TRAINING UPDATE

Lab Location:	WOMC	**Date Distributed:**	2/3/2023
Department:	Core Lab	**Due Date:**	3/1/2023

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Title: Hemoglobin A1c by Dimension Vista® System (WOMC.2000)

Description of change(s):

- Updated QC information
- Updated freezer temps

This revised SOP will be implemented March 1st of, 2023

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

WOMC.2000 Hemoglobin A1c by Dimension Vista® System

Copy of version 3.0 (approved, not yet effective)

Last Approval or Periodic Review Completed	1/18/2023
Next Periodic Review Needed On or Before	1/18/2025
Effective Date	3/1/2023

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Printed By Demetra Collier (110199)

Organization Adventist HealthCare

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	1/18/2023	3.0	Nicolas Cacciabeve MD Nicolas Cacciabeve	
Approval	Core lab approvals	1/17/2023	3.0	Robert SanLuis Robert SanLuis	
Approval	Lab Director	8/30/2021	2.0	Nicolas Cacciabeve	
Approval	Core lab approvals	8/29/2021	2.0	Robert SanLuis Robert SanLuis	
Approval	QA approval	8/25/2021	2.0	Leslie Barrett	

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
3.0	Approved, Not Yet Effective	Major revision	1/17/2023	3/1/2023	Indefinite
2.0	Approved and Current	Initial version	8/25/2021	10/19/2021	3/1/2023

Technical SOP

Title	**Hemoglobin A1c by Dimension Vista® System**	
Prepared by	Demetra Collier	Date: 12/19/2019
Owner	Robert SanLuis	Date: 12/19/2019

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	Dimension Vista® System	A1C

Synonyms/Abbreviations
Glycohgb, HbA1c, A1C

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Dimension Vista A1C assay measures both HbA1c and total hemoglobin. The HbA1c measurement is based on a turbidimetric inhibition immunoassay (TINIA) principle, and the measurement of total hemoglobin is based on a modification of the alkaline hematin reaction. Using the values obtained for each of these two analytes, the relative proportion of the total hemoglobin that is glycated is calculated and reported. Pre-treatment to remove the labile fraction is not necessary as only the Amadori rearranged form of HbA1c is detected. All hemoglobin variants that are glycated at the beta-chain N-terminus and have epitopes identical to that of HbA1c are measured by this assay.

Total Hemoglobin Measurement: A sample of whole blood is added to a reaction vessel containing lysing reagent. This reagent lyses the red blood cells and simultaneously converts the released hemoglobin to a derivative that has a characteristic absorbance spectrum. An aliquot of the lysed whole blood is transferred from the reaction vessel to a cuvette where total hemoglobin concentration is measured at 405 nm and 700 nm.

Whole blood + lysing agent -------> Released hemoglobin -------> hemoglobin derivative (measured at 405 nm)

Hemoglobin A1c Measurement: The same aliquot of the lysed whole blood that is transferred from the reaction vessel to the cuvette for the Hb measurement is also used for the measurement of HbA1c. The cuvette contains an anti-HbA1c antibody in a buffered reagent. Hemoglobin A1c in the sample reacts with anti-HbA1c antibody to form a soluble antigen-antibody complex. A polyhapten reagent containing multiple HbA1c epitopes is then added to this cuvette. The polyhapten reacts with excess (free) anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex. The rate of this reaction is measured turbidimetrically at 340 nm and blanked at 700 nm and is inversely proportional to the concentration of HbA1c in the sample.

hemoglobin A1c + anti-HbA1c antibody ----------> hemoglobin A1c-anti-HbA1c antibody complex

anti-HbA1c antibody (excess) + polyhapten ----------> Ab/polyhapten complex (absorbs at 340 nm)

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
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 **-Other Acceptable**	EDTA Whole blood None
Collection Container	Lavender Top Tube
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL
Transport Container and Temperature	Collection container or plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 3 days (15 - 25ºC)
	Refrigerated: 7 days
	Frozen: Not recommended
Timing Considerations	No more than one rack should be loaded every 5 minutes.
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	Samples should be mixed gently by inversion (gently invert the tube 10 times) or in a rocker mixer to obtain uniform distribution of the erythrocytes prior to testing. Avoid the formation of foam.
Other Considerations	Load no more than 6 samples at a time and only one rack every 5 minutes, to prevent increased settling of the red cells.

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
A1C Kit	Siemens, Flex® reagent cartridge, Cat. No. K3105B Includes A1C flex reagent cartridge and calibrator

Note: Each kit lot contains matched sets of HbA1c Flex® reagent cartridges and calibrators. These components are not interchangeable between kits with other lot numbers.

