

# Hemoglobin A1C

## 1.0 INTENDED USE

This reagent is intended 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.

## 2.0 BACKGROUND

### 2.1 METHOD AND HISTORY

Throughout the circulatory life of the red cell, HbA1c 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, (10.1) showed HbA1c in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended HbA1c serve as an indicator of metabolic control of the diabetic, since HbA1c levels approach normal levels for diabetics in metabolic control. (10.2,10.3,10.4) HbA1c has been defined operationally as the fast fraction hemoglobins (HbA<sub>1a</sub>, A<sub>1b</sub>, A<sub>1c</sub>) 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 HbA<sub>0</sub>. The present procedure utilizes an antigen and antibody reaction to directly determine the concentration of HbA1c.

### 2.2 TEST PRINCIPLE

This method utilizes the interaction of antigen and antibody to directly determine HbA1c in whole blood. Total hemoglobin and HbA1c have the same specific 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 the latex particles. The amount of agglutination is measured as absorbance. The HbA1c valued is obtained from a calibration curve.

## 3.0 SPECIMEN COLLECTION AND HANDLING

### 3.1 PATIENT PREPARATION

No special patient preparation is required. Fasting specimens are not required.

### 3.2 SPECIMEN COLLECTION.

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.).

### 3.3 SPECIMEN STORAGE

HbA1c in whole blood collected with EDTA is stable for one week at 2-8°C. (10.5)

Interferences: Bilirubin to 50 mg/dl, ascorbic acid to 50 mg/dl, triglycerides to 2000 mg/dl, carbamylated Hb to 7.5 mmol/L and acetylated Hb to 5.0 mmol/L do not interfere with this assay. See also the LIMITATION SECTION (6.2).

## 4.0 MATERIALS

Catalog No 78050 (40 ml)

Reagents necessary for the determination of HbA1c are included in the kit.

### 4.1 REAGENT

4.1.1 Reagent 1 (R1): Latex 0.13%, glycine buffer 20 mmol/L.

4.1.2 Reagent 2a (R2a): Glycine buffer 80 mmol/L

4.1.3 Reagent 2b (R2b): Mouse anti-human AbA1c monoclonal antibody 0.05 mg/ml, goat antimouse IgG polyclonal antibody 0.08 mg/dl, stabilizers.

4.1.4 Hemolysis Reagent (Lyse): water and stabilizers.

### 4.2 WARNINGS AND PRECAUTIONS

4.2.1 This reagent kit is intended for in vitro diagnostic use only.

4.2.2 Reagent is not for internal or external use in humans or animals.

### 4.3 REAGENT PREPARATION

R1 and the hemolysis reagent are ready to use as is. R2 is prepared by pouring the entire contents of R2b vial into the R2a vial. Mix gently

### 4.4 REAGENT STORAGE AND STABILITY

4.4.1 Store reagent at 2-8°C.

4.4.2 Unopened reagent is stable until the expiration date.

4.4.3 R1 and R2 are stable for at least one month after opening when store at 2-8°C.

## 4.5 REAGENT DETERIORATION

4.5.1 Alterations in the physical appearance of the reagent or values of control materials outside of the acceptable range may be an indication of reagent instability.

## 4.6 ADDITIONAL MATERIALS REQUIRED

4.6.1 Spectrophotometer

4.6.2 10µl and 1ml pipettes.

4.6.3 HbA1c calibrator

4.6.4 HbA1c controls

## 5.0 TEST PROCEDURE

The following is a procedure for use on Hitachi 717. Call Biotron for specific instrument applications. (Hitachi 717 is a registered trademark of Nissei Sangyo Co., Ltd, Japan)

### 5.1 HEMOLYSATE PREPARATION

A hemolysate must be prepared for each sample.

5.1.1 Dispense 1 ml of hemolysis reagent into tubes labeled: Control, Patient, Calibrator, etc. (Plastic or glass tubes of appropriate sizes are acceptable.)

5.1.2 Dispense 10µl of controls or calibrators into the appropriately labeled lyse reagent tube. Mix

5.1.3 Centrifuge whole blood specimens at 2000rpm for 2 minutes and place 10µl of packed cells into the appropriately labeled lyse reagent tube. Mix.

