HbA1c Reagents Type-W

For In Vitro Diagnostic use

■ Intended Use

This test provides a convenient, quantitative assay for measuring concentrations of hemoglobin A1c (HbA1c) in blood. The level of HbA1c is a useful index in controlling blood glucose levels in patients with diabetes. This test is based on latex immunoagglutination where HbA1c in the test sample is absorbed onto latex particles, and then Cross-linked anti-HbA1c is added to form an Antigen-Antibody reaction. Finally the percentage of HbA1c is obtained from the dose response curve.

■ Specification

1. Since concentrations (%) of hemoglobin A1c (HbA1c) can be determined directly from the dose response curve, it is not necessary to measure hemoglobin concentrations of a test sample.
2. Latex agglutination assay makes it suitable for automation.
3. A good correlation with HPLC
4. Not affected by labile hemoglobin A1c, carbamylated hemoglobin or acetylated hemoglobin.

■ Components

Product code	Cat #	Reagent	Form
A1c-F R1W	M0220000900ZZ000	Latex	Liquid
A1c-F R2W	M0220001000ZZ000	Anti-HbA1c Cross linked anti-human hemoglobin A1c mouse monoclonal antibody	Liquid

Associated Reagent

Product code	Cat #
HbA1c Calibrator set	M0220000800ZZ000
HbA1c Control set	M0220000700ZZ000

■ Usage and Dosage

1. Preparation

No.	Reagent	Preparation	Shelf Life
R-1	Latex	Ready for use	Refer to the date printed on the outside of the bottle
R-2	Anti-HbA1c	Ready for use	Refer to the date printed on the outside of the bottle

＊ Mix each reagent well before use

Preparation of HbA1c calibrators and/or controls in “HbA1c Calibrator Set” and/or “HbA1c Control Set”

Dissolve with 0.5 mL DW for each vial

2. Materials Required but not Provided

1. "HbA1c Calibrator Set" (5 concentrations x 1)
2. Pipette (1mL)
3. Micropipette (2-20 ,100 ,300 ,500 uL)
4. Test tube
5. Mixer for test tube
6. Autoanalyzer

3. Procedural Precautions

1) Collect blood samples with EDTA.
2) High lipid (hormagine concentration of 2700 degrees) or billrubin, (conjugated type 20 mg/dL, free type 20 mg/dL) in blood sample does not affect the test result.
3) Use thoroughly washed test tubes to avoid errors in the assay.
4) The dose response curve should be prepared for each set of assays since the reaction is affected to some extent by time and temperature, etc.
5) Blood samples should be stored at 2-10 °C and tested within two weeks. Do not freeze blood samples to avoid hemolysis.
6) Change tips each time to avoid contamination between test samples

4. Test Procedures

For the assay, please refer to the operation manual of the autoanalyzer (Please refer to attached documents for examples of the parameter in the autoanalyzer).

1) Preparation of test samples

   Take 10 uL of whole blood, as a test sample, add 500 u L of DW to hemolyze the sample.

2) 1st reaction

   Add 150 uL of R-1 Latex to 6 uL of the hemolyzed test sample or each HbA1c calibrator, and after mixing the solution, incubate it for five minutes at 37°C.

3) 2nd reaction

   Add 50 uL of R-2 solution and after mixing, incubate for five minutes at 37 °C.

4) Measurement

   Measure absorbance (turbidity) of each test sample and respective HbAlc calibrators (at 660nm/800nm) according to the operating procedures for the autoanalyzer.

5) Calculation of HbA1c concentration

According to the operating procedures for the autoanalyzer, calculate the concentration of HbA1c in the test sample.