4.2 Reagent Preparation and Storage

Reagent	**A1C Kit**
Container	Reagent cartridge and calibrator (5 levels)
Storage	Store at 2-8°C
Stability	• Stable until expiration date stamped on reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Open wells: 5 days for wells 1 - 12
Preparation	All reagents are liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
A1C CAL	Siemens Dimension Vista®, Cat. No. K3105B

5.2 Calibrator Preparation and Storage

Calibrator	A1C CAL
Preparation	• Remove vials from refrigerator and proceed to the next step. • Remove stopper, avoid the loss of lyophilized material. • Volumetrically add 2.0 mL ± 0.01mL of reagent grade water to each vial. Water should be at room temperature. • Replace stopper, and let stand for 5 minutes. Do not invert the vials at this time. • Swirl vials gently for 30 seconds, then gently invert 10 times • Let vials stand for 30 minutes, then invert gently 10 times.

Storage/Stability	• Store at 2-8°C • **Unopened Calibrator:** until expiration date on the box. • **Opened Calibrator:** Reconstituted calibrator is stable for 8 hours at 25ºC, 48 hours at 2-8ºC and 2 months at -15 to -25ºC. Do not refreeze.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	A1C CAL
Assay Range	3.8 – 14.0 %
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values.
Frequency	• Every new reagent cartridge lot. • Every 30 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	5 levels, n = 3 for A1C

5.4 Calibration Procedure

Note: Calibration cups must be filled with 300 – 500 µL of calibrator.

Manual Calibration:

1. Verify that calibrators and reagents are in inventory on the instrument.
2. Press **System > Method Summary > Calibration**.
3. Select a method from the sidebar menu. Press the **Order Calibration** button on the screen.
4. Verify that the information on the screen is correct. Verify that the calibrator lot is correct using the drop-down menu.
   a. When calibrating using Vials press **OK**.
   b. When calibrating using Cups, check the Use Cups box. This displays the rack and cup position fields. For additional cups use the positions in ascending order. Be sure to use the number of calibration levels and cups as specified in the method IFU. Scan the rack barcode and place calibrator cups in an adapter in position 1 on a rack. Press **OK** and load the rack on the instrument.
5. The status field in the calibration screen changes sequentially to Awaiting Scheduling, Preparing Calibrators and Processing.

5.5 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
Liquichek Diabetes Control, Levels 1 and 3	Bio-Rad Laboratories Cat. No. 12011343 and 12011345

6.2 Control Preparation and Storage

Control	Liquichek Diabetes Controls, Level 1 and 3
Preparation	Allow the frozen product to thaw at room temperature (18 to 25°C) for approximately 15 minutes or until completely thawed prior to use. Before use, gently swirl the contents of the product to ensure homogeneity. After each use promptly replace the stopper and return to 2 to 8°C storage.
Storage/Stability	**Frozen:** stable until the expiration date at -20 to -70°C. **Thawed:** all analytes will be stable for 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 Dimension Vista® QC Schedule and the Dimension Vista® 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** • 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. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • 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

Step	Action
	Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • 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

Dimension Vista® System

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C for QC product.
- Freezer capable of sustaining range not to exceed -15 to -25°C for calibrator.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

A1C Kit Cat. No. K3105B is required to perform this test.

Hemoglobin A1c is performed on the Dimension Vista® System after the method is calibrated (see Reference Material in Calibration section) 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	Sample Processing
1.	A sample rack holding tubes or cups is placed on the rack input lane.
2.	The sample shuttle moves the rack to the barcode reader which identifies the rack and samples to the system.
3.	The rack moves into the sample server and to the rack positioner.
4.	At the same time, aliquot plates move from the aliquot loader into position.
5.	The aliquot probe aspirates the sample from the tubes or cups and dispenses it into the wells of the aliquot plates.
6.	After each aspirate-dispense action, the probe is thoroughly rinsed inside and out to prevent sample carryover.
7.	When sample aspiration is completed, the sample server moves the rack back to the sample shuttle, where it is placed on the output lane and can be removed by the operator.

8.2	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension Vista® QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension Vista® Operator's Manual
3.	Additional testing instructions for A1C: a. Gently mix sample by inversion 10x b. Remove stopper and make sure there are no bubbles or film

8.2	Specimen Testing
	c. Pipet the appropriate volume of sample into a sample cup or an SSC cup • Sample Cups: Minimum fill volume is 200µL • SSC cups, Minimum fill volume is 400µL d. Use ONLY 'Surplus" (gray) racks e. Run samples as STAT f. Load NO MORE than one rack every 5 minutes to avoid increased RBC settling.
4.	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension Vista® system manual "Error messages" section for troubleshooting.
5.	Follow protocol in Section 10.5 "Repeat criteria and resulting" for samples with results above or below the Analytical Measurement Range (AMR). Investigate any failed delta result and repeat, if necessary.
6.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

