

# 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: ADVIA 1200

### 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. reeze once only.

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

## Instrument settings

### Hb

ANALYTICAL PARAMETERS		Reanalysis conditions		Multi-Standards setting			
<b>Analytical Conditions</b>		Serum reac. smp. vol. (µ)	3.00	Formula	Linear correction	Axis conv.	No convert.
R1 volume	90.00	Serum dilut. method (µ)	None	Points			
R2 volume	0.00	Serum reac. smp. vol. (d)	3.00				
R3 volume	0.00	Serum dilut. method (d)	None				
R4 volume	0.00			FV	MEAN		
R1 diluent vol.	0.00	<b>Standards setting</b>		BLK	0.00	...	
R2 diluent vol.	0.00	BLK H	9.9999	1	*	...	
R3 diluent vol.	0.00	BLK L	-9.9999				
R4 diluent vol.	0.00	STD H	9.9999				
Serum reac. s. vol.	15	STD L	-9.9999				
Serum dil. method	Standard	FV	0.0000				
Reaction time	10 min.	Abnml. (serum) H	40.00	Blank: Make reagent blank with sodium chloride 154 mmol/L (NaCl 0.9%).			
Reagent 1 stir.	Weak	Abnml. (serum) L	1.10	(*) Assigned value of the Standard S4.			
Reagent 2 stir.	Weak						
Reagent 3 stir.	Weak	<b>Calculation method setting</b>					
Reagent 4 stir.	Weak	M-DET. P. I	0	Prozone		<b>Reaction rate method</b>	
		M-DET. P. m	40	Prozone form.	None	Cycle	3
		M-DET. P. n	42	Prozone limit	9.999	Factor	3.0
		S-DET. P. p	0	Prozone judge	Upper limit	Reac. Type	Inc.
		S-DET. P. r	0	Judge limit	9.999	E2 corre.	Not do
				M-DET. P. m.	0	Blank (µ)	9.9999
				M-DET. P. n.	0	Blank (d)	-9.999
		Check D.P.I.	0	M-DET. P. n.	0	Sample (µ)	9.9999
		Limit value	0.003	S-DET. P. p.	0	Sample (d)	-9.999
		Variance	10.0	S-DET. P. r.	0		
						<b>Endpoint method</b>	
						Re. Absorb (µ)	9.9999
						Re. Absorb (d)	-9.999
<b>Sub-analy. conditions</b>							
Name	Hb						
Digits	1						
M-wave. L.	545 nm						
S-wave. L.	751 nm						
Analy. mthd.	EPA						
Calc. mthd	STD						
Qualit. judg.	Not do						

HbA1c

ANALYTICAL PARAMETERS		Reanalysis conditions		Multi-Standards setting			
<b>Analytical Conditions</b>		Serum reac. smp. vol. (μ)	0.00	Formula	Logit Log 3	Axis conv.	No convert.
R1 volume	100.00	Serum dilut. method (μ)	None	Points	5		
R2 volume	0.00	Serum reac. smp. vol. (d)	0.00				
R3 volume	20.00	Serum dilut. method (d)	None	FV	Dil. Method	Dil. Smp. Vol	Diluent Vol.
R4 volume	0.00			BLK			
R1 diluent vol.	0.00	<b>Standards setting</b>		1	*	None	0.0
R2 diluent vol.	0.00	BLK H	9.9999	2	*	None	0.0
R3 diluent vol.	0.00	BLK L	-9.9999	3	*	None	0.0
R4 diluent vol.	0.00	STD H	9.9999	4	*	None	0.0
Serum reac. s. vol.	4.0	STD L	-9.9999				
Serum dil. method	Standard	FV	0.0000	Blank: Make reagent blank with sodium chloride 154 mmol/L (NaCl 0.9%).			
		Abnml. (serum) H	2.50	(*) Calibrator assigned values S1-S4			
		Abnml. (serum) L	0.05				
Reaction time	10 min.	<b>Calculation method setting</b>		<b>Prozone</b>		<b>Reaction rate method</b>	
Reagent 1 stir.	Weak	M-DET. P. I	0	Prozone form.	None	Cycle	3
Reagent 2 stir.	Weak	M-DET. P. m	96	Prozone limit	9.999	Factor	3.0
Reagent 3 stir.	Weak	M-DET. P. n	98	Prozone judge	Upper limit	Reac. Type	Inc.
Reagent 4 stir.	Weak	S-DET. P. p	40	Judge limit	9.999	E2 corre.	Not do
		S-DET. P. r	42	M-DET. P. m.	0	Blank (μ)	9.9999
		Check D.P.I.	0	M-DET. P. n.	0	Blank (d)	-9.999
		Limit value	0.003	S-DET. P. p.	0	Sample (μ)	9.9999
		Variance	10.0	S-DET. P. r.	0	Sample (d)	-9.999
						<b>Endpoint method</b>	
						Re. Absorb (μ)	9.9999
						Re. Absorb (d)	-9.999
<b>Sub-analy. conditions</b>							
Name	HbA1c						
Digits	2						
M-wave. L.	340 nm						
S-wave. L.							
Analy. mthd.	EPA						
Calc. mthd	MSTD						
Qualit. judg.	Not do						

Vers. 0704

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:

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