Brown Clinic Northridge
511 14th Ave. NE
Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Hemoglobin A1C (HB1C)

PURPOSE:
The HB1C assay on the Dimension® clinical chemistry system is an in vitro diagnostic assay for the quantitative determination of hemoglobin A1c (HbA1c) in human anticoagulated whole blood. Measurements of hemoglobin A1c are effective in monitoring long-term glucose control in individuals with diabetes mellitus

PRINCIPLE:
The Dimension HbA1C assay measures both HbA1C and total hemoglobin. The HbA1v measurement is based on a turbidimetric inhibition immunoassay 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 the first cuvette 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 first cuvette to a second cuvette where total hemoglobin concentration is measured at 405 nm and 700 nm.[4]

Hemoglobin A1C Measurement:
The same aliquot of the lysed whole blood that is transferred from the first cuvette to the second cuvette for the HB measurement is also used for the measurement of HBA1C. The second 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 reacts with excess(free) anti-HBA1c antibodies to form an insoluble antibody-polyhapten complex. The rate of this reaction is measured turbidimetrically at 340nm and blanked at 700nm and is inversely proportional to the concentration of HBa1c in the sample.

Risk and Safety:
Harmful to aquatic live with long lasting effects. Avoid release to the environment. Dispose of contents and container in accordance with local, regional, and national regulation.

Precautions:

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.

Safety Data Sheets (MSDS/SDS) can be found at www.siemens.com/healthcare

For *in vitro* diagnostic use

REAGENT PREPARATION

All reagents are liquid and ready to use

Reagent Storage Instructions: Store at 2 - 8°C.

Expiration: Refer to carton for expiration date of the kit. All components are stable until the expiration date when stored unopened at 2-8C.

- Sealed cartridge wells on the instrument are stable 30 days.
- Open well stability 5days for wells 1-2 and 5-6. 10 days for well 3.

SPECIMEN

Specimen collection: Recommended specimen type is Whole Blood treated with EDTA

Specimen: Specimens should be collected using recommended procedures for collection of diagnostic blood specimens by venipuncture. Follow the instructions provided with your specimen collection device for use and processing.

- Samples for the HB1C method can only be assayed from a sample cup or SSC.
- Samples **cannot** be assayed directly from primary collection tubes.
- Samples should be mixed gently by inversion (gently invert the tube ten times) or in a rocker mixer prior to pipetting into the sample cup or SSC in order to obtain uniform distribution of the erythrocytes prior to testing. Avoid the formation of foam.
- Samples containing clots should not be used.
- Pipette **300 – 500 µL** of the whole blood sample into the sample cup or SSC.
- A maximum of two determinations from a single sample cup may be used.
- Sample can sit in sample cup on instrument for up to one hour.

Specimen Stability: Samples are stable when stored for no greater than:

3 days at 15 – 25 °C
7 days at 2 – 8 °C
4 months at -20 °C (freeze only once)

The purpose of specimen storage information is to provide guidance to users; however, users may validate their own procedures for storing samples.

PROCEDURE

Materials Needed

Materials Provided

HB1C Kit, Cat. No. DF105A: Includes HB1C Flex® reagent cartridges and HB1C calibrator (5 levels)

Materials Required But Not Provided

Quality Control Materials

Test Steps Test Conditions

Sampling, reagent delivery, mixing, and processing are automatically performed by the Dimension® clinical chemistry system. For details of this processing, refer to your Dimension® Operator's Guide.
The sample container must contain 300 – 500 µL whole blood to ensure proper re-mix.

	Cuvette 1	Cuvette 2
Sample Volume:	3ul	19ul
Hemolyzing reagent volume	300ul	0ul
Antibody/Buffer volume	0ul	320ul
Polyhapten volume	0ul	52ul
Temperature		37C
Test Wavelength	349 and 700 nm for hemoglobin A1c	
	405 and 700 nm for hemoglobin	
Type of Measurement	Turbidimetric for hemoglobin A1c	
	Colorimetric for hemoglobin	

Backup:
Refer to Brown Clinic Backup Policy

Calibration:

General calibration procedure is described in the Dimensions Operators Guide. HB1C requires lot specific scalers which must be entered in the Calibrations Set Up Screen prior to calibration. The scaler values are provided on the Flex reagent cartridge carton. These scalers are applied to all QC and patient results to maintain accuracy. Failure to enter the lot specific scalers will cause inaccurate results.

