**TRAINING UPDATE**

| | | | |
|---|---|---|---|
| **Lab Location:** | SGMC | **Date Distributed:** | 7/1/2021 |
| **Department:** | Core Lab | **Due Date:** | 8/1/2021 |

**DESCRIPTION OF PROCEDURE REVISION**

**Name of procedure:**

# Hemoglobin A1c (A1c-E) by Atellica CH Analyzer SGMC.C3029 v2

**Description of change(s):**

| Section | Reason |
|---|---|
| 6, 17 | Updated Diabetes Control information (QC product) |
| 10.6 | Added Error message processes |

This revised SOP was implemented on June 16, 2021

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

| | |
|---|---|
| **Title** | **Hemoglobin A1c (A1c-E) by Atellica CH Analyzer** |
| **Prepared by** | Ashkan Chini Date: 4/30/2021 |
| **Owner** | Robert SanLuis Date: 4/30/2021 |

| **Laboratory Approval** | **Local Effective Date:** | |
|---|---|---|
| **Print Name and Title** | **Signature** | **Date** |
| *Refer to the electronic signature page for approval and approval dates.* | | |

## TABLE OF CONTENTS

## 1. TEST INFORMATION

| Assay | Method/Instrument | Test Code |
|---|---|---|
| Hemoglobin A1c | Atellica CH Analyzer | A1C |

| Synonyms/Abbreviations |
|---|
| Enzymatic Hemoglobin A1c, Glycohgb, HbA1c, A1C |

| Department |
|---|
| Chemistry |

## 2. ANALYTICAL PRINCIPLE

The Atellica CH A1c_E assay consists of two separate measurements: glycated hemoglobin (A1c_E) and total hemoglobin (tHb_E). The two measurements are used to determine the %HbA1c (NGSP units) or the hemoglobin A1c_E/tHb_E ratio in mmol/mol (IFCC units). The individual concentration values of A1c_E and tHb_E generated by this assay are used only for calculating the %HbA1c or A1c_E/tHb_E ratio, and must not be used individually for diagnostic purposes.

The anticoagulated whole blood specimen is lysed on the system using the Atellica CH A1c_E pretreatment solution to obtain hemolysate for the Atellica CH A1c_E assay.

The Atellica CH A1c_E assay is an enzymatic method that specifically measures N-terminal fructosyl dipeptides on the beta-chain of HbA1c. In the pretreatment step, the erythrocytes are lysed and the hemoglobin is oxidized to methemoglobin by reaction with sodium nitrite. In the first step of the reaction (the Atellica CH A1c_E reagent 1 (R1) + sample), the N-terminal fructosyl dipeptide fragment is cleaved from the hemoglobin beta chain with a protease. Concurrently, methemoglobin is converted into stable azide-methemoglobin in the presence of sodium azide and the total hemoglobin concentration is determined by measuring the absorbance at 478/694 nm. In the second step of the reaction, fructosyl peptide oxidase (FPOX) is added to react with the fructosyl dipeptide to generate hydrogen peroxide. The hydrogen peroxide reacts with the chromogen in the presence of peroxidase to develop a color that is measured at 658/805 nm. The Atellica CH A1c_E assay incorporates a turbidity normalization mechanism (cHb_E) that is measured at 805 nm to effectively remove any sample turbidity which could impact the tHb_E measurement.

## 3. SPECIMEN REQUIREMENTS

### 3.1 Patient Preparation

| Component | Special Notations |
|---|---|
| **Fasting/Special Diets** | N/A |

| Component | Special Notations |
|---|---|
| **Specimen Collection and/or Timing** | Normal procedures for collecting and storing whole blood may be used for samples to be analyzed by this method. |
| **Special Collection Procedures** | N/A |
| **Other** | N/A |

### 3.2 Specimen Type & Handling

| Criteria | | |
|---|---|---|
| **Type -Preferred** | $K_2$-EDTA or $K_3$-EDTA Whole Blood | |
| **-Other Acceptable** | None | |
| **Collection Container** | Lavender Top Tube | |
| **Volume - Optimum** | 1.0 mL | |
| **- Minimum** | 0.5 mL | |
| **Transport Container and Temperature** | Collection container or plastic vial at room temperature | |
| **Stability & Storage Requirements** | Room Temperature: | 48 hours |
| | Refrigerated: | 7 days |
| | Frozen: | 21 months |
| **Timing Considerations** | N/A | |
| **Unacceptable Specimens & Actions to Take** | Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS. | |
| **Compromising Physical Characteristics** | Thoroughly mix all samples immediately prior to testing. Avoid the formation of bubbles or foam. Ensure samples are free of fibrin or particulate matter. | |
| **Other Considerations** | None | |

**NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.**

## 4. REAGENTS

**The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.**

### 4.1 Reagent Summary

| Reagents | Supplier & Catalog Number |
|---|---|
| Enzymatic Hemoglobin (A1c-E) – Contains<br>• Reagent Pack 1 (P1), 2 each<br>• Reagent Pack 2 (P2), 2 each<br>• A1c-E PRE (Vial 1), 2 each | Siemens, Atellica CH, Cat. No. 11097536 |

### 4.2 Reagent Preparation and Storage

| **Reagent** | **Enzymatic Hemoglobin (A1c-E)** |
|---|---|
| **Storage** | • Store at 2-8°C<br>• Protect from heat and light sources. |
| **Stability** | Reagents are stable onboard the system for 63 days. |
| **Preparation** | Reagent is liquid and ready to use. |
| **Instructions** | Reagents Pack 1 and 2 are loaded onboard like other reagents.<br>A1c-E PRE is loaded in a different place. To load A1c-E PRE onboard the CH Module cover needs to open:<br>• From the CH Module Screen select **Home – Module State**<br>• Press **Pause** and wait until the system status changes to **Stopped**<br>• Select **Unlock Front Cover**<br>• Once the front cover is unlocked open the cover and load A1c-E PRE onboard<br>• Close the front cover and reset the instrument. |

## 5. CALIBRATORS/STANDARDS

### 5.1 Calibrators/Standards Used

| Calibrator | Supplier and Catalog Number |
|---|---|
| Enzymatic Hemoglobin A1c-E Calibrator (A1c-E CAL), 3 levels | Siemens Atellica CH, Cat. No. 11099338 |

### 5.2 Calibrator Preparation and Storage

| **Calibrator** | Enzymatic Hemoglobin A1c-E Calibrator (A1c-E CAL) |
|---|---|
| **Preparation** | **Note:** A1c-E CAL Level 1 is light-sensitive. When handling the reagent, protect it from light and ensure that the CH Analyzer top cover is closed when running the A1c-E assay.<br>A1c-E CAL Level 1 is liquid and ready to use.<br>Prepare Levels 2 and 3 using the following steps: |

| | |
|---|---|
| | 1. Add 1.0 mL of reagent grade water into each vial using a calibrated pipette.<br>2. Let the vials stand for 30 minutes at room temperature to allow the lyophilized material to dissolve.<br>3. Gently swirl and invert the vials to ensure homogeneity of the material. |
| **Storage/Stability** | • Store at 2-8°C<br>• Protect from heat and light sources<br>• **Unopened:** stable until expiration date stamped on the box.<br>• **Level 1:** 214 days when recapped immediately after use.<br>• **Levels 2 & 3:** stable for 11 days after reconstitution. |

### 5.3 Calibration Parameter

| Criteria | Special Notations |
|---|---|
| **Reference Material** | Enzymatic Hemoglobin A1c-E Calibrator (A1c-E CAL) |
| **Assay Range** | See Package Insert for specific assay ranges. |
| **Suggested Calibration Level** | See Reagent Package Insert for lot specific assigned values in % HbA1c |
| **Frequency** | • When changing lot numbers of primary reagent packs.<br>• At the end of the lot calibration interval (180 days), for a specified lot of calibrated reagent on the system.<br>• At the end of pack calibration interval (63 days), for calibrated reagent packs on the system.<br>• When indicated by quality control results.<br>• After major maintenance or service.<br><br>At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded. |
| **Calibration Scheme** | See Package Insert for specific calibration scheme. |
| **Procedure** | Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions. |

### 5.4 Tolerance Limits

| IF…… | THEN…… |
|---|---|
| If result fall within assay-specific specification, and QC values are within acceptable limits, | proceed with analysis |
| If result falls outside assay-specific specification, or QC values are out of Acceptable limits, | troubleshoot the assay and/or instrument and repeat calibration |

## 6. QUALITY CONTROL

### 6.1 Controls Used

| Controls | Supplier and Catalog Number |
|---|---|
| InteliQ Diabetes Control, Levels 1 & 2 | Bio-Rad Laboratories<br>Cat. No. 12008309 & 12008310 |

### 6.2 Control Preparation and Storage

| | |
|---|---|
| **Control** | InteliQ Diabetes Control, Levels 1 & 2 |
| **Preparation** | Allow the control to reach room temperature (18-25°C) for approximately 60 minutes or until completely thawed. Gently invert the tube several times to ensure homogeneity.<br>Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage. |
| **Storage/Stability** | **Frozen:** stable until the expiration date at -20 to -70ºC.<br>**Thawed and stored off-board:** 10 days at 2-8°C<br>**Thawed and stored on-board Atellica:** 45 days at 2-8°C |

### 6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Siemens Atellica QC Schedule and the Siemens Atellica Quick Reference Guide.

