HbA1c Direct with Calibrator
(Latex Turbidimetric Method)

Code	Product Name	Pack Size
SE001A	HbA1c Direct with Calibrator	40 ML
SE001B	HbA1c Direct with Calibrator	80 ML
SE001C	HbA1c Direct with Calibrator	5 LTR

Intended Use
For the quantitative determination of Hemoglobin A1c (HbA1c) in human blood. The determination of HbA1c is most commonly performed for the evaluation of glycemic control in diabetes mellitus. HbA1c values provide an indication of glucose levels over the preceding 4-8 weeks. A higher HbA1c value indicates poorer glycemic control. For in vitro diagnostic use only.

Summary and Explanation of Test
Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the adduction of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. In a classical study, Trivelli et al[1] showed Hemoglobin A1c in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that Hemoglobin A1c serve as an indicator of metabolic control of the diabetic, since Hemoglobin A1c levels approach normal values for diabetics in metabolic control.[2, 3, 4]
Hemoglobin A1c has been defined operationally as the "Fast Fraction" hemoglobins (HbA1a, A1b, A1c) that elute first during column chromatography with cation-exchage resins. The non-glycosylated hemoglobin which consists of the bulk of the hemoglobin has been designated HbA0. The present procedure utilizes a antigen and antibody reaction to directly determine the concentration of the HbA1c.

Principle
This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (R2), latex-HbA1c-mouse antihuman HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

Contents
Reagent 1 : Latex Reagent
Reagent 2 : Antibody Reagent.
Reagent 3 : Hemolysis Reagent
Reagent 4 : Calibrator
[4 Level Lyophilized 4x1.0ml]

Reagent Storage
Store all reagents refrigerated at 2-8°C.

Reagents Preparation
R1, R2 and Hemolysis reagents are supplied as ready to use liquids, Mix gently before use.

Calibrator : 1. Reconstitute the Calibrator with 1.0ml distilled water
2. Mix Thoroughly by gentle vortexing for 30 seconds .
3. Stand for 60 minutes at room temperature and protect from light. Meanwhile ,shake and flip over the brownbottle gently to ensure the content is fully dissolved .aviod air bubbles.
4. take 10 µL of Reconstituted calibrator for testing directly.

Stable for 30 days at 2-8°C. **Do Not Freeze**

Reagent Deterioration
Alterations in the physical appearance of the reagents or values of control materials outside of the manufacture's acceptable range may be an indication of reagent instability.

Specimen Collection and Preparation
Special preparation of the patient is unnecessary. Fasting specimens are not required. No special additives or preservatives other than anticoagulants are required. Collect venous blood with EDTA using aseptic technique. All human specimens should be regarded as potentially biohazardous. Therefore, universal precautions should be used in specimen handling (gloves, lab garments, avoid aerosol production, etc.).

To determine HbA1c, a hemolysate must be prepared for each sample :
1. Dispense 1ml Hemolysis Reagent into tubes labeled : Samples
Note : Plastic or glass tubes of appropriate size are acceptable.

2. Place 20ul of well mixed whole blood into the appropriately labeled lyse reagent tube, Mix.

3. Allow to stand for 5 minutes or until complete lysis is evident. Hemolysates may be stored up to 10 days at 2-8°C.

Instruments
Refer to specific instrument application for suggested settings.

Precautions
1. This reagent is for in vitro diagnostic use only.
2. Not for internal or external use in humans or animals.

Storage and Stability
1. All reagents are stable to the expiration date stated on the labels. Do not use the reagents past their expiration date.
2. Hemoglobin A1c in whole blood collected with EDTA is stable for one week at 2-8°C.[5]

GENERAL SYSTEM PARAMETER

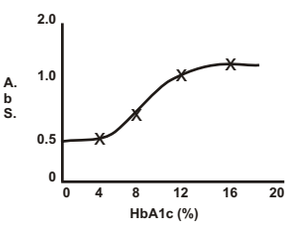
Mode of reaction	End point
Slope of reaction	Increasing
Wavelength	660nm (600-660nm)
Temperature	37°C
Calibrator Concentration	Refer Kit Insert
Linearity	16%
Blank	Reagent blank
Sample volume	10 µL
Reagent 1 Volume	360 µL
Reagent 2 Volume	120 µL
Light Path	1 cm

PROCEDURE

	Blank	Calibrator	Sample/control
Latex Reagent	360 µL	360 µL	360 µL
Calibrator	-	10 µL	-
Hemolysate (sample)	-	-	10 µL
Mix & Incubate for 5 min at 37°C.			
Antibody Reagent	120 µL	120 µL	120 µL

Mix and incubate for 5 min at 37°C and read absorbance(A) at **660 nm**.

