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 917**

Samples

Capillary or venous blood collected by standard procedures and with heparin or EDTA as anticoagulants .
HbA1C in blood is stable 3 days at 15-25ºC, 7 days at 2-8ºC and 6 months at –20ºC. 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 15-25ºC, 24 hours at 2-8ºC and 6 months at –20ºC. 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 ºC, 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

ANALIZE	
Assay/Time/Point	End Point / 10 / 16 - 0
Wavelength (Second./Main)	660 / 570
Sample Volume (Normal)	20 / 0 / 0
(Decreased)	10 / 0 / 0
(Increased)	20 / 0 / 0
Diluent/Expiration	951 / 99
Reagent (R1) T1	230 / 0 / .../ 28
(R2) T2	0 / 0 / ... / 0
(R3) T3	0 / 0 / ... / 0
(R4) T4	0 / 0 / ... / 0
Abs. Limit	32000 – Increase
Prozone Limit	-32000 – Lower
Washing Solution	Detergent 1

RANGE	
Application Nº / Units	... / **g/dL**
Name	Hemoglobin
Control Interval	1000
Instrument Factor	a = 1.0 b = 0.0
Technical Limit	1.10 - 40
Repetition Limit	1.10 - 40
References Values Male	... - ...
Female	... - ...

CALIB	
Calibration Type	Linear
Points/Span Point	2 / 2
Weigth	0
SD Limit	0.1
Duplicate Limit % / Abs	10 / 50
Sensitivity Limit	-9999 / 9999
Abs. S1 Limit	-32000 / 32000

OTHERS

	Calib. Code	*Concentration*	*Position*
(1)	...	0.0	...
(2)	...	*	...

	Sample volume	*Diluted*	*Diluent*
(1)	20	0	0
(2)	20	0	0

Blank: Make reagent blank with sodium cloride 154 mmol/L.
Calibrator: Standard S4

... Data entered by the operator
(*) Enter S4 Standard value

HBA1C

ANALIZE	
Assay/Time/Point	2 POINT END / 10 / 16 - 34
Wavelength (Second./Main)	700 / 340
Sample Volume (Normal)	10 / 0 / 0
(Decreased)	5 / 0 / 0
(Increased)	20 / 0 / 0
Diluent/Expiration	951 / 99
Reagent (R1) T1	250 / 0 / .../ 28
(R2) T2	0 / 0 / ... / 0
(R3) T3	50 / 0 / ... / 28
(R4) T4	0 / 0 / ... / 0
Abs. Limit	32000 – Increase
Prozone Limit	-32000 – Lower
Washing Solution	Detergent 1
RANGE	
Application Nº / Units	... / **g/dL**
Name	Hemoglobin A1c
Control Interval	1000
Instrument Factor	a = 1.0 b = 0.0
Technical Limit	0.05 – 2.5
Repetition Limit	0.05 – 2.5
References Values Male	... - ...
Female	... - ...

... Data entered by the operator

CALIB	
Calibration Type	Logit-Log (4P)
Points/Span Point	5 / 4
Weigth	0
SD Limit	250
Duplicate Limit % / Abs	10 / 50
Sensitivity Limit	-9999 / 9999
Abs. S1 Limit	-32000 / 32000

OTHERS

	Calib. Code	*Concentration*	*Position*
(1)	...	0.00	...
(2)	...	*	...
(3)	...	*	...
(4)	...	*	...
(5)	...	*	...

	Sample volume	*Diluted*	*Diluent*
(1)	10	0	0
(2)	10	0	0
(3)	10	0	0
(4)	10	0	0
(5)	10	0	0

Blank: Make reagent blank with sodium cloride 154 mmol/L.
(*) Calibrators 2-5: Standards S1-S4

Version 0704

CALCULATION

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

The HbA_{1c} 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:

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