HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Chemistry)		
Document:	Internal Policy and Procedure		
Title:	Analysis of Haemoglobin A1c Level		
Applies To:	All Laboratory Staff		
Preparation Date:	January 06, 2025	**Index No:**	LB-IPP-163
Approval Date:	January 20, 2025	**Version :**	2
Effective Date:	February 20, 2025	**Replacement No.:**	LB-IPP-163(1)
Review Date:	February 20, 2028	**No. of Pages:**	04

1. PURPOSE:

1.1 The purpose of this policy & procedure is to provide all information related to the analysis of Haemoglobin A1c level in blood on Dimension machines.

2. DEFINITONS:

2.1 HbA1c is formed in two steps by the non-enzymatic reaction of glucose with the N -terminal amino group of the 13 -chain of normal adult Hb (HbA).

3. POLICY:

3.1 This policy provides instructions for performing the quantitative determination of HbA1C in patient blood on Dimension machines.

3.2 Haemoglobin (Hb) consists of four protein subunits, each containing a haem moiety, and is the red-pigmented protein located in the erythrocytes. Its main function is the transport of oxygen and carbon dioxide in blood. Each Hb molecule can bind four oxygen molecules. Hb consists of a variety of sub fractions and derivatives. Among this heterogeneous group of haemoglobins HbA1C is one of the glycated haemoglobins, a sub fraction formed by the attachment of various sugars to the Hb molecule. HbA1C is formed in two steps by the non-enzymatic reaction of glucose with the N -terminal amino group of the 8 -chain of normal adult Hb (HbA). The first step is reversible and yields labile HbA1C. This is rearranged to form stable HbA1C in a second reaction step.

3.3 Haemoglobin A1C increased in untreated or poorly controlled diabetes, lead toxicity, alcoholism and iron deficiency anaemia and decreased in haemolyticanaemia, decreased RBCs survival, pregnancy, acute or chronic blood loss, chronic renal failure, insulinoma, congenital spherocytosis and HbS, HbC, HbD disease

4. PROCEDURE:

4.1 **Specimen:**

- 4.1.1 Type:
  - 4.1.1.1 Blood
- 4.1.2 Tube Type:
  - 4.1.2.1 EDTA Tube
- 4.1.3 Amount Required:
  - 4.1.3.1 2.0 to 3.0 ml
- 4.1.4 Delivery Arrangements:
  - 4.1.4.1 Sample to be delivered to the lab as soon as possible.
- 4.1.5 Temperature Restrictions:
  - 4.1.5.1 At room temperature
- 4.1.6 Unacceptable Specimen:
  - 4.1.6.1 See sample rejection criteria policy

4.1.7 Specimen Retention:

4.1.7.1 Period of retention: up to one week after separation of the sample.

4.1.7.2 Storage condition: store at 2-8C

4.1.8 Safety Precaution:

4.1.8.1 Treat all samples material as infectious and handled in accordance with the OHSA standard on blood borne pathogens.

4.2 **Principle:**

4.2.1 Whole blood + lysing agent —›Released haemoglobin —› Haemoglobin derivative

4.2.2 The rate of this reaction is measured turbid imetrically at 340 nm and blanked at 700 inversely proportional to the concentration of HbA1C in the sample.

4.3 **Calibration:**

4.3.1 Calibration is stable approximately 30 days and required with each change in reagent lot number. Calibration curve with at least two levels of controls according to the established Quality requirements for your laboratory. Calibration must be done when:

4.3.1.1 A complete change of reagents that affects the range used to report patient results or QC value

4.3.1.2 A reagent kit with new lot number is used

4.3.1.3 A new assay file that requires a calibration is installed

4.3.1.4 QC fails to meet the established criteria

4.3.1.5 After major maintenance or service

4.3.1.6 When recommended by the manufacturer

4.3.1.7 Documentation accompanying a new version of an existing file states calibration is required

4.3.1.8 At least every 6 months

4.3.2 Calibrator retention:

4.3.2.1 48 hours at 2-8 °C and 2 months at -15°C to -25° C

4.3.3 Calibration Procedure:

4.3.3.1 Calibration is performed by running Distilled Water and 3 levels of HB1C CAL for machine

4.3.3.2 Verify that the correct calibrator values have been entered into the calibration file. For details refer to Operator Guide of DimensionEXL200.

4.3.3.3 Allow calibrator to come to room temperature.

4.3.3.4 Mix bottle 10 times by inversion.

4.3.3.5 Open the bottle, place a minimum of 300 ul of each level in separate sample cup, and place on the assigned positions.

