol/mol	1	240	43.82	0.152	0.3	0.400	0.9	0.811	1.9
	2	240	43.93	0.214	0.5	0.422	1.0	0.804	1.8
	3	240	43.97	0.192	0.4	0.382	0.9	0.891	2.0
Panel near 63.9 mmol/mol	1	240	62.94	0.145	0.2	0.396	0.6	0.886	1.4
	2	240	63.07	0.238	0.4	0.456	0.7	0.905	1.4
	3	240	63.13	0.175	0.3	0.385	0.6	0.959	1.5

[a] Within-Laboratory variability contains within-run, between-run, and between-day variance components.

[b] Contains within-run, within-day, between-day, and between-lot variance components.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.[23] Testing was conducted using 3 lots of the Alinity c Hemoglobin A1c Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB) and Limit of Detection (LoD) are summarized below.

Whole Blood

	Glycated Hemoglobin (HbA1c) µmol/L	Total Hemoglobin (THb) µmol/L
LoB[a]	43.8095	0.0000
LoD[b]	48.1568	6.7745

[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.

Hemolysate

	Glycated Hemoglobin (HbA1c) µmol/L	Total Hemoglobin (THb) µmol/L
LoB[a]	39.0741	12.2147
LoD[b]	41.0713	16.3614

[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.

Linearity

A study was performed based on guidance from CLSI EP06-A.*24*

This assay is linear across the measuring interval of **4.0 to 14.0** %HbA1c (20.22 to 129.51 mmol/mol HbA1c).

Analytical Specificity

Hemoglobin Derivatives

This study was performed on the ARCHITECT c System.

A specificity study was performed based on guidance from CLSI EP07-A2.*25* Specificity was assessed by comparing test samples containing the potential interferents listed below to reference samples. No interference was observed for:

- Acetylated Hemoglobin with ≥ 50 mg/dL of acetylsalicylic acid (ASA) (aspirin)
- Carbamylated Hemoglobin with ≥ 10 mmol/L of Cyanate
- Labile Hemoglobin with ≥ 1000 mg/dL of Glucose

IFCC

The Alinity c Hemoglobin A1c assay had a difference within ± 7% for samples with concentrations ≥ 38.78 mmol/mol HbA1c.

NGSP

The Alinity c Hemoglobin A1c assay had a difference within ± 5% for samples with concentrations ≥ 5.7 %HbA1c.

Hemoglobin Variants

A study was performed based on guidance from CLSI documents EP07-A2.*25*

Specificity was assessed by comparing samples containing abnormal hemoglobins to a comparator assay**. Heterozygous** hemoglobin variants (HbS, HbC, HbD, HbE, HbA2) do not interfere with the Alinity c Hemoglobin A1c assay.

IFCC

The Alinity c Hemoglobin A1c assay had a difference within ± 7%.

NGSP

The Alinity c Hemoglobin A1c assay had a difference within ± 5%.

Heterozygous Hemoglobin Variant	Relative % Difference from Reference Concentration			
	~ 6.0 %HbA1c (5.5 to 6.5 %HbA1c)		~9.0 %HbA1c (8.0 to 10.0 %HbA1c)[a]	
	Relative % Difference	Range[b]	Relative % Difference	Range[b]

Heterozygous Hemoglobin Variant	Relative % Difference from Reference Concentration			
	~ 6.0 %HbA1c (5.5 to 6.5 %HbA1c)		~9.0 %HbA1c (8.0 to 10.0 %HbA1c)[a]	
	Relative % Difference	Range[b]	Relative % Difference	Range[b]
HbC	-4.2	-8.9, -1.6	-2.5	-6.2, 0.0
HbD	1.9	-1.7, 4.8	2.3	1.1, 3.5
HbE	1.4	-3.2, 6.6	-0.9	-4.1, 2.5
HbS	-0.3	-5.5, 1.8	-1.9	-4.9, 1.1
HbA2	-0.8	-4.9, 3.6	-0.6	-3.7, 5.4
HbF	Difference exceeds -5% when the amount of HbF in the sample exceeds ~5%.[c] See the Heterozygous Hemoglobin Variant HbF interference in the following table.			

[a] The HbA2 results at ~9.0 %HbA1c consisted of samples between 7.1 to 14.0 %HbA1c.

[b] The range is defined as the minimum and maximum relative % difference at each concentration level (~6.0 and ~9.0 %HbA1c).

[c] Refer to the LIMITATIONS OF THE PROCEDURE section of this package insert for further information.

NOTE: The presence of multiple variants in a sample may impact the % difference.

Heterozygous Hemoglobin Variant HbF interference is summarized in the following table.

A negative % difference with HbF is proportional in magnitude to the % HbF present in the sample. For example, when the amount of HbF in the sample was 20.4%, the % difference was -21.3% on the Alinity c analyzer.

Heterozygous Hemoglobin Variant	% HbF	Individual % Interference from Comparator
HbF	3.2	-3.1
	4.6	-4.9
	6.2	-4.6
	8.6	-7.4
	11	-9.8

Refer to the LIMITATIONS OF THE PROCEDURE section of this package insert for further information regarding the specific concentrations of HbF that were found to interfere with the Alinity c Hemoglobin A1c assay.