When concentration (%) of test samples exceed the measurable range, dilute the test samples by 2-3 times with HbA1c calibrator STD-2, or another test sample with a known concentration (%) of HbA1c. Then run the test again and calculate the concentration (%) of HbA1c from the following calculation formula;

Concentration(%) of HbA1c
= [retested HbA1c (%) x n] - [HbA1c (%) of the sample/ a calibrator used for the dilution x (n-1)]
n = Dilution factor of the test sample (2-3)

[Calculation example for a test sample which exceeds the measurable range]

When adding 200 uL of a known sample (HbA1c 4.5%) to 100 uL of a test sample with approximately 17%, obtained by using the test on the undiluted test sample. This means three times dilution. If at retest, 9.1 % was obtained, the correct concentration (%) of the test sample is calculated by following the calculation formula;

Concentration (%) of HbA1c = (9.1 x3)-(4.5x(3 - 1)) = 27.3 - 9.0 = 18.3 %

■ Reference Normal values

4.6-6.2% (NGSP)

■ Performance

1. Sensitivity

   (1) When absorbance of HbA1c calibrator STD-1(0%) is measured according to the test procedure described in the package insert, the absorbance (turbidity) at 660 nm should be 0.50 or less.

   (2) When absorbance of HbA1c calibrator STD-5(15.6%) is measured according to the test procedure described in the package insert, the absorbance (turbidity) at 660 nm should be 1.00 or more.

2. Specificity

   When 2 HbA1c control samples with a known concentration (%) (high, middle, low) are tested according to the test procedure described in the package insert, each test result should be within ± 20% of respective known concentrations (%) of the HbA1c control sample.

3. Reproducibility

   When 2 positive samples with a known concentration (high, low) are tested 10 times respectively according to the test procedure described in the package insert, the respective CV should be less than 15%.

4. Measuring range 3.3- 14.5% (NGSP)

   The concentration (%) of HbA1c calibrator STD-5 is 14 % or more.

5. Influence of interfering substances

   The criteria for no significant interference are recovery within 10% of the initial value. No interferences have been observed by free bilirubin up to 20 mg/dL, conjugated bilirubin up to 21 mg/dL, high lipid up to 1400 formazin turbidity units, and ascorbic acid up to 100 mg/dL.

5. Correlation

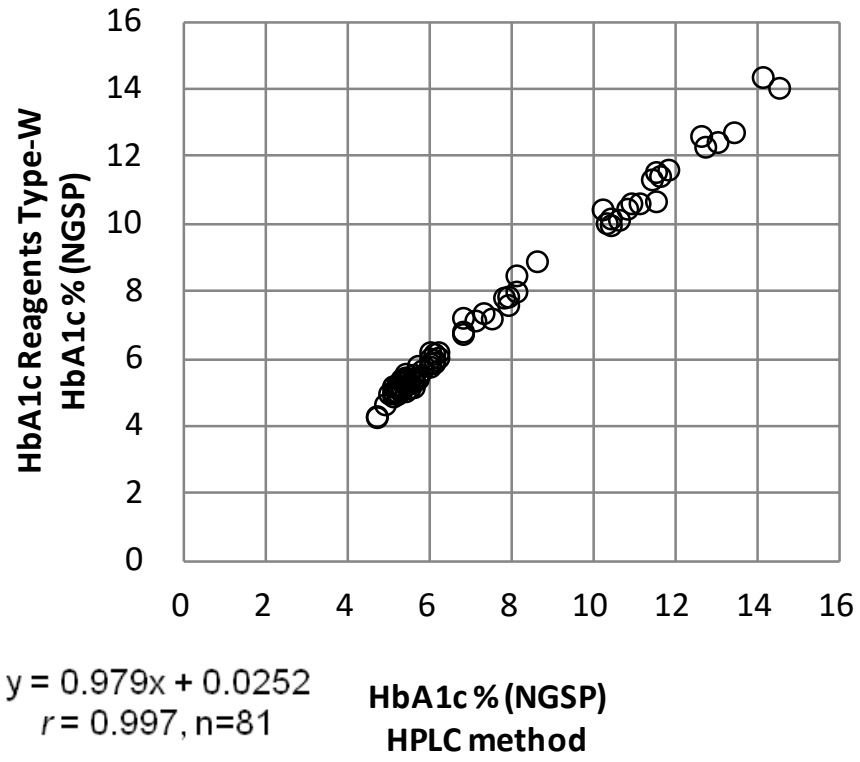
Study results in comparison with HPLC method.