Test Conditions		
	Vessel	Cuvette
Sample Volume:	1.5 µL	8 µL
Hemolyzing Reagent Volume:	123 µL	0 µL
Antibody/Buffer Volume:	0 µL	140 µL
Polyhapten Volume:	0 µL	28 µL
Diluent Volume:	16 µL	23 µL
Reaction Time:		14 minutes
Temperature:		37°C
Wavelength:	340 and 405 nm	
Type of measurement:	Turbidimetric	

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 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

%

10.4 Clinically Reportable Range (CRR)

3.8 – 14.0 %

10.5 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 % (or Hg < 5g/dL)	A "Below Assay Range" error will be generated and the % HbA1C result will either not be printed or a result of < 3.8% will be reported by the instrument. Dispense a new aliquot of whole blood and re-assay the sample once. If the error persists notify your supervisor/tech in charge before releasing result. With supervisor approval, report as "<3.8 %-REP"
>14.0 %	An "Above Assay Range" error will be generated and the % HbA1C result will either not be printed or a result of > 14 % will be reported by the instrument. Repeat the sample using the Manual Dilution method below. **Manual Dilution**: Mix one part reagent grade water and one part well-mixed whole blood patient sample. Re-assay the diluted specimen to obtain results within the analytical measurement range. Do not multiply result. Correction factor is not appropriate for this assay because the diluted result is calculated based on the ratio between HbA1c and Hb. If the errors persist after dilution, report as "> 14.0 %-REP". Bring to the attention of the Group Lead or Tech in Charge (TIC) prior to releasing result.

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 (reported 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. Refer to your Dimension Vista Operator's Guide.

The expected maximum observed standard deviations for repeatability using n = 5 replicates at the following Hemoglobin A1c concentrations are:

HbA1c Concentration	Acceptable S.D. Maximum
5.5%	0.27%
9.3%	0.43%

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

3.8 – 14.0 %

14.2 Precision

Material N=720	Mean %	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Whole Blood Pool 1	5.1	0.06 (1.3)	0.09 (1.7)
Whole Blood Pool 2	6.5	0.05 (0.8)	0.11 (1.8)
Whole Blood Pool 3	7.9	0.09 (1.1)	0.17 (2.1)
Whole Blood Pool 4	11.7	0.11 (0.9)	0.13 (1.1)

14.3 Interfering Substances

Various substances other than sugars can form aggregates with hemoglobin and potentially interfere with the assay causing false results. Examples include individuals with opiate addiction, lead poisoning and alcoholism.
The antibody reagent used in the Dimension Vista® A1C assay will measure any glycosylated hemoglobin variants that are glycated at the beta-chain N-terminus and have epitopes identical to that of HbA1c. This includes HbS, Hbc, HbD, and HbE. Other hemoglobinopathies may give incorrect results with this test. Care must be taken when interpreting any HbA1c result from patients with Hb variants. Abnormal hemoglobins might affect the half-life of the red cells or the in vivo glycation rates. In these cases, even analytically correct results do not reflect the same level of glycemic control that would be expected in patients with normal hemoglobin.

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

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.

HbA1c Flex® Reagent Cartridge is harmful to aquatic life with long lasting effects. Contains: 2-methyl-4-isothiazolin-3-one. Avoid release into the environment.

16. RELATED DOCUMENTS

1. Dimension Vista® Clinical Chemistry System Operator's Manual
2. Dimension Vista® Calibration/Verification Procedure
3. Dimension Vista® Cal Accept Guidelines
4. Dimension Vista® Calibration summary
5. Dimension Vista® Sample Processing, Startup and Maintenance procedure
6. Laboratory Quality Control Program
7. Vista QC Schedule
8. Laboratory Safety Manual
9. Safety Data Sheets (SDS)
10. Dimension Vista® Limits Chart (AG.F200)
11. Retention of Records and Materials (Lab Policy)
12. Dimension Vista® System Error Messages Chart
13. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
14. Specimen Acceptability Requirements (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
17. Current package insert A1C Kit 3105B

17. REFERENCES

1. Package Insert, A1C Kit K3105B, Siemens Healthcare Diagnostics Inc., 4/1/2019
2. Package Insert, Liquichek Diabetes Control, Bio-Rad Laboratories, 09/2022
3. A1C Calibrator package insert, Siemens, 06/2019

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
1	8/24/21	Header Footer	Retired SGMC SOP re-instated for WOMC	L Barrett	R SanLuis
1	8/24/21	6.1, 6.2	Changed QC to levels 1 & 3	A Chini	R SanLuis
1	8/24/21	8.2	Updated testing conditions	A Chini	R SanLuis
1	8/24/21	14.3	Updated Interfering Substances	A Chini	R SanLuis
1	8/24/21	17	Updated insert dates	A Chini	R SanLuis
2	1/16/23	6.1, 6.2	Updated QC Information	A Chini	R SanLuis
2	1/16/23	7.2	Updated freezer temp. range	A Chini	R SanLuis
2	1/16/23	17	Updated package Insert dates	A Chini	R SanLuis

19. ADDENDA

None