Interference

This study was performed on the ARCHITECT c8000 and c4000 Systems.

Potentially Interfering Substances

A study was performed based on guidance from CLSI EP07-A2.[25] Interference effects were assessed by comparing test samples containing the potential interferents listed below to reference samples.

IFCC

The Alinity c Hemoglobin A1c assay had a difference within ± 7% for samples with concentrations ≥ 38.78 mmol/mol HbA1c.

NGSP

The Alinity c Hemoglobin A1c assay had a difference within ± 5% for samples with concentrations ≥ 5.7 %HbA1c. The data in %HbA1c (NGSP) showing the highest level of interference observed on the ARCHITECT c8000 and c4000 Systems is presented in the table.

Potentially Interfering Substance	**Interferent Concentration**		**% Interference[a]**	
	Conventional Units	**SI Units**	**6.0 to 7.0 %HbA1c**	**≥ 8.0 %HbA1c**
Ascorbic Acid	3.0 mg/dL	0.15 mmol/L	0.0	0.0
Bilirubin (Conjugated)[b]	15.0 mg/dL	180 µmol/L	-3.2	-3.3
Bilirubin (Unconjugated)[c]	10.0 mg/dL	171 µmol/L	-3.0	-2.7
Glucose	1000 mg/dL	55.5 mmol/L	0.0	0.0
Rheumatoid Factor	200 IU/mL	200 IU/mL	0.0	0.0
Triglycerides	3000 mg/dL	33.9 mmol/L	-1.6	-4.5
Total Protein	22 g/dL[d]	220 g/L	0.0	-1.1
Urea	667 mg/dL	111.06 mmol/L	0.0	0.0
Vitamin E	8.6 mg/dL	200 µmol/L	1.6	0.0

[a]

$$\% \text{ Interference} = \frac{\text{Test Result - Control Result}}{\text{Control Result}} \times 100$$

[b] Samples containing conjugated bilirubin at > 15.0 mg/dL (180 µmol/L) demonstrated interference. Refer to the LIMITATIONS OF THE PROCEDURE section of this package insert for further information.

[c] Samples containing unconjugated bilirubin at > 10.0 mg/dL (171 µmol/L) demonstrated

interference. Refer to the LIMITATIONS OF THE PROCEDURE section of this package insert for further information.

[d] The total protein concentration of 22 g/dL includes serum protein as well as hemoglobin.

Potentially Interfering Substance	Interferent Concentration		% Interference[a]	
	Conventional Units	SI Units	6.0 to 7.0 %HbA1c	≥ 8.0 %HbA1c
Acarbose	50 mg/dL	0.77 mmol/L	0.0	0.0
Acetaminophen	200 mg/L	1324.5 µmol/L	-0.59	-0.80
N-acetyl-4-benzoquinone imine	20 mg/L	134.2 µmol/L	-1.5	-0.8
N-acetyl-L-cysteine	1600 mg/L	9816 µmol/L	-3.4	-2.7
Acetylsalicylate	50.8 mg/dL	2.82 mmol/L	0.0	0.0
Atorvastatin	0.06 mg/dL	600 µg Eq/L	1.6	0.0
Captopril	0.5 mg/dL	23 µmol/L	-1.5	-1.1
Chlorpropamide	74.7 mg/dL	2.7 mmol/L	0.0	-1.1
Cyanate	50 mg/dL	6.16 mmol/L	0.0	1.1
Dipyrone	100 mg/L	300.3 µmol/L	0.2	0.1
Furosemide	6.0 mg/dL	181 µmol/L	0.0	1.1
Gemfibrozil	7.5 mg/dL	300 µmol/L	0.0	0.0
Ibuprofen	50 mg/dL	2425 µmol/L	0.0	1.1
Insulin	450 micro units per mL	450 micro units per mL	0.8	0.0
Losartan	5 mg/dL	0.11 mmol/L	0.0	0.0
Metformin	5.1 mg/dL	310 µmol/L	0.0	0.0
Nicotinic Acid	61 mg/dL	4.95 mmol/L	-1.5	-0.5
Propranolol	0.2 mg/dL	7.71 µmol/L	0.0	-0.5
Repaglinide	0.006 mg/dL	132.57 nmol/L	0.8	0.0
4-acetamido antipyrine	40 mg/L	163.3 µmol/L	-0.4	-0.4

Potentially Interfering Substance	Interferent Concentration		% Interference[a]	
	Conventional Units	SI Units	6.0 to 7.0 %HbA1c	≥ 8.0 %HbA1c
4-aminoantipyrine	40 mg/L	197.0 µmol/L	-0.4	-0.2
4-formylamino antipyrine	40 mg/L	173.2 µmol/L	-0.1	-0.6
4-methylamino antipyrine	40 mg/L	184.3 µmol/L	-0.3	-0.4

a

$$\% \text{ Interference} = \frac{\text{Test Result - Control Result}}{\text{Control Result}} \times 100$$

Refer to the LIMITATIONS OF THE PROCEDURE section of this package insert for further information.