n = 81

Correlation coefficient: r = 0.997

Regression equation: y = 0.979x + 0.0252

(x, HPLC method; y, HbA1c Reagents Type-W)



■ Handling Precaution

1. A test procedure for HbA1c Reagent should conform to explanations detailed in the packaging insert.
2. Test samples should be handled with care, considering possible infection by HBV, HIV or HCV and the apparatus or equipments used should be sterilized with hypochlorite, etc or autoclave processing (at 121℃ for 1 hour or more).
3. Although Blood materials used for HbA1c calibrators have been tested and found negative for HBs antigen, antibody to HIV and antibody to HCV, it should be considered potentially infectious and handled in accordance with good laboratory practice using appropriate precautions.
4. Avoid R-1 Latex from freezing or drying because it may cause non-specific agglutination.
5. Sodium azide is contained as a preservative for reagents of this kit. Waste disposed of in the drain should be flushed with a large quantity of water for safety.

   In case reagents accidentally contacted either with eyes, mouth or skin, clean up the contacted parts with plenty of water as a first-aid treatment, consult a doctor, if such is required.
6. Reagent containers should be incinerated or disposed of in line with regulations on medical or industrial waste law.
7. Do not use the reagents in this kit for purposes other than HbA1c measurement.
8. Test results should not be used in isolation but used in conjunction with the patient's clinical symptoms, clinical history and any other available data to produce an overall clinical diagnosis.

■ Storage Condition

Storage at 2-10 °C.

■ Shelf Life

Refer to the date printed on the outside of the package.

■ Associated Reagent

"HbA1c Calibrator Set" (5 concentrations x 1) and "HbA1c Control Set" (2 concentrations x 1) are separately available.

ACTIF

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APPENDIX

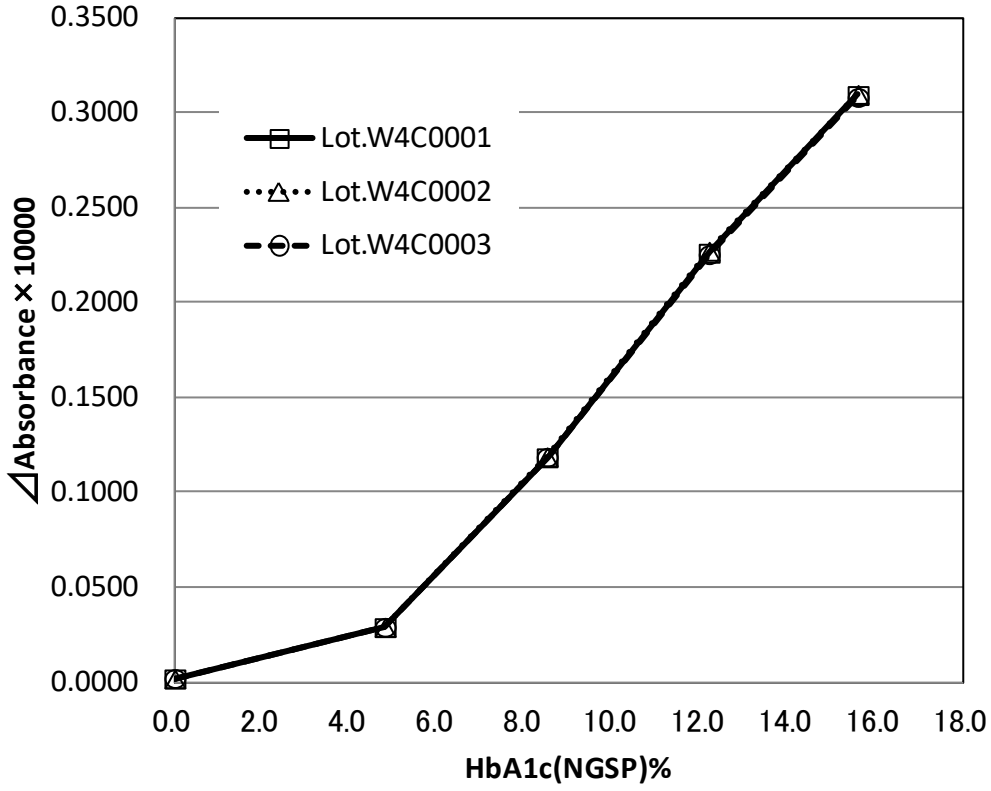
■ Refference Data

[Condition of Analysis]

