Hemoglobin A1C

Purpose

This procedure provides instructions for performing HEMOGLOBIN A1C on Alinity c analyzers.

The Alinity c Hemoglobin A1c assay is used in clinical laboratories for the quantitative in vitro measurement of percent hemoglobin A1c (NGSP) in human whole blood 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.

Policy Statements

This procedure applies to all personnel responsible for operating the Abbott Alinity c at Children's Minnesota Laboratory. This assay is also referred to as HbA1c.

Principle

Method: Enzymatic

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.

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

$$HbA1c\,\% = \left(\frac{HbA1c}{THb}\right) \times 91.48 + 2.152$$

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

Clinical Significance

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. 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.

For monitoring diabetic patients, it is recommended that glycemic goals are individualized following current professional society recommendations. 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.
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.
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.

Test Code

HBA1C

Materials

Product Description	*Product Code*	*Stability*

Hemoglobin A1c Reagent (1300 tests per box; each box contains 5 reagent sets of 260 tests). Each reagent set consists of 2 cartridges, including R1, R2, and diluent. **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%). **Diluent:** Active ingredient: sodium nitrite (> 0.05 to < 0.3%). Preservative: ProClin 300 (0.01%). Abbott Diagnostics	MFR# 08P4320 CHC# 35280	**Store at:** 2-8C **Unopened:** Stable until lot expiration date. **Opened:** 50-day onboard stability is tracked internally by the analyzer. When stored capped and refrigerated off the instrument, the reagent remains stable until lot expiration date.
Hemoglobin A1C Calibrator Abbott Diagnostics	MFR# 08P4301 CHC# 35281	**Store at:** 2-8C **Unopened:** Stable until lot expiration date. **Opened:** Once opened and reconstituted, calibrators are stable for up to 10 days while stored at 2-8C.
Liquicheck Diabetes Control BioRad, Cardinal	Level 1: MFR# BR12011343 CHC# 35363 Level 2: MFR# BR12011344 CHC# 35364	**Store at:** -20 to -70C **Unopened:** Stable until lot expiration date. **Opened:** Stable at 2-8C for 45 days after opening, not to exceed the lot expiration date.

Conical bottom tubes 12x75 mm, 1,000 per bag, Sarstedt	MFR# 57.512 CHC# 35283	**Store at:** RT **There is no expiration date**
Tube Plugs (Caps) 12 mm diameter for conical bottom tubes, 1,000 per bag. Sarstedt	MFR# 65.809.306 CHC# 19424	**Store at:** RT **There is no expiration date**

Sample

Sample: Preferred 1 mL whole blood EDTA, (minimum 200uL)

- Do not centrifuge.
- Whole blood samples greater than 78 mm in height from the bottom of tube will result in an instrument error. Do not over fill vacutainer tubes.

Stability:

- Whole blood samples are stable at room temperature up to 8 hours.
- Whole blood samples are stable at 2-8C up to 7 days.

Rejection criteria: Unlabeled tube, over/under filled, incorrect temperature storage conditions

Preparation:

- Inspect sample for fibrin clots; remove any clots with a clean applicator stick.
- Samples of 200-600uL must use the conical bottom tubes; samples >600uL may be tested directly from the vacutainer tube. *Do NOT use Alinity sample cups for whole blood samples.*
- Gently invert the tube 10 times before running on the analyzer.

Special Safety Precautions

The following warnings and precautions apply to the **R1 Reagent**	
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.
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, clothing, and 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

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

The following warnings and precautions apply to the **A1c Diluent** 	
WARNING	Contains sodium nitrite*, maleic acid and methylisothiazolones.
H317	May cause an allergic skin reaction.
H402*	Harmful to aquatic life.
Prevention	
P 261	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.

Calibration

Assay Range	4% - 14%
Reference Material	**Hemoglobin A1C Calibrator,** Abbott Diagnostics, 08P4301
Suggested Calibration Levels	See lot specific documentation for assigned calibrator values.
Calibration Scheme	Two levels. The Alinity c Hemoglobin A1c assay utilizes the Linear data reduction method to generate a calibration and results.
Calibration Frequency	Calibration is due every 50 days, with each new reagent lot, or as needed for troubleshooting.

Calibrator preparation	Add 1.6ml CLRW water to each vial. Let sit at room temperature at least 10 minutes, then mix by gentle inversion. Decant to Alinity sample cups for testing. Stable 10 days at 2-8C once opened.
AMR	AMR is verified twice annually using the Maine StandardsHbA1c Linearity Kit, product number 605. Run all applicable levels in triplicate. Results are reviewed and approved by the Technical Specialist. Any questionable results are investigated and corrective actions documented

Quality Control

Material: BioRad LiquiCheck Diabetes Control, levels 1 and 2
Frequency: Two levels each day of use
Preparation: When removing from frozen storage, thaw at room temperature at least 15 minutes and until completely thawed. Mix gently and thoroughly. Decant into barcoded conical bottom tube.
Gently swirl before each use. Keep capped & refrigerated between uses.
Acceptable Ranges: Acceptable QC ranges are defined within Unity Real Time (URT) software.

Procedure

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

Whole blood samples over 600 uL may be sampled directly from the vacutainer tube.
Whole blood samples 200 uL to 600 uL must be sampled from the 12 x 75 mm conical bottom tubes.

Calibrators are lyophilized and are the only samples to be used with Alinity sample cups.

Dilutions

Do not dilute. See result reporting.

Limitations

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.
- 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.

• 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.
• 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:

- 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 and unconjugated bilirubin at > 10.0 mg/dL.
• 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 consequently, specimens containing high amounts of HbF (> 5%) may result in lower than expected mmol/mol HbA1c values (IFCC) and %HbA1c values (NGSP).

Reference Intervals

A1c Diagnostic:

≤5.6% Normal
5.7-6.4% At risk for diabetes mellitus
≥6.5% Suitable for diagnosis of diabetes mellitus

A1c Monitoring: Goals must be individualized and reassessed over time. An A1c of <7% is appropriate for many children and adolescents.[2]

Result Reporting

Results will be reported to one decimal place.
Results between 4.0 and 14.0% without error messages are released.
Results below 4% without error are reported as <4.0%.
Results above 14% without error are reported as >14.0%

References	1. Alinity c Hemoglobin A1C Reagent Kit Package Insert, REF 08P4320, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, March 2022 *2.* American Diabetes Association. Standards of Care in Diabetes – 2024. *S20-S42, S263* 3. Rifai, Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th Edition, Elsevier, AACC, 2018 4. Bio-Rad Liquichek Diabetes Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618, November 2023

Historical Record	**Version**	**Written/Revised by:**	**Effective Date:**	**Summary of Revisions**
	1	Matt Johnson	6/10/24	Initial Version