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[26]

		Units	N	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity c Hemoglobin A1c vs NGSP Secondary	Whole Blood	%HbA1c (NGSP)	134	0.99	-0.10	1.00	4.4 - 14.0
	Hemolysate	%HbA1c (NGSP)	134	0.99	-0.10	1.00	4.4 - 14.0
Alinity c Hemoglobin A1c vs ARCHITECT Hemoglobin A1c	Whole Blood	%HbA1c (NGSP)	130	1.00	0.10	1.00	4.5 - 13.1
		mmol/mol (IFCC)	130	1.00	0.10	1.01	25.72 - 119.99
	Hemolysate	%HbA1c (NGSP)	133	1.00	0.00	1.00	4.7 - 13.1
		mmol/mol (IFCC)	133	1.00	-0.39	1.02	28.22 - 119.44

Predicted Bias

IFCC

The Alinity c Hemoglobin A1c assay is designed to have a predicted bias of ≤ 5% at 42.06, 47.53, and 53.00 mmol/mol HbA1c using Deming regression.

NGSP

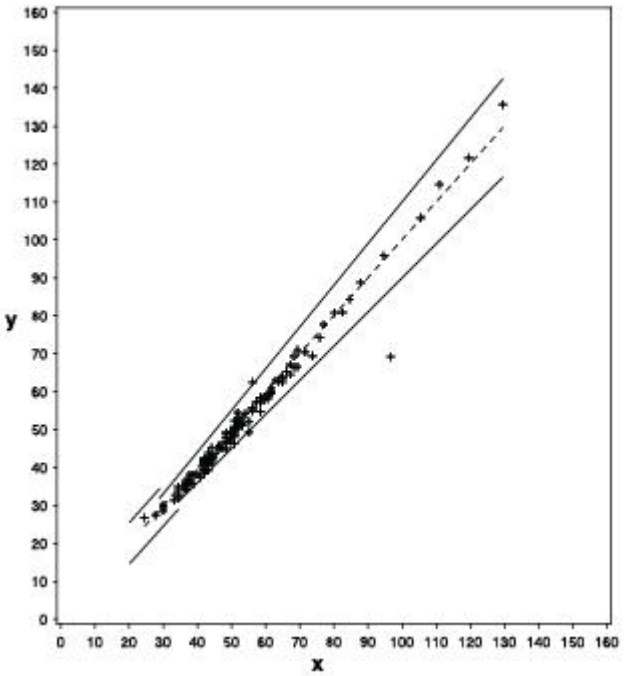
The Alinity c Hemoglobin A1c assay is designed to have a predicted bias of ≤ 3% at 6.0, 6.5, and 7.0 %HbA1c using Deming regression. The predicted bias in %HbA1c (NGSP) ranged from -2.0% to -1.5%.

Allowable Total Difference (ATD) Zone

The Alinity c Hemoglobin A1c assay is designed to have > 95% of observations in the ATD zone and the low limit of the two-sided 95% Confidence Interval (CI) > 89.5%. The percentage of observations in the ATD zone was 99.3% (133/134) and the lower limit of the two-sided 95% CI was 95.9%. The ATD zone plots are presented below.

ATD Zone- Alinity c analyzer

Hemoglobin A1c in Whole Blood, mmol/mol (IFCC)

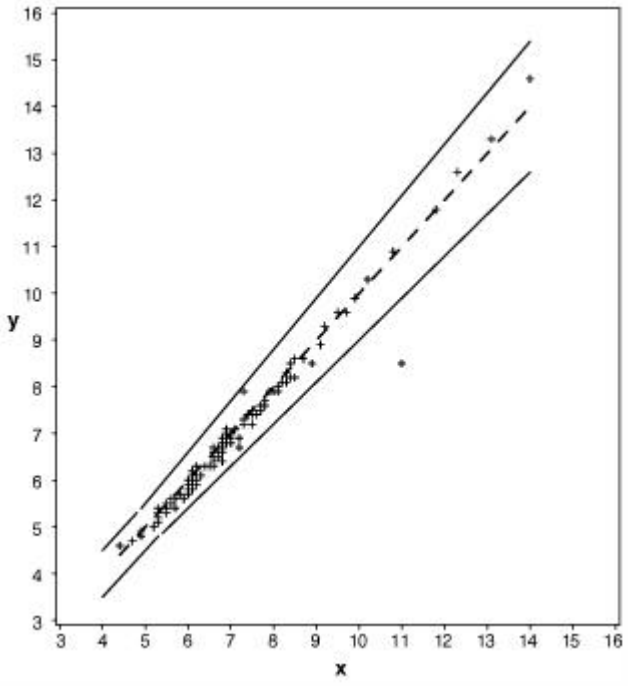


x = NGSP Secondary Reference Laboratory (mmol/mol)

y = Alinity c Hemoglobin A1c (mmol/mol)

ATD Zone- Alinity c analyzer

Hemoglobin A1c in Whole Blood, %HbA1c (NGSP)



x = NGSP Secondary Reference Laboratory (%HbA1c)

y = Alinity c Hemoglobin A1c (%HbA1c)

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