Analyzer	Hitachi 7180
Sample/R1/R2(μL)	6.0/150/50
Assay method	2 pointend
Point	34-19 point
Wavelength(sub/main)	800 nm/660 nm
Calibration curve	Spline

1. Calibration curve

Calibrator	HbA1c(NGSP)%	⊿Abs. x10,000		
		Lot W4C0001	Lot W4C0002	Lot W4C0003
STD-1	0.0	19	19	21
STD-2	4.8	289	291	291
STD-3	8.5	1184	1189	1186
STD-4	12.2	2261	2272	2254
STD-5	15.6	3092	3098	3082



2. Sensitivity

	Abs. 660 at STD-1	Abs. 660 at STD-5
Criteria	≦ 0.5	≧ 1.00
Lot W4C0001	0.343	1.440
Lot W4C0002	0.346	1.449
Lot W4C0003	0.341	1.433

3. Specificity

<Fujikurakasei Quality Control>

Sample	Predetermined Concentration (%) (NGSP)	Lot W4C0001		Lot W4C0002		Lot W4C0003	
		Measure-ments (%)	Ratio to expectation	Measure-ments (%)	Ratio to expectation	Measure-ments (%)	Ratio to expectation
CTL-L	5.0	5.00	99.9	5.01	100.1	5.01	100.2
CTL-M	9.0	9.03	100.3	9.06	100.7	9.05	100.5
CTL-H	13.0	12.83	98.7	12.91	99.3	12.95	99.6

<BIO-RAD Liquichek™ Diabetes Control>

Sample	Lot	Indicated values(%) (NGSP)	Range	Lot.W4C0001		Lot.W4C0002		Lot.W4C0003	
				Measure-ments(%)	Difference to indicated values(%)	Measure-ments(%)	Difference to indicated values(%)	Measure-ments(%)	Difference to indicated values(%)
Level 1	38431	5.17	4.13～6.21	5.06	−0.12	5.06	−0.11	5.08	−0.10
Level 2	38432	9.34	7.47～11.2	9.47	0.13	9.53	0.19	9.53	0.19
Level 3	38433	13.7	11.0～16.5	13.73	0.03	13.78	0.08	13.80	0.10

4. Reproducibility

<Intra-Day>

N	Lot W4C0001			Lot W4C0002			Lot W4C0003		
	CTL-L	CTL-M	CTL-H	CTL-L	CTL-M	CTL-H	CTL-L	CTL-M	CTL-H
1	5.0	9.0	12.8	5.0	9.0	12.9	5.0	9.0	13.0
2	5.0	9.0	12.8	5.0	9.0	12.9	5.0	9.0	13.0
3	5.0	9.0	12.8	5.0	9.0	12.9	5.0	9.1	13.0
4	5.0	9.0	12.8	5.0	9.0	12.9	5.0	9.0	13.0
5	5.0	9.0	12.8	5.0	9.0	12.9	5.0	9.1	12.9
6	5.0	9.0	12.8	5.0	9.0	12.9	5.0	9.0	12.9
7	5.0	9.0	12.8	5.0	9.0	13.0	5.0	9.0	12.9
8	5.0	9.0	12.8	5.0	9.0	13.0	5.0	9.0	12.9
9	5.0	9.0	12.8	5.0	9.0	13.0	5.0	9.0	12.9
10	5.0	9.0	12.8	5.0	9.0	12.9	5.0	9.0	12.9
Mean	5.0	9.0	12.8	5.0	9.0	12.9	5.0	9.0	12.9
SD	0.01	0.02	0.02	0.01	0.01	0.03	0.01	0.02	0.03
CV(%)	0.25	0.21	0.17	0.27	0.15	0.23	0.25	0.17	0.24