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

### 5.2 PROCEDURE CONDITIONS

TEST NAME	HbA1c
ASSAY CODE	[1-POINT]:[50]-[0]
SAMPLE VOLUME	[7] [3]
R1 VOLUME	[240] [50] [NO]
R2 VOLUME	[80] [20] [NO]
WAVELENGTH	[ ] [660]
CALIBRATION	[NONLINEAR] [4] [5]
STD(1) CONC-POS (saline)	[0.0] [1]
STD(2) CONC-POS	[user def d] [2]
STD(3) CONC-POS	[user def d] [3]
STD(4) CONC-POS	[user def d] [4]
STD(5) CONC-POS	[user def d] [5]
SD LIMIT	[999]
DUPLICATE LIMIT	[1000]
SENSITIVITYLIMIT	[0]
ABS LIMIT (INC/DEC)	[32000] [INCREASE]
PROZONE LIMIT	[-] [-]
EXPECTED VALUE	[-] [-]
PANIC VALUE	[-] [-]
INSTRUMENT FACTOR	[1.0]

### 5.3 CALCULATION AND RESULTS

Results for the samples are calculated by referencing the absorbance of the sample with the calibration curve.

## 6.0 INTERPRETATION OF RESULTS

### 6.1 EXPECTED VALUES (10.11)

Recommended values:

Less than 6% for non-diabetic

Less than 7% for glycemic control of a person with diabetes

These values are suggested guidelines. Each laboratory should establish its own expected values. In using HbA1c 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 lag time before HbA1c reflects changes in blood sugar level.

### 6.2 LIMITATIONS OF PROCEDURE

6.2.1 This assay should not be used for the diagnosis of diabetes mellitus.

6.2.2 Specimens should always be assayed using a calibration curve.

6.2.3 It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead-poisoning, uremia (carbamylated Hb), alcoholism, ingest large doses of aspirin. (10.6,10.7,10.8,10.9)

6.2.4 It has been reported that elevated levels of HbF may lead to underestimation of HbA1c and, that uremia does not interfere with HbA1c determination by immunoassay. (10.10)

6.2.5 It has been reported that hemoglobin variants HbS and HbA2 are not detected by immunoassay, leading to possible inaccurate determination. Also it has been reported that labile intermediates (Schiff base) are not

detected and therefore do not interfere with HbA1c determination by immunoassay.

6.2.6 Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

## 7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Biotron's HbA1c controls can be used to monitor the daily acceptable variations. These controls should be assayed daily with the run of patient samples, whenever a new reagent lot is used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

## 8.0 CALIBRATION PROCEDURES

Use Biotron HbA1c calibrator set . Use saline for 0. The calibration is stable for 7 days.

The assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrators. Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

## 9.0 PERFORMANCE CHARACTERISTICS

### 9.1 PRECISION

Within-Run: The intra assay precision was established by assaying bloods with 3 levels of HbA1c twenty times each.

<u>Mean (%)</u>	<u>SD (%)</u>	<u>CV (%)</u>
4.76	0.06	1.26
7.29	0.08	1.10
10.9	0.168	1.47

Between Run: The inter run precision was established by assaying bloods with 3 levels of HbA1c for ten runs conducted over a five day period.

<u>Mean (%)</u>	<u>SD (%)</u>	<u>CV (%)</u>
4.72	0.06	1.27
7.36	0.08	1.09
11.0	0.17	1.55

### 9.2 CORRELATION

A study on human specimens between this HbA1c procedure and a another immunoassay procedure (Roche Diagnostics) yielded a correlation coefficient of 0.995 and a linear regression equation of  $y=1.05x-0.36$ .(n=45).

### 9.3 LINEARITY

The HbA1c assay range is 2.0% to 16.0%

### 9.4 SENSITIVITY

This HbA1c procedure has an approximate 0.073 absorbance change for 1.0% of HbA1c under the conditions as described in the PROCEDURE CONDITIONS (5.2) section. Saline showed little or no drift.

## 10.0 REFERENCES

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