Results reported in % HbA1c c using the following equation:

$$\%HbA1c = 100 \times [HbA1c\ (mmol/L)/Hb\ (mol/L)]$$

Calibrator values (g/dL) are required for hemoglobin A1c and total hemoglobin. These values are obtained from the Table for Assigned Values provided in the Dimension HB1C Calibrator Instructions for Use (IFU). Subsequently the Dimension clinical chemistry system calculates an NGSP standardized %HbA1c result based on the calculation shown below. This calculation provides a %Hb1c value that is standardized to the DCCT study result, which is printed on the Dimension report slip. The values of the polynomial coefficients may vary by Flex reagent cartridge lot.

$$\text{NGSP Standardized } \%HbA1c = (b \times (\%HbA1c)^2) + (c \times \%HbA1c) + d$$

HB1C lots are produced under controlled conditions to meet established product specifications. Annually, one lot is tested on each Dimension model to confirm standardization to the Nation Glycohemoglobin Standardization Program (NGSP). Copies of certificates may be accessed at

www.Siemens.com/diagnostics. Additional information concerning NGSP certification may be obtained at www.ngsp.com.

Hb Assay Range	5.0-25 g/dL Hb
HbA1c Assay Range	0.3-2.6 g/dL HbA1c
Calibration Material	Secondary Calibrators such as HB1C calibrators
Calibration Scheme	2 levels, n=2 for Hb
	5 levels, n=2 for HbA1c
Units	%
Typical Calibration levels	5.0-25.0 g/dL Hb
	0.3-2.6 g/dL HbA1c
Calibration Frequency:	Every new reagent cartridge lot
	Every 30 days for any one lot
	For each new lot of Flex reagent cartridges
	After major maintenance or service, if indicated by quality control results
	As indicated in laboratory quality control procedures
	When required by government regulations
Assigned Coefficients C0	145.6
	C1 -147.5
	C2 -1.60
	C3 1.00
	C4 0.5
Scalers	See Flex reagent cartridge carton for lot-specific scaler values.

Controls:

At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

Results:

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

The Analytical Measurement Range (AMR) for Calculated HbA1C Ratio is 3.6 to 16.0%

Samples with results in excess of 16.0%, 25 g/dL hemoglobin or 2.6 g/dL HbA1c will be reported as "above Assay Range" and should be repeated on dilution. If the message persists on the diluted sample, report the results as "greater than 16.0%."

> Manual Dilution: Mix one part of clinical laboratory reagent water and on part of well mixed whole blood. Reassay the dilution mixture to obtain results within the analytical range.
>
> NOTE: The resulting readout (%HB1C {mmol/mol}) is the reportable result. The result must not be corrected for dilution as it is a calculated result based on the ratio between HB1C and Hb. Therefore, a dilution factor must not be used in the HB1C method.

Results with results less than 3.6%, hemoglobin less than 5.0 g/dL or HbA1c less than 0.3 g/dL will be reported as "Below Assay Range" by the instrument. Dispense a new 300-500 uL aliquot of whole blood and re-assay the sample. If the message persists, contact the Technical Solutions center for assistance.

Limitations of Procedure

The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in HB1C results. Refer to your dimension Operator's Guide for the meaning of report flags and comments. Any report containing flags and/or comments should be addressed according to your laboratory's procedure manual and not reported. A system malfunction may exist if the following 5-test precision is observed:

HB1C Concentration %	S.D. %
5.5	>0.2
9.6	>0.3

As with any other laboratory procedures, a large discrepancy between the clinical impression and the tests results usually warrants investigation. Some of the following test limitations should be considered.

Any cause of shortened red cell survival will reduce the exposure of red cells to glucose with a consequent decrease in HB1C values, e.g. hemolytic anemia or other hemolytic diseases, pregnancy, recent significant blood loss, etc. Results of HbA1c are not reliable in patients with chronic blood loss and consequent variable erythrocyte lifespan.

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 HB1C method will measure any glycosylated hemoglobin variants that are glycated at eh the beta-chain N-terminus and have epitopes identical to that of HbA1c. This includes HbS, HbC, HbD and HbE. Other hemoglobinopathies my give incorrect results with this test. Care must be taken when interpreting any HbA1c result from patient with Hb Variants.

Reporting:

report format: reported at %

Expected Values

4.5 – 6.2 % HbA1c [24 – 43 mmol/mol]

This range is consistent with the recommended limit (4.0 – 6.0%) from the American Diabetes Association (ADA) for a non-diabetic population. Hemoglobin A1c values in healthy individuals may vary across reference populations.
Elevated levels of HbA1c suggest the need for more aggressive treatment of glycemia. The American Diabetes Association recommends that a primary goal of therapy should be a HbA1c of <7% and that physicians should reevaluate the treatment regimen in patients with HbA1c values consistently >8%.

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

Reference: Siemens Dimension HB1C REF DF105A product insert. 2015-3-9

Origination Date: 9-19-16
Implementation Date: 11-1-16
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Written By: _Lori Murray, MT(ASCP)_____ Date: _9-22-16

Approved By: __Aaron Shives, MD___ Date: __10/23/2017___
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
10/17/17	Heather Hall	Added to Risk and Safety, Modified Backup process