<Inter-Day>

Day	Lot W4C0001			Lot W4C0002			Lot W4C0003		
	CTL-L	CTL-M	CTL-H	CTL-L	CTL-M	CTL-H	CTL-L	CTL-M	CTL-H
1	5.0	9.0	12.8	5.0	9.1	12.9	5.0	9.0	13.0
2	5.0	9.0	12.9	5.0	9.1	13.0	5.0	9.1	13.0
3	5.0	9.0	12.9	5.0	9.1	13.0	5.0	9.1	13.0
4	5.0	9.0	13.0	5.0	9.1	13.0	5.0	9.1	13.0
5	5.0	9.0	13.0	5.0	9.1	13.0	5.0	9.1	13.0
Mean	5.0	9.0	12.9	5.0	9.1	13.0	5.0	9.1	13.0
SD	0.02	0.02	0.06	0.02	0.01	0.04	0.02	0.01	0.02
CV(%)	0.32	0.19	0.45	0.30	0.16	0.29	0.34	0.11	0.13

5. Linearity

Ratio		Expected	Lot.W4C0001		Lot.W4C0002		Lot.W4C0003	
STD-2	Sample		Measure-ment	Difference	Measure-ment	Difference	Measure-ment	Difference
1.0	0.0	4.8	4.8	0.0	4.7	−0.1	4.8	0.0
0.9	0.1	5.9	6.1	0.2	5.9	0.0	5.9	0.1
0.8	0.2	7.0	7.1	0.1	7.0	0.1	7.0	0.1
0.7	0.3	8.0	8.3	0.2	8.0	0.0	8.0	0.0
0.6	0.4	9.1	9.3	0.1	9.1	−0.1	9.1	−0.1
0.5	0.5	10.2	10.3	0.1	10.1	−0.1	10.1	−0.1
0.4	0.6	11.3	11.4	0.1	11.2	−0.1	11.2	−0.1
0.3	0.7	12.4	12.4	0.1	12.2	−0.2	12.2	−0.1
0.2	0.8	13.4	13.5	0.1	13.4	0.0	13.4	0.0
0.1	0.9	14.5	14.6	0.0	14.5	0.0	14.5	0.0
0.0	1.0	15.6	15.4	−0.2	15.5	−0.1	15.5	−0.1

6. Influence of interfering substances

Bilirubin F

(mg/dL)	Measure-ments (%)	Difference to Blank
0	4.96	–
4	4.99	0.02
8	5.00	0.04
12	5.02	0.06
16	5.04	0.08
20	5.04	0.08

Bilirubin C

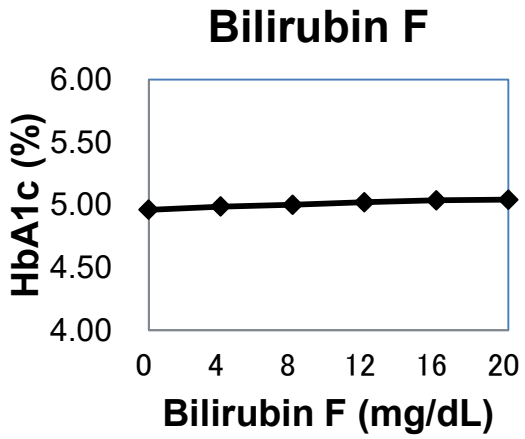
(mg/dL)	Measure-ments (%)	Difference to Blank
0	4.86	–
4	4.86	0.00
8	4.89	0.03
13	4.97	0.12
17	5.06	0.20
21	5.17	0.32