4.3.3.6 Cap the bottle tightly and store at 2-8°C. Immediately after use.

4.3.3.7 Perform calibration as indicated in Operator Guide of DimensionEXL200 and SynchronDXC600.

4.3.4 Calibration Expected Values:

4.3.4.1 Refer to HB1C CAL for Dimension

4.4 **Quality Control:**

4.4.1 Normal and pathological control. Run before running the samples

4.4.2 If more frequent control monitoring is required, follow the established quality control procedures for your laboratory

4.4.3 If quality control results do not fall within an acceptable range defined by your laboratory, patient values may be affected, and corrective action should be taken

4.4.4 Quality Control retention:

4.4.4.1 Unopened control vial is stable up to expiry date printed on the label

4.4.4.2 Opened control vial is stable for 7 days after reconstituting and tightly capped at 2-8°C.

4.4.5 QC Procedure:

4.4.5.1 Verify that the correct QC values have been entered into the QC file. For details refer to Operator Guide of Dimension

4.4.5.2 Allow QC to come to room temperature.

4.4.5.3 Gently remove the stopper to avoid loss of the lyophilized pellet and add exactly 5.0 ml distilled or de-ionized water.

4.4.5.4 Leave to stand for 20 minutes

4.4.5.5 Mix bottle several times by inversion to allow homogeneity
4.4.5.6 Gently invert just prior to use. Avoid foaming
4.4.5.7 Open bottle, place a minimum of 1000 ul of each level in separate sample cup, and place on the assigned positions.
4.4.5.8 Cap bottle tightly and store at 2-8°C. Immediately after use.
4.4.5.9 Perform QC as indicated in Operator Guide of DimensionEXL200 and SynchronDXC600 machines.

4.4.6 QC Expected Values:
4.4.6.1 Refer to the BioradLyphochek diabetes controls value sheet for Dimension

4.5 **Method:**
4.5.1 See policy of loading sample on machine (Ref: Operative Manuals' of DimensionEXL200)

4.6 **Calculation:**
4.6.1 Instrument system automatically calculates the Analytic activity and gives results in the form of print outs.

4.7 **Status:**
4.7.1 Routine

4.8 **Reference range:**
4.8.1 4.3-6 %

4.9 **Dilution Information:**
4.9.1 Specimens with values exceeding the linearity range are flagged and may be diluted with manual dilution. Manual Dilution should be performed as follows:
4.9.1.1 Use saline (0.85% to 0.90%) to dilute the sample.
4.9.1.2 The operator must enter the dilution factor in the patient order screen.
4.9.1.3 The system uses this dilution factor to automatically correct the concentration by multiplying the result by the entered factor.
4.9.1.4 If the operator does not enter the dilution factor, the result must be multiplied by the appropriate dilution factor before reporting the result.
4.9.1.5 If a diluted sample result generates a Linear Low (LL) result error code, do not report the result. Prepare an appropriate dilution/concentration and rerun.

5. MATERIALS AND EQUIPMENT:

5.1 **Reagent:**
5.1.1 HB1C flex contains 6 wells, mixing and dilution are automatically performed by the Dimension system, Estimated test per cassette, 20 tests.

Analytical Range: Hb 5.0 — 25.0 g/dL HbAlc 0.3 —2.6 g/dL Calculated haemoglobin Ratio 3.6 — 16.0 %

5.2 **Regents retention:**
5.2.1 The unopened reagents are stable until the expiration date when stored at 2-8°C. Reagent stability is 30 days if the reagent is unopened and for 5 days if the reagent is opened wells (1-2) and (5-6) and well 3 for 10 days.

5.3 **Calibrator for Dimension** (HB1C CAL)

5.4 **Control Materials:** BIORAD LYPHOCHEK DIABTES CONTROL
5.4.1 Level 1
5.4.2 Level 2

5.5 Dimension Sample Cups for Dimension machine

5.6 Disposable plastic transfer pipettes

6. RESPONSIBILITIES:

6.1 Chemistry shift on charge is responsible for, running calibration and control and samples of HBA1C

6.2 Chemistry staff are responsible for running HBA1C samples all over the day

7. APPENDICES:

7.1 N/A

8. REFERENCES:

8.1 Tietze Text Book of clinical chemistry and molecular diagnostics 4th Edition,2006
8.2 Company Leaflets of reagents

9. APPROVALS:

	Name	Title	Signature	Date
Prepared by:	Dr. Talal Abdelgawad	Clinical Pathologist		January 06, 2025
Reviewed by:	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 13, 2025
Reviewed by:	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 13, 2025
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 13, 2025
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 13, 2025
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 20, 2025