

# HEMOGLOBIN A1C

Turbidimetry

The application parameters comprised here constitute a guide to facilitate the validation of our reagents by the instrument. It is advisable to validate the use when there is any change in software or reagent versions.

## Instrument: HITACHI 902 (=HITACHI 7020)

### Samples

Capillary or venous blood collected by standard procedures and with heparin or EDTA as anticoagulants.

HbA1C in blood is stable 3 days at 15-25°C, 7 days at 2-8°C and 6 months at -20°C. Freeze once only.

### Hemolysate preparation

The calibrators do not require pretreatment.

1. Bring the reagent A to room temperature.
2. Pipette into a test tube:

Blood	10 µL
Reagent (A)	1000 µL

3. Shake thoroughly. Avoid the formation of foam. The hemolysate can be used after the solution has changed color from red to brownish-green (approximately 3 minutes).

The hemolysate is stable 4 hours at 15-25°C, 24 hours at 2-8°C and 6 months at -20°C. Freeze once only.

### Reagent preparation

Reagents (A), (B), (C) and (D) are provided ready to use.

HbA1C Standards (S1-S4): Reconstitute with 2.0 mL of distilled water. Stable for 8 hours at 15-25 °C, 2 days at 2-8°C and 3 months at -20°C. Freeze once only.

Hb Reagent 1: Reagent B  
HbA1c Reagent 1: Reagent C Reagent 2: Reagent D

## Instrument settings

HB

1 Test Name	HB	29 Calib. Conc. 4	0
2 Assay Code (Mthd)	1 point	30 Calib. Position 4	0
3 Assay Code (2. Test)	0	31 Calib. Conc. 5	0
4 Reaction Time	10	32 Calib. Position 5	0
5 Assay Point 1	17	33 Calib. Conc. 6	0
6 Assay Point 2	0	34 Calib. Position 6	0
7 Assay Point 3	0	35 S1 ABS	
8 Assay Point 4	0	36-40 K-K5 Factor	0
9 Wavelength (SUB)	660	41 A Factor	0
10 Wavelength (MAIN)	570	42 B Factor	0
11 Sample Volume	20	43 C Factor	0
12 R1 Volume	230	44 SD Limit	0.1
13 R1 Position	...	45 Duplicate Limit	300
14 R1 Bottle size	Small	46 Sensitivity Limit	0
15 R2 Volume	0	47 S1 ABS. Limit (L)	-32000
16 R2 Position	0	48 S1 ABS. Limit (H)	32000
17 R2 Bottle size	Small	49 ABS. Limit	0
18 R3 Volume	0	50 ABS. Limit (D/I)	Increase
19 R3 Position	0	51 Prozone Limit	0
20 R3 Bottle size	Small	52 Prozone Limit (U/D)	Lower limit
21 Calib. Type (Type)	Linear)	53 Prozone (End Point)	35
22 Calib. Type (Weight)	0	54 Expected Value (L)	...
23 Calib. Conc. 1	0.0	55 Expected Value (H)	...
24 Calib. Position 1	...	56 Instrument Factor (a)	1.0
25 Calib. Conc. 2	(*)	57 Instrument Factor (b)	0.0
26 Calib. Position 2	...	58 Key Setting	...
27 Calib. Conc. 3	0		
28 Calib. Position 3	0		
		Blank: make reagent blank with sodium chloride 154 mmol/L	
		Calibrator: Standard S4	
		... Data entered by the operator	

**HbA1C**

1 Test Name	HbA1C	29 Calib. Conc. 4	(*)
2 Assay Code (Mthd)	2 point	30 Calib. Position 4	...
3 Assay Code (2. Test)	0	31 Calib. Conc. 5	(*)
4 Reaction Time	10	32 Calib. Position 5	...
5 Assay Point 1	17	33 Calib. Conc. 6	0
6 Assay Point 2	35	34 Calib. Position 6	0
7 Assay Point 3	0	35 S1 ABS	
8 Assay Point 4	0	36 K Factor	
9 Wavelength (SUB)	700	37 K2 Factor	0
10 Wavelength (MAIN)	340	38 K3 Factor	0
11 Sample Volume	10	39 K4 Factor	0
12 R1 Volume	250	40 K5 Factor	0
13 R1 Position	...	41 A Factor	0
14 R1 Bottle size	Small	42 B Factor	0
15 R2 Volume	0	43 C Factor	0
16 R2 Position	0	44 SD Limit	350
17 R2 Bottle size	Small	45 Duplicate Limit	300
18 R3 Volume	50	46 Sensitivity Limit	0
19 R3 Position	...	47 S1 ABS. Limit (L)	-32000
20 R3 Bottle size	Small	48 S1 ABS. Limit (H)	32000
21 Calib. Type (Type)	Logit-Log (4P)	49 ABS. Limit	0
22 Calib. Type (Weight)	0	50 ABS. Limit (D/I)	Increase
23 Calib. Conc. 1	0.00	51 Prozone Limit	0
24 Calib. Position 1	...	52 Prozone Limit (U/D)	Lower limit
25 Calib. Conc. 2	(*)	53 Prozone (End Point)	35
26 Calib. Position 2	...	54 Expected Value (L)	...
27 Calib. Conc. 3	(*)	55 Expected Value (H)	...
28 Calib. Position 3	...	56 Instrument Factor (a)	1.0
		57 Instrument Factor (b)	0.0
		58 Key Setting	...
Blank: make reagent blank with sodium chloride 154 mmol/L Calibrators 2-5: Standards S1-S4		... Data entered by the operator	

Version 0603

**CALCULATION**

$$\% \text{ HbA1C - IFCC} = \frac{\text{HbA1C (g/dL)}}{\text{Hb (g/dL)}} \times 100$$

The HbA<sub>1c</sub> percentage in the sample is calculated using the following general formula. The values are traceable to IFCC Reference Method:

The traceable values to Reference Method as described by the US National Glycohemoglobin Standardization Program (NGSP) are calculated using the following general formula:

$$\% \text{ HbA1C-NGSP} = 0.915 \times \% \text{ HbA1C-IFCC} + 2.15$$