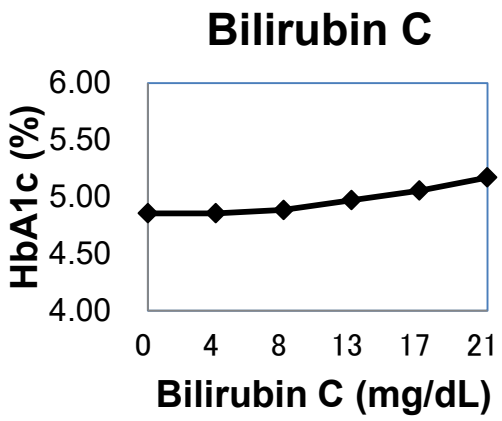
Chyle

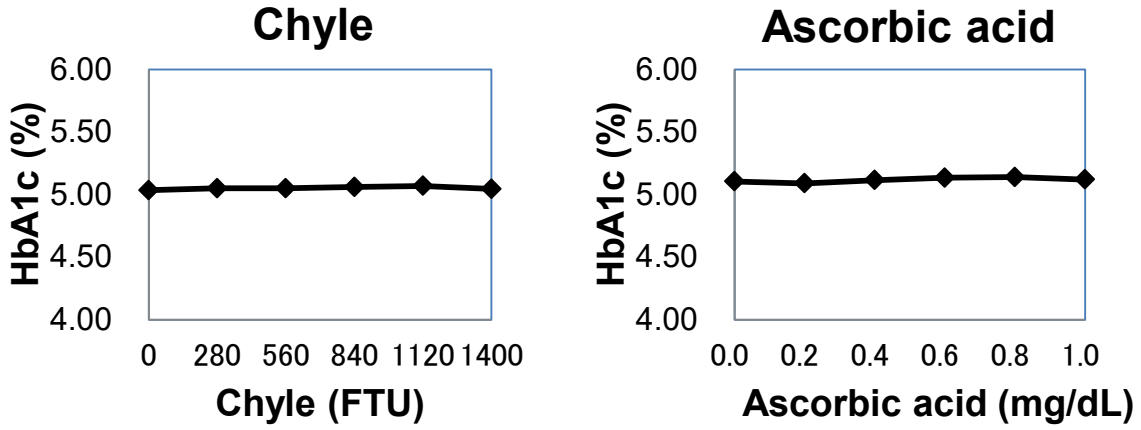
(FTU)	Measure-ments (%)	Difference to Blank
0	5.04	–
280	5.05	0.01
560	5.05	0.01
840	5.06	0.03
1120	5.07	0.04
1400	5.05	0.01

Ascorbic acid

(mg/dL)	Measure-ments (%)	Difference to Blank
0	5.11	–
0.2	5.09	−0.02
0.4	5.12	0.01
0.6	5.14	0.03
0.8	5.14	0.04
1.0	5.12	0.01



