

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: COBAS MIRA / MIRA Plus

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

GENERAL			CALCULATION		
MEASUREMENT MODE	ABSORB		SAMPLE LIMIT	NO	SPACE
REACTION MODE	R- S	1	REACTION DIRECTION	INCREASE	1
CALIBRATION MODE	SLOPE AVG	2	CHECK	ON	1
REAGENT BLANK	REAG/SOL	3	CONVERSION FACTOR		1
CLEANER	NO	1	OFFSET		0
WAVELENGTH	600 nm	5	TEST RANGE LOW		1.1 g/dL
DECIMAL POSITION		1 (g/dL)	HIGH		40 g/dL
UNIT		11 (g/dL)	NORM RANGE LOW		...
			HIGH		...
ANALYSIS			NUMBER OF STEPS		1
POST DIL. FACTOR	NO	2	CALCULATION STEP A	ENDPOINT	1
CONC. FACTOR	NO	SPACE	READING FIRST		T1
SAMPLE CYCLE		2	LAST		13
VOLUME		20 µL			
DILUTION NAME	H2O	0	CALIBRATION		
VOLUME		10 µL	CALIB. INTERVAL	ON REQUEST	3
REAGENT CYCLE		1	TIME	NO	SPACE
VOLUME		230 µL	REAGENT RANGE LOW	NO	SPACE
			HIGH	NO	SPACE
			BLANK RANGE LOW	NO	SPACE
			HIGH	NO	SPACE
			STANDARD POSITION	...	
			Std. 1	(*) S4	

Blank: Make reagent blank with sodium chloride 154 mmol/L (NaCl 0.9%).
 ... Value entered by the operator
 (*) Enter the Standard S4 value.

HbA1C

GENERAL			CALCULATION		
MEASUREMENT MODE	ABSORB		SAMPLE LIMIT	NO	SPACE
REACTION MODE	R- S-SR1	3	REACTION DIRECTION	INCREASE	1
CALIBRATION MODE	LOGIT/LOG 5	6	CHECK	ON	1
REAGENT BLANK	NO BLANK	0	CONVERSION FACTOR		1
CLEANER	NO	1	OFFSET		0
WAVELENGTH	340 nm	1	TEST RANGE LOW		0.05 g/dL
DECIMAL POSITION		2	HIGH		2.5 g/dL
UNIT	11 (g/dL)		NORM RANGE LOW		...
ANALYSIS			HIGH		...
POST DIL. FACTOR	NO	2	NUMBER OF STEPS		1
CONC. FACTOR	NO	SPACE	CALCULATION STEP A	ENDPOINT	1
SAMPLE CYCLE		1	READING FIRST		11
VOLUME		8 µL	LAST		24
DILUTION NAME	H2O	0	CALIBRATION		
VOLUME		10 µL	CALIB. INTERVAL	ON REQUEST	3
REAGENT CYCLE		1	TIME	NO	SPACE
VOLUME		200 µL	REAGENT RANGE LOW	NO	SPACE
START R1 CYCLE		12	HIGH	NO	SPACE
VOLUME		40 µL	BLANK RANGE LOW	NO	SPACE
DILUTION NAME	H2O	0	HIGH	NO	SPACE
VOLUME		10 µL	STANDARD POSITION		
			Std. 1	...	(*) S1
			Std. 2		(*) S2
			Std. 3		(*) S3
			Std. 4		(*) S4
			Std. 5		0.00
... Value entered by the operator (*) Enter the corresponding S1, S2, S3, S4 standards value.					

Version 0704

CALCULATION

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

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:

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