

Hemoglobin A1c

For the Quantitative Determination of Human Hemoglobin A1c in Blood

Cat. No. KAI-196 / KAI-197

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.

INTRODUCTION AND SUMMARY

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 (HbA_{1a}, A_{1b}, A_{1c}) that elute first during column chromatography with cation-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbA₀. The present procedure utilizes an antigen and antibody reaction to directly determine the concentration of the HbA1c.

PRINCIPLE OF TEST

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 anti-human HbA1c monoclonal antibody is added (R2), latex-HbA1c-mouse anti-human 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.

KIT COMPOSITION

Reagents (Liquid Stable)

KAI-196, Hemoglobin A1c

R1: 30 mL Latex 0.13%, buffer, stabilizer.
R2: 10 mL Mouse anti-human HbA1c monoclonal antibody
0.05 mg/mL, goat anti-mouse IgG polyclonal antibody
0.08 mg/dL, buffer, stabilizers.
Hemolysis reagent: 125 mL Water and stabilizers.

KAI-197, Hemoglobin A1c (L)

R1: 90 mL Latex 0.13%, buffer, stabilizer.
R2: 30 mL Mouse anti-human HbA1c monoclonal antibody
0.05 mg/mL, goat anti-mouse IgG polyclonal antibody
0.08 mg/dL, buffer, stabilizers.
Hemolysis reagent: 500 mL Water and stabilizers.

WARNINGS AND PRECAUTIONS

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

REAGENT PREPARATION

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

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. R1 and R2 are stable for at least one month after opening when stored at 2-8°C.
3. Hemoglobin A1c in whole blood collected with EDTA is stable for one week at 2-8°C.⁵
4. Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability.

INSTRUMENT

Refer to specific instrument application for suggested settings.

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 1 mL Hemolysis Reagent into tubes labeled: Control, Patients, etc. Note: Plastic or glass tubes of appropriate size are acceptable.
2. Place 20 µL 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.

PROCEDURE

Materials Supplied

Refer to "KIT COMPOSITION"

Materials Required But Not Supplied

1. Pipettes to dispense 20 µL and 1 mL and Test Tubes to hold 1.02 mL.
2. Hemoglobin A1c calibrator set (cat. no. KAI-098C) and Hemoglobin A1c control set (cat. no. K29C-4M).

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Hitachi 717
TEMPERATURE	37°C
TEST	(HbA1c)
ASSAY CODE	(1 POINT) : (50) - (0)
SAMPLE VOLUME	(5) (3)
R1 VOLUME	(180) (50) (NO)
R2 VOLUME	(60) (20) (NO)
WAVELENGTH	() (660)
CALIB. METHOD	(NONLINEAR) (4) (5)
STD.(1) Conc.-POS.	(0.0) - (1)
STD.(2) Conc.-POS.	(*2) - (2)
STD.(3) Conc.-POS.	(*3) - (3)
STD.(4) Conc.-POS.	(*4) - (4)
STD.(5) Conc.-POS.	(*5) - (5)
STD.(6) Conc.-POS.	() - ()
SD LIMIT	(999)
DUPLICATE LIMIT	(1000)
SENSITIVITY LIMIT	(0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(0) (LOWER)
EXPECTED VALUE	(-99999) (99999)
PANIC VALUE	(-99999) (99999)
INSTRUMENT FACTOR	(1.0)

Use isotonic saline as STD (1)

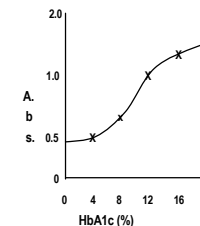
* 2-5 Input concentration of calibrators

Parameters for other automated analyzers are available.

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CALIBRATION AND CALCULATIONS

HbA1c results for the unknowns and controls are determined using the prepared calibration curve. An example curve is illustrated below:



QUALITY CONTROL

The reliability of test results should be monitored whenever patient samples are assayed using a standard and quality control materials analyzed in the same manner employed for the unknowns. We suggest the use of commercially available Hemoglobin A1c controls with an assayed range. If controls do not fall into the assayed range, patient values from that run should not be reported. The run should be repeated, making sure that all mixing and handling instructions are strictly followed.

Linearity of the assay should be verified with a commercial linearity check set, or dilutions of a high specimen, at least every six months.

LIMITATIONS OF PROCEDURE

1. This assay should not be used for the diagnosis of diabetes mellitus.
2. Patient specimens should always be assayed using a calibration curve.
3. 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}
4. It has been reported that elevated levels of HbF may lead to underestimation of HA1c and, that uremia does not interfere with HbA1c determination by immunoassay.¹⁰ It has been reported that labile intermediates (Schiff base) are not detected and therefore, do not interfere with HbA1c determination by immunoassay.⁵
5. It has determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
6. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

PERFORMANCE

Sensitivity

Sensitivity was investigated by reading the change in absorbance at 660 nm 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 0.056 absorbance change is approximately equivalent to 1.0% HbA1c.

Precision

Within Run: The within run precision was established by assaying blood samples following NCCLS protocol EP5 on a Hitachi 717.

	Sample 1	Sample 2	Sample 3
N	20	20	20
Mean (%)	5.97	8.49	12.21
S.D.	0.138	0.072	0.152
C.V.	2.31 %	0.85 %	1.24 %

Day to Day: The between day precision was established by assaying blood samples following NCCLS protocol EP5 on a Hitachi 717.

	Sample 1	Sample 2	Sample 3
N	20	20	20
Mean (%)	5.95	8.34	12.15
S.D.	0.190	0.093	0.179
C.V.	3.19 %	1.12 %	1.47 %

Method Comparison / Correlation

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 $y = 0.983x + 0.140$. ($Syx = 0.230$)

Linearity

The Hemoglobin A1c assay range is 2.0-16.0%.

INTERFERENCE

1. Bilirubin to 50 mg/dL, ascorbic acid to 50 mg/dL, triglycerides to 2,000 mg/dL, carbamylated Hb to 7.5 mmol/L and acetylated Hb to 5.0 mmol/L do not interfere with 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 HA1c.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 variants HbA2, HbC and HbS do not interfere with this method.
5. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

EXPECTED VALUES¹¹











Recommended Values: less than 6% for a non-diabetic, less than 7% for glyceric 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.

REFERENCES

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11. American Diabetes Association: Clinical Practice Recommendations (Position Statement). Diabetes Care 24 (Suppl. 1): S33-S55 (2001).

LABELING SYMBOLS

	Lot Number
	Reagent
	Expiration or "Use By" Date
	Catalog Number
	For <i>In Vitro</i> Diagnostic Use
	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use
	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE



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