Enzymatic HbA1c

Enzymatic assay for the quantitative direct determination of Glycated HbA1c in human whole blood

CE IVD

ORDER INFORMATION

REF	Kit size
GD5418 00	1x21 + 1x9 + 1x13 + 1x75 ml
KL5418 00	1x21 + 1x9 + 1x13 + 1x75 ml

INDICATION

Glycohemoglobin is produced by non-enzymatic addition of glucose to amino groups in hemoglobin. HbA1c refers to glucose-modified hemoglobin A (HbA) specifically at N-terminal valine residues of hemoglobin beta chains.
This process reflects the average exposure of hemoglobin to glucose over an extended period.
In diabetic subjects hemoglobin A1c fraction is found 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; in fact HbA1c is a good indicator of glycemic control in the preceding 2-3 months. HbA1c test is used both as an index of mean glycemia and as measure of risk for the development of diabetes complications[1-4].
Currently, the HbA1c is recommended for patients with diabetes every 2-3 months as part of the patient Diabetes management program.

PRINCIPLE

Lysed whole blood samples are subjected to extensive protease digestion with Bacillus *sp* protease. This process releases amino acids including glycated valines from the hemoglobin beta chains. Glycated valines then serve as substrates for specific recombinant fructosyl valine oxidase (FVO) enzyme, produced in *E. coli*. The recombinant FVO specifically cleaves N-terminal valines and produces hydrogen peroxidase (H_2O_2). This, in turn, is measured using horseradish peroxidase (POD) catalyzed reaction and suitable chromogen. No separate measurement for total Hemoglobin (Hb) is needed in this direct assay.

COMPOSITION

REAGENT A1 (RA1):

MES buffer, pH 7.0	5 mmol/l
Protease	4 KU/ml
Triton-X-100	0.5%
Redox agents	>10 µmol/l

REAGENT A2 (RA2):

MES buffer, pH 6.3	1 mmol/l
Redox agents	<3 µmol/l

REAGENT B (RB):

Tris buffer, pH 8.0	15 mmol/l
FVO enzyme	>10 U/ml
POD	90 U/ml
Chromogenic substrate	0.8 mmol/l

REAGENT C (RC):
(Hemolysis reagent)

CHES buffer, pH 8.7	100 mmol/l
Triton-X-100	1%
SDS	0.45%
Redox agents	0.5 mmol/l

BLANKING SOLUTION (CAL 0):
(Blanking Solution)
To be used only if the analyzer require the reagent blank.

REAGENTS PREPARATION

Enzymatic HbA1c reagents are supplied as liquid ready to use reagents:

- for analyzers capable of handling 3 reagents, RA1, RA2 and RB are ready to use and used as R1, R2 and R3;
- for analyzers capable of handling only 2 reagents, reagents A1 and A2 should be mixed in a 7:3 ratio (e.g. 21 ml of RA1 + 9 ml of RA2) to form R1, while Reagent B is used as R2.

Storage and stability

All reagents are stable up to expiry date stated on the label, if stored at 2-8 °C.
Working solution prepared by mixing RA1 with RA2 (R1 on automatic analyzers) is stable one month at 2-8 °C.
RA1, RA2 and RB are light sensitive: protect from direct sunlight.

REAGENT DETERIORATION

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.

AUXILIARY MATERIAL

- 20 to 500 µl variable volume pipettes
- Sample cups
- HbA1c Enzymatic Calibrator, REF GD5420 00
- HbA1c Controls, REF GD5422 00
- Spectrophotometer capable to read absorbances at 700 nm
- Reaction microcuvettes (500 µl reading capacity)

SPECIMEN

Anti coagulated whole blood.
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 infectious.
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A37.[12]

Stability

Hemoglobin A1c in whole blood collected with EDTA is stable for one week at 2-8 °C. [5] Do not freeze samples! This may lead to a natural hemolysis. If necessary, store lysed samples at -20 °C instead of whole blood.

INTERNAL 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.

Control material

Minias Globe Diagnostics Enzymatic HbA1c assay is based upon redox and enzymatic reaction. Some commercial artificially-made control materials contain unbalanced redox component and artificially-glycated hemoglobin that are not suitable for testing. These controls may gain abnormally higher results. Please check the compatibility of the control material prior to use, especially during EQA (External Quality Assessment, VEQ).

PROCEDURE

Hemolysate preparation

1. Dispense 250 µl of hemolysis reagent (RC) into labeled sample cups. Plastic or glass tubes of appropriate size are acceptable.
2. Prior to testing, whole blood samples should be mixed by gentle inversion at least 5 times to resuspend settled erythrocytes.
3. Place 20 µl of well-mixed, fully resuspended whole blood into the appropriately labeled hemolysis reagent tube. Mix well the solution.
4. Allow to stand for 10 minutes or until complete lysis is evident. Complete lysis is observed when the mixture becomes a clear dark red solution without any particulate matter.
5. Mix well again.
6. The calibrators, Blanking Solution (CAL 0) and controls should be treated exactly as patient samples.

Hemolysate stability

Hemolysates may be stored up to 10 days at 2-8 °C, or at -20 °C for longer period (stability is unknown).

Manual Procedure

Allow the reagents to reach working temperature before using.