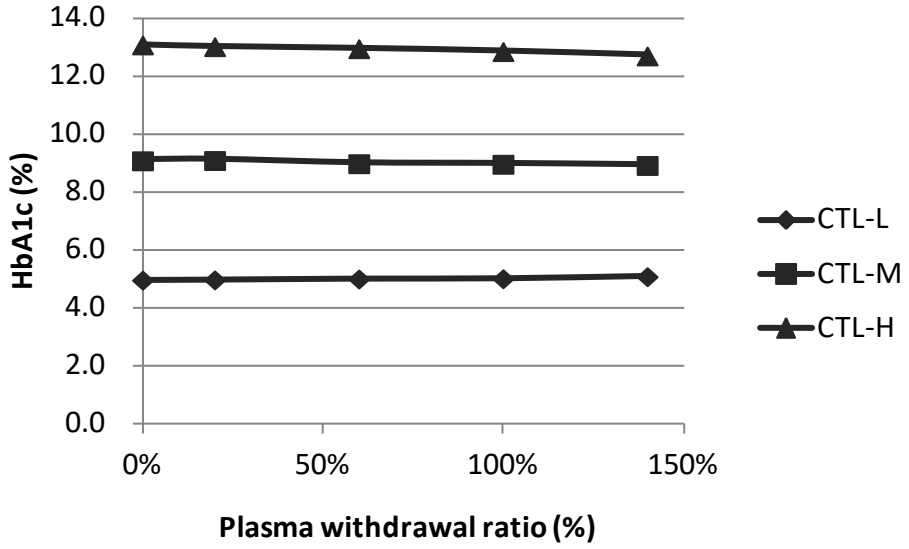




7. Influence of plasma

Sample		Target Value(%)	Plasma withdrawal ratio* (Fixed Hb Conc. at 2.5mg/mL)				
			0%	20%	60%	100%	140%
CTL-L	HbA1c(%)	5.0	5.0	5.0	5.0	5.0	5.1
	Difference(%)	–	0.0	0.0	0.0	0.0	0.1
CTL-M	HbA1c(%)	9.0	9.1	9.1	9.0	9.0	8.9
	Difference(%)	–	0.1	0.1	0.0	0.0	−0.1
CTL-H	HbA1c(%)	13.0	13.1	13.0	13.0	12.9	12.7
	Difference(%)	–	0.1	0.0	0.0	−0.1	−0.3

* Plasma withdrawal ratio is described as the ratio of plasma to packed red cell.
* The ratio of 100% means the mixture ratio between plasma and packed red cell is 1:1.



8. Realtime Stability

Reagent Lot		W15C001			W15C002			W15C003		
Sample		CTL-L	CTL-M	CTL-H	CTL-L	CTL-M	CTL-H	CTL-L	CTL-M	CTL-H
Target value		5.5	9.0	12.5	5.5	9.0	12.5	5.5	9.0	12.5
0 month	Measure-ments(%)	5.6	9.1	12.7	5.6	9.0	12.6	5.6	9.0	12.6
	Difference to target(%)	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.0	0.1
	CV(%) (n=5)	0.32	0.25	0.40	0.2	0.5	0.27	0.41	0.41	0.62
6 months	Measure-ments(%)	5.6	9.1	12.7	5.6	9.2	12.7	5.6	9.1	12.7
	Difference to target(%)	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.2
	CV(%) (n=5)	0.37	0.32	0.37	0.27	0.23	0.25	0.24	0.34	0.37
12 months	Measure-ments(%)	5.6	9.1	12.7	5.5	9.1	12.7	5.6	9.1	12.7
	Difference to target(%)	0.1	0.1	0.2	0.0	0.1	0.2	0.1	0.1	0.2
	CV(%) (n=5)	0.45	0.12	0.12	0.20	0.17	0.57	0.41	0.17	0.31
18 months	Measure-ments(%)	5.6	9.0	12.4	5.6	9.1	12.5	5.6	9.1	12.5
	Difference to target(%)	0.1	0.0	−0.1	0.1	0.1	0.0	0.1	0.1	0.0
	CV(%) (n=5)	0.27	0.16	0.22	0.32	0.24	0.26	0.47	0.39	0.18
21 months	Measure-ments(%)	5.5	9.0	12.5	5.6	9.1	12.5	5.5	9.0	12.4
	Difference to target(%)	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	−0.1
	CV(%) (n=5)	0.30	0.20	0.15	0.40	0.28	0.25	0.27	0.20	0.14

9. On-board Stability/ Severe Stability

Storage Temp.	4℃		4℃		37℃	
Calibration	Each day		Only day 0 (on-board)		Each day	
Sample	CTL-L	CTL-H	CTL-L	CTL-H	CTL-L	CTL-H
Target Value(%)	5.0	13.0	5.0	13.0	5.0	13.0
Day 0	5.0	12.9	5.0	13.0	5.0	12.9
Day 1	5.0	13.0	5.0	13.1	5.1	13.2
Day 2	5.0	13.0	5.0	13.1	5.0	12.9
Day 3	5.0	13.2	5.0	13.2	5.0	13.0
Day 6	5.0	13.1	5.0	13.1	5.0	12.9
Day 7	5.0	13.1	5.0	13.2	5.0	13.0
Day 10	4.9	13.0	5.0	13.1	4.9	12.8
Day 14	5.0	13.1	5.0	13.2	5.0	13.0
Day 21	5.0	13.0	5.0	13.1	5.0	12.9
Day 28	5.0	13.2	5.1	13.3	5.0	13.2