Calculation
Calibration curve
Calculate the Abs of calibrators = Abs calibrator – Abs Blank.
Plot the Δ Abs of each calibrator verus assigned concentration (HbA1c %) on a linear graph paper. HbA1c results according to NGSP for the samples and controls are determined using the prepared calibration curve.
An example curve is illustrated below.



Calculate
Δ Abs of sample i.e. abs sample - abs blank
HbA1c % in the sample is calculated by interpolation of Abs of sample on the calibration curve. For calculation of results according to IFCC, use IFCC calibrator values (see calibrator insert), or use following equation.
NGSP = (0.915 x IFCC) + 2.15

Interferences & Limitations
1. Bilrubin to 50mg/dL, ascorbic acid to 50mg/dl, triglycerides to 2000 mg/dl, Carbamylated Hb to 7.5 mmol/L and acetylated Hb to 5.0 mmol/L do not interfere in this assay.
2. It has been reported that results may be inconsistent in patients who have the following conditions : opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin.[6, 7, 8, 9]
3. It has been reported that elevated levels of HbF may lead to underestimation of HbA1c.[10] Also, it has been reported that labile intermediates (Schiff base) are not detected and do not interfere with HbA1c determination by immunoassay.[5]
4. It has been determined that Hemoglobin varients HbA2, HbC and HbS do not interfere with this method.
5. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

6. Patient specimens should always be assayed using a calibration curve.

Expected Values[11]

Recommended values: less than 6% for a non-diabetic,less than 7% for glycemic control of a person with diabetes.

Each laboratory should establish its own expected values. In using hemoglobin A1c to monitor diabetic patients ,results should be interpreted individually. That is, the patient should be monitored against him or herself. There is a 3-4 week time lag before Hemoglobin A1c reflects changes in blood glucose level.

Linearity

This Procedure linear up to 16% (NGSP)(Up to maximaim value of Calibrator)

Performance

1. Linearity : The Hemoglobin A1c assay range is 4.0% - 16.0%
2. Comparison : A study using 40 human specimens between this Hemoglobin A1c procedure and an automated HPLC procedure (Tosoh) yielded a correlation coefficient of 0.988 and a linear regression equation of y = 1.050x - 0.481. (syx = 0.332)
3. Precision :

   Within Run : The within run precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

Level	Mean	Std. Dev.	% C.V.
Low	5.48	0.078	1.43
High	10.28	0.176	1.72

   Day to Day : The between day precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

Level	Mean	Std. Dev.	% C.V.
Low	5.48	0.152	2.77
High	10.28	0.275	2.68

4. Sensitivity : Sensitivity was investigated by reading the change in absorbance at 660 mm for a saline sample and a whole blood sample with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used (Hitachi 717), the HbA1c reagent showed little or no drift on the zero sample. Under the reaction conditions described, a 0.073 absorbance change is approximately equivalent to 1.0% HbA1c.

Reference :

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11. american Diabetes Association: Clinical practice recommendations (Position statement). Diabetes care 24 (Suppl. 1):S33-S55,(2001).

SYMBOLS USED ON LABELS

REF Catalogue Number | Manufacturer | See Instruction for Use

LOT Lot Number | CONT Content | Storage Temperature

Expiry Date | IVD In Vitro Diagnostics

BEA/24/HBA/SE/IFU-04 12/07/2023

ASSIGNED VALUES ACCORDING TO NGSP (%)

Lot No. :

INSTRUMENT	CALIBRATOR 1(%)	CALIBRATOR 2(%)	CALIBRATOR 3(%)	CALIBRATOR 4(%)
Vchem Plus/ Turbicon Plus/ DS 140/DS 302				