### 6.4 Tolerance Limits and Criteria for Acceptable QC

| Step | Action |
|---|---|
| 1 | Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime. |
| 2 | **Run Rejection Criteria**<br>• Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported.<br>• The technologist must follow the procedure in the Laboratory QC Program to resolve the problem. |
| 3 | **Corrective Action**:<br>• All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory |

| Step | Action |
|---|---|
| | QC Program. Follow corrective action guidelines in the Laboratory QC Program.<br>• Corrective action documentation must follow the Laboratory Quality Control Program. |
| 4 | **Review of QC**<br>• QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee.<br>• If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions. |

### 6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

### 6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

## 7. EQUIPMENT and SUPPLIES

### 7.1 Assay Platform

Siemens Atellica CH Analyzer

### 7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

### 7.3 Supplies

- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

## 8. PROCEDURE

Atellica CH Enzymatic Hemoglobin A1c (A1c-E) is required to perform this test.

A1c-E is performed on the Atellica CH Analyzer after the method is calibrated and Quality Controls are acceptable.

**NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.**

| 8.1 | Instrument Set-up Protocol |
|---|---|
| 1. | Perform any required instrument maintenance. |
| 2. | Ensure that the instrument has sufficient primary and ancillary reagents. |
| 3. | Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate. |
| 4. | Check calibration status and re-calibrate as needed. |

| 8.2 | Specimen Testing |
|---|---|
| 1. | Thoroughly mix the specimens immediately before testing. |
| 2. | Load A1C samples in the Atellica STAT rack **ONLY** and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system |
| 3. | Refer to the general operating procedure for detailed steps. |
| 4. | Follow protocol in section 10.6 “Repeat Criteria and Resulting” for samples with results above the analytical measurement range (AMR).<br>Investigate any flagged results and repeat as necessary. |
| 5. | Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors. |

**NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.**

## 9. CALCULATIONS

The instrument automatically calculates the concentration of Enzymatic Hemoglobin A1c.

# 10. REPORTING RESULTS AND REPEAT CRITERIA

## 10.1 Interpretation of Data

None required

## 10.2 Rounding

No rounding is necessary. Instrument reports results up to one decimal point.

## 10.3 Units of Measure

% HbA1c

## 10.4 Clinically Reportable Range (CRR)

3.8 – 14.0 % HbA1c

## 10.5 Review Patient Data

Each result is reviewed for error messages. Resolve any problems noted before issuing patient reports.

## 10.6 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa listing for specific information.

Values that fall below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

| IF the result is … | THEN… |
|---|---|
| < 3.8 % HbA1c | Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 3.8 % HbA1c |
| > 14.0 % HbA1c | Report as: "> 14.0 % HbA1c -REP"<br>Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results. |
| Error | DI will display "Check current HGB. If HGB less than 8g/dL, result A1C with A1QST and release. If greater than 8g/dL, REPEAT". Continue with appropriate scenario below - |
| Error<br>**and**<br>HGB < 8g/dL | In DI, right click on the result and select A1QST from the drop down.<br>• The Test Comment field must be left blank<br>• Release the result of A1QST *<br>• SQ will add the order of XHA1C to the accession<br>• Take sample to Processing for send out<br>*A1QST expands to "Unable to perform on the Atellica. Testing sent to Quest." |

| IF the result is … | THEN… |
|---|---|
| Error<br>**and**<br>HGB > 8g/dL | Repeat the test. If the result is acceptable, report it.<br>If the rerun result is still = "Error", DI will set the result for A1C to A1QST, and it will display "Send A1C to Quest".<br>• The Test Comment field needs to be left blank<br>• Release the result of A1QST<br>• SQ will add the order of XHA1C to the accession<br>• Take sample to Processing for send out |

| Message | Code |
|---|---|
| Verified by repeat analysis | Append –REP to the result. |

## 11. EXPECTED VALUES

### 11.1 Reference Ranges

< 5.7 %

### 11.2 Critical Values

None established

### 11.3 Standard Required Messages

The following comment is automatically added to the report by the LIS:
"Reference range and Suggested Diagnosis:

HbA1c (%)
Diabetic ≥6.5
Prediabetes 5.7 – 6.4
Normal <5.7

The frequency of HbA1c testing should depend on the clinical situation, the treatment regimen, and the clinician's judgment. The American Diabetes Association recommends a reasonable HbA1c goal for many nonpregnant adults is <7%. Less stringent HbA1c goals may be appropriate for some patients with diabetes and other risk factors, such as severe hypoglycemia or extensive comorbid conditions.