Pipette into one reaction cuvette for each specimen	
Reagent A1	224 µl
Hemolysate (sample, calibrator or control)	50 µl
Reagent A2	96 µl
Mix and incubate for **5 minutes** at **37 °C.** After 5 minutes of incubation read absorbance A_1 at 700 nm. Then add:	
Reagent B	140 µl
Mix and incubate for **5 minutes** at **37 °C** After 5 minutes of incubation read absorbance A_2 at 700 nm. Calculate $\Delta A = A_2 - A_1$.	

Instrument Procedure

Please refer to the specific analyzers information sheet for applications parameters.

On Board Stability

Stability of open vials on board of most common automatic analyzers is 4 weeks at 2-8 °C.
Stability of Reagent 1 (mixed RA1+RA2) is 4 weeks at 2-8 °C.

Calibration Stability

On most common automatic analyzers calibration is stable up to 3 weeks. This information is given *as is*, and is recommended to verify the stability of the calibration by means of an appropriate quality control program.

Calibration remarks

Minias Globe Diagnostics Enzymatic HbA1c reagents are comprised of redox balanced components. Mixed reagent blank or water as blank sample should not be used in this assay. For the reagent blank use exclusively the Blanking Solution (CAL 0).

CALCULATION OF RESULTS

Plot the ΔA calculated for each Calibrator against its concentration (concentrations are reported in a value sheet).
Results are found by comparing the Sample ΔA against the plotted curve.
A curve fitting system software it is suggested to achieve more precise results.
Depending upon which standardization is used while assessing the calibration, results are expressed accordingly.

To convert results into other Designated Comparison Method (DCM) standardization, apply the following calculations[8-11]:

National DCM	**From IFCC to DCM**
NGSP (USA)	NGSP = 0.09148 IFCC + 2.152
JDS/JSCC (Japan)	JDS = 0.09274 IFCC +1 .724
Mono-S (Sweden)	Mono-S = 0.09890 IFCC + 0.884

National DCM	**From DCM to IFCC**
NGSP (USA)	IFCC = 10.93 NGSP - 23.50
JDS/JSCC (Japan)	IFCC = 10.78 JDS - 18.59
Mono-S (Sweden)	IFCC = 10.11 Mono-S - 8.94

EXPECTED VALUES[5-7]

IFCC / DCM	**Clinical Condition**	
	Upper level of non-diabetic reference range	ADA target for patients with diabetes
IFCC	43 mmol/mol	53 mmol/mol
NGSP	6.1%	7.0%
JDS/JSCC	5.7%	6.6%
Mono-S	5.1%	6.1%

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 weeks time lag before Hemoglobin A1c reflects changes in blood glucose level.

CALIBRATION TRACEABILITY

Please refer to the Enzymatic HbA1c Calibrator (REF GD5420 00) package insert for information about traceability.

ANALYTICAL PERFORMANCES

Precision

The intra assay precision was established by assaying blood with two Hemoglobin A1c levels per NCCLS EP-5 procedure. The results are reported in the following table:

Calculated Precisions	**Level 1 mmol/mol**	**Level 2 mmol/mol**
Mean value	38.8	89.1
Within run SD	0.39	0.62
Within run %CV	1.0%	0.7%
Between run SD	0.70	1.70
Between run %CV	1.8%	1.8%

Reportable Range

Assay reportable range is 20.2 - 107.7 mmol/mol.
Sensitivity is 20.2 mmol/mol.
Linearity is up to 107.7 mmol/mol.

Correlation

A study using 66 human specimens between this Hemoglobin A1c procedure and a reference procedure (HPLC) yielded the following results:
y = 0.9912x – 0.215 mmol/mol r=0.9943

Interferences

1. The following other components at the indicated concentrations do not interfere with this analytical method:
   - Total Bilirubin: 15 mg/dl
   - Conjugated Bilirubin: 13 mg/dl
   - Ascorbic Acid: 12 mg/dl
   - Triglycerides: 4000 mg/dl
   - Glucose: 4000 mg/dl
   - Uric Acid: 30 mg/dl
   - Urea: 80 mg/dl
2. Stable glycated hemoglobin serves as substrate for enzymatic reaction used by the present test. Acetylated, carbamylated and labile HbA1c does not adversely affect the enzymatic reaction used in this assay.

3. Variant hemoglobin S, C and E do not significantly interfere with the assay.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium Azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labeling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended [13].

Waste management

Please refer to local legal requirements.

HITACHI 717 SUGGESTED PARAMETERS

R1: Mix 7 parts of RA1 with 3 parts of RA2
R2: use RB

TEST	[HbA1c]
ASSAY CODE	[2 POINT] : [24]-[40]
SAMPLE VOLUME	[20] [20]
R1 VOLUME	[200] [50] [NO]
R2 VOLUME	[85] [20] [NO]
WAVE LENGHT	[800][700]
CALIB METHOD	[LINEAR] [0] [0]
STD. (1) CONC - POS.	[5.4] [6]
STD. (2) CONC - POS.	[11.4] [7]
STD. (3) CONC - POS.	[0] [0]
STD. (4) CONC - POS.	[0] [0]
STD. (5) CONC - POS.	[0] [0]
STD. (6) CONC - POS.	[0] [0]
SD LIMIT	[32000]
DUPLICATE LIMIT	[500]
SENSITIVITY LIMIT	[0]
ABS. LIMIT (INC/DEC)	[32000] [INCREASE]
PROZONE LIMIT	[0] [LOWER]
EXPECTED VALUE	[4.0] - [6.0]
PANIC VALUE	[3.0] - [8.0]
INSTRUMENT FACTOR	[1.00]

REFERENCES

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12. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999);
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