10. Correlation

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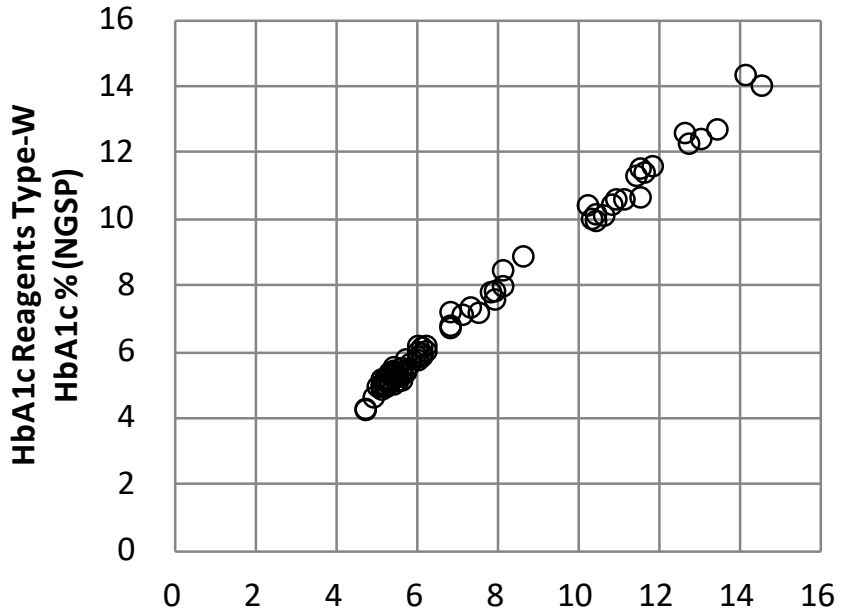

$y = 0.979x + 0.0252$
$r = 0.997$, n=81

Whole Blood Sample#	HbA1c % (NGSP)	
	HPLC method	Fujikura HbA1c
1	4.7	4.3
2	4.7	4.3
3	4.9	4.7
4	5.0	5.0
5	5.1	5.0
6	5.1	5.2
7	5.1	5.2
8	5.1	5.0
9	5.1	4.9
10	5.2	5.2
11	5.2	5.2
12	5.2	5.2
13	5.2	5.0
14	5.2	5.0
15	5.2	5.0
16	5.3	5.4
17	5.3	5.4
18	5.3	5.2
19	5.3	5.3
20	5.3	5.1
21	5.3	5.1
22	5.4	5.6
23	5.4	5.3
24	5.4	5.5
25	5.4	5.4
26	5.4	5.2
27	5.4	5.1

Whole Blood Sample#	HbA1c % (NGSP)	
	HPLC method	Fujikura HbA1c
28	5.5	5.4
29	5.5	5.3
30	5.5	5.1
31	5.6	5.5
32	5.6	5.4
33	5.6	5.4
34	5.6	5.4
35	5.6	5.4
36	5.6	5.3
37	5.6	5.2
38	5.7	5.8
39	5.7	5.4
40	5.7	5.5
41	5.8	5.7
42	6.0	5.8
43	6.0	6.2
44	6.0	5.9
45	6.0	6.0
46	6.1	5.9
47	6.1	6.0
48	6.1	6.2
49	6.2	6.2
50	6.2	6.1
51	6.8	6.8
52	6.8	7.2
53	6.8	6.8
54	7.1	7.2

Whole Blood Sample#	HbA1c % (NGSP)	
	HPLC method	Fujikura HbA1c
55	7.3	7.4
56	7.5	7.2
57	7.8	7.8
58	7.9	7.9
59	7.9	7.6
60	8.1	8.5
61	8.1	8.0
62	8.6	8.9
63	10.2	10.5
64	10.3	10.0
65	10.4	10.2
66	10.4	10.0
67	10.6	10.1
68	10.8	10.5
69	10.9	10.6
70	11.1	10.6
71	11.4	11.3
72	11.5	10.7
73	11.5	11.6
74	11.6	11.4
75	11.8	11.6
76	12.6	12.6
77	12.7	12.3
78	13.0	12.5
79	13.4	12.7
80	14.1	14.4
81	14.5	14.1