American Diabetes Association, Diagnosis and Classification of Diabetes Mellitus, Diabetes Care 2017; 40 (Supplement 1): S11-S24."

## 12. CLINICAL SIGNIFICANCE

HbA1c refers to the product of a non-enzymatic reaction between glucose and hemoglobin A1. The human erythrocyte is freely permeable to glucose, which can non-enzymatically combine with hemoglobin to form HbA1c. This non-enzymatic reaction between the alpha-amino group of the N-terminal valine of the hemoglobin beta-chain and glucose takes place to form an unstable aldimine or Schiff base intermediate (labile fraction). This reaction is

slow and reversible and occurs at a rate that is proportional to the glucose concentration in the blood. The aldimine intermediate subsequently undergoes a non-reversible Amadori rearrangement to form the stable ketoamine 1 – glucofrutovaline product. Since the reaction is driven by the concentration of reactants, the degree of glycosylation (resported as HbA1c relative to the total hemoglobin) is proportional to the average concentration of blood glucose over the circulating life span of hemoglobin in the red cell (approximately 120 days).

## 13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up.

## 14. LIMITATIONS OF METHOD

### 14.1 Analytical Measurement Range (AMR)

3.8 – 14.0 % HbA1c

### 14.2 Precision

| Material | Mean % HbA1c | Standard Deviation (%CV) | |
|---|---|---|---|
| | | Repeatability | Within-Lab |
| Control 1 | 4.62 | 0.03 | 0.6 |
| Control 2 | 8.94 | 0.03 | 0.3 |
| 5.0 % HbA1c | 5.28 | 0.02 | 0.5 |
| 6.5 % HbA1c | 6.49 | 0.03 | 0.4 |
| 8.0 % HbA1c | 7.89 | 0.03 | 0.3 |
| 12.0 % HbA1c | 11.79 | 0.03 | 0.3 |

### 14.3 Interfering Substances

- The A1c_E assay has significant interference with fetal hemoglobin (HbF) and samples may produce a negative bias (lower than actual results).
- This assay should not be used to diagnosis diabetes during pregnancy. Hemoglobin A1c reflects the average blood glucose levels over the preceding 8-12 weeks and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased RBC survival.
- This assay should not be used to diagnose or monitor diabetes in patients with the following conditions: hemoglobinopathies except as demonstrated to produce acceptable performance (sickle cell trait), abnormal RBC turnover (such as anemias with hemolysis and iron deficiency), malignancies, and severe chronic hepatic and renal disease.

- Do not use sodium fluoride / potassium oxalate collection tubes as they may interfere with results.

**HIL Interference:**
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

| Substance tested | Substance Concentration | Analyte Concentration 6.5 % HbA1c | Analyte Concentration 8.0 % HbA1c |
|---|---|---|---|
| Bilirubin (conjugated) | 10 mg/dL | NSI* | NSI* |
| Bilirubin (unconjugated) | 10 mg/dL | NSI* | NSI* |
| Lipemia Intralipid® | 1000 mg/dL | NSI* | NSI* |

*NSI = No significant interference. A percentage effect > 5% is considered a significant interference.

### 14.4 Clinical Sensitivity/Specificity/Predictive Values

**Detection Capability**
The assay is designed to have a limit of blank (LoB) < 3.8 %HbA1c. The assay is designed to have a limit of detection (LoD) ≤ 3.8 %HbA1c.

## 15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

Atellica A1c-E reagent and Calibrator level 1 may cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention.
**Contains:** 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol- 3-one (R1 and A1c_E PRE); Maleic acid (A1c_E PRE)

## 16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Retention of Records and Materials (Lab policy)
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Enzymatic Hemoglobin A1c (A1c-E) Reagent

## 17. REFERENCES

1. Package Insert, A1c-E Reagent, Siemens Healthcare Diagnostics Inc., 09/2019
2. Package Insert, A1c-E CAL, Siemens Healthcare Diagnostics Inc., 01/2020
3. Package Insert, InteliQ Diabetes Controls, Bio-Rad Laboratories, 03/2021

## 18. REVISION HISTORY

| Version | Date | Section | Reason | Reviser | Approval |
|---|---|---|---|---|---|
| 1 | 6/9/21 | 6, 17 | Updated Diabetes Control information | A Chini | R SanLuis |
| 1 | 6/9/21 | 10.6 | Added Error message processes | L Barrett | R SanLuis |

## 19. ADDENDA

None