Automation Hitachi™ 911/912
HbA1c enzymatic

IVD

Enzymatic assay, for the quantitative determination of Hemoglobin A1 fraction in whole blood.

REF		Reagents
1011039E3013HE	187 test auto AUTOMATION	R1-A : 21 mL R1-B : 9 mL R2 : 13 mL R3 : 75 mL

Hitachi 912/911, 717 …… 187 test
Hitachi 704 …… 150 test

1.0 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 al1 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-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbAo. The present procedure utilizes a antigen and antibody reaction to directly determine the concentration of the HbA1c.

2.0 PRINCIPLE

Samples of whole blood lysate were subjected to extensive enzymatic digestion using protease from Bacillus sp.. This process releases amino acids including valine glycated by hemoglobin beta chains. The Valine glycated therefore serves as a substrate for the enzyme recombinant E. Coli fructosil-valine oxidase (FVO). The FVO acts specifically on glycated valine producing hydrogen peroxide (H2O2). This is ultimately measured by the action of peroxidase (POD) and a chromogenic substrate.

The dosage separate total hemoglobin (Hb) is not necessary.

3.0 SPECIMEN COLLECTION

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 .

	Temperature (°C)		
	20-25	4-8	- 20
Serum/plasma	-	2 days	-

4.0 REFERENCE VALUES

HbA1C	
Not diabetic	4.0 – 6.0 %
Target value	7.0 %
Medium diabetic control	6.0 – 8.0 %

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.

5.0 WASTE MANAGENETS

Please refer to local legal requirements.

6.0 REAGENTS

R1-A Liquid ready to use	MES buffer, pH 7.0 5 mmol/l, Protease 4 KU/ml, Triton-X-100 0.5%, Redox agents >10 µmol/l.
R1-B Liquid ready to use	MES buffer, pH 6.3 1 mmol/l, Redox agents <3 µmol/l
R2 Liquid ready to use	Tris buffer, pH 8.0 15 mmol/l, FVO enzyme >10 U/ml, POD 90 U/ml, Chromogenic substrate 0.8 mmol/l
R3 Liquid ready to use	(Hemolysis reagent). CHES buffer, pH 8.7 100 mmol/l, Triton-X-100 1%, SDS 0.45%, Redox agents 0.5 mmol/l
R4 Liquid ready to use	blanking solution (CAL 0). To be used only if the analyzer require the reagent blank.

7.0 PREPARATION AND STABILITY OF REAGENTS

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

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

7.1 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 R1-A with R1-B (R1 on automatic analyzers) is stable one month at 2-8 °C.

R1-A, R1-B and R2 are light sensitive: protect from direct sunlight.

7.2 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.

8.0 PRECAUTIONS IN USE

This reagent is for *in vitro* diagnostic use only.Not for internal or external use in humans or animals.The reagents contain inactive components such as preservatives, 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 labelling 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.

9.0 MATERIAL REQUIRED BUT NOT PROVIDED

1. Pipettes to dispense 20 ul and 1 ml and Test Tubes to hold 1.02 ml.
2. Hemoglobin A1c calibrator set, control set.

10.0 PROCEDURE AUTOMATION . HITACHI 912

TEST :	**[A1C] [*]**
TEST NAME:	**[HBA1C]**
APPLICATION CODE:	**[312]**
UNIT:	**[mmol/mol]**
DATA MODE :	**[ON BOARD]**
REPORT NAME :	**[HBA1C]**
CONTROL INTERVAL :	**[*]**
INSTRUMENT FACTOR (Y=aX+b):	**a[1.0] b[0]**

EXPECT VALUE	**< Serum >**		**< Urine >**
Age	(M)	(F)	
[100] [A]	**[20] - [43]**	**[20] - [43]**	**[0] - [9999]**
[100] [A]	**[0] - [*]**	**[0] - [*]**	
[*] [*]	**[0] - [*]**		

TECHNICAL LIMIT **<Serum>** **[20] - [107]** **< Urine >** **[0] - [99999]**

STD.	Conc	Pos	Sample	Pre.	Dil	Calib	Lot.No	Qualit	No
(1)	[***]	**	[20]	[0]	[0]	[**]	000001	(1) [0]	[ ]
(2)	[***]	**	[20]	[0]	[0]	[**]	000002	(2) [0]	[ ]
(3)	[0]		[0]	[0]	[0]	[ ]	000000	(3) [0]	[ ]
(4)	[0]		[0]	[0]	[0]	[ ]	000000	(4) [0]	[ ]
(5)	[0]		[0]	[0]	[0]	[ ]	000000	(5) [0]	[ ]
(6)	[0]		[0]	[0]	[0]	[ ]	000000	(6) [0]	[ ]

ASSAY CODE :		**[2 POINT-END] [10] [ ]**
WAVELENGHT(2nd /Primary):		**[800] / [700]**
ASSAY POINT :		**[14] – [24] – [0] – [0]**
DILUENT :		**[00301] / [0]**
	<Serum>	<Urine>
S.Vol (Normal)	**[25] [0] [0]**	**[10] [0] [0]**
S.Vol (Decrease)	**[25] [0] [0]**	**[10] [0] [0]**
S.Vol (Increase)	**[25] [0] [0]**	**[10] [0] [0]**
ABS Limit	**[0] [0]**	**[Incr.]**
Prozone Limit	**[0] [0]**	**[Inf./Lower]**
Reagent T1	**[112] [0]**	**[00369] [0]**
Reagent T2	**[48] [0]**	**[00369] [0]**
Reagent T3	**[70] [0]**	**[00369] [0]**
Reagent T4	**[0] [0]**	**[00000] [0]**
Calibration Type	**[Linear] [2]**	**[2] [0] [ ]**
Auto Time out Blank	**[0]**	
SD limit	**[999]**	
Span	**[0]**	
Duplicate limit	**[500]**	
2 point	**[0]**	
Sensitivity limit	**[0]**	
Full	**[0]**	
S1 ABS limit	**[-32000]**	**[32000]**
Compensation Limit	[ ]	

***** *user defined*
****** *STD position on the sample tray*
******* *concentration stated in the package insert of calibrator (Ref. 1011040H)*

11.0 CALCOLATION 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-IFCCtoDCM
NGSP(USA)	NGSP=0.09148IFCC+2.152
JDS/JSCC(Japan)	JDS=0.09274IFCC+1.724
Mono-S(Sweden)	Mono-S=0.09890IFCC+0.884

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

12.0 PERFORMANCE

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

Calculated-Precisions	Level1-mmol/mol	Level2-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%

12.2 Reportable Range. Assay reportable range is 20.2 - 107.7 mmol/mol.
12.3 Sensitivity is 20.2 mmol/mol.
12.4 Linearity is up to 107.7 mmol/mol.
12.5 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
12.6 Interferences . 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
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.Variant hemoglobin S, C and E do not significantlyinterfere with the assay.

13.0 REFERENCES

1. Goldstein, D.E. *et al.*, Diabetes Care. 27(7):1761-73 (2994);
2. United Kingdom Prospective study, Lancet 352:837-53 (1998);
3. The Diabetes Control and Complications Trial Research Group, N. Engl. J. Med. 329:977-86 (1993);
4. Little, R. *et al.*, Clin Chemistry, 47:1985-1992 (2001);
5. American Diabetes Association (ADA). Clinical practice recommendation: standards of medical care for patients with diabetes mellitus. Diab Care 22 (supp): S32-41 (1999);
6. American Diabetes Association (ADA). Clinical Practice recommendation, Diab Care 16S2 (93):10-13 (1992);
7. American Diabetes Association (ADA). Clinical Practice recommendation, Diabetes 42:1555-58 (1993);
8. NGSP, http://www.missouri.edu/
9. Goldstein *et al.*, Clin Chem 32:B64-B70 (1986);
10. Hoelzel, W. *et al.*, IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan and Sweden: a method comparison study. Clin Chem 50:166-74 (2004);
11. Sacks, D (ed). Global Harmonization of Hemoglobin A1c.Clin Chem 51(4):681-83 (2005);
12. NCCLS Document, “Procedures for the collection of arterial blood specimens”, Approved Standard, 3rd Ed. (1999);
13. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

14.0 LEGENDA SIMBOLI – DIRETTIVA 98/79/CE

	Attenzione, consultare le istruzioni per l’uso	Σ	N°determinazioni per kit		Fabbricante
IVD	Solo per uso diagnostico *in vitro*		Usare entro		Non riutilizzare
30°C 2°C	Conservare a 2-30°C	LOT	Numero del lotto	REF	Codice #

MEDIA DIAGNOSTICI s.r.l.
Via Costiera 31 D/E
47100 FORLI (FC)
Telefono 0543 782.786
Fax 0543 782.787
e-mail info@mediagnostic.biz
web http://www.mediagnostic.biz