

# Comparison of methods of analysis of HbA1c

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**JESSA**  
Z I E K E N H U I S

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ASO klinische biologie

# Diabetes mellitus

- Diabetes is a chronic metabolic disease characterized by elevated levels of blood glucose, which leads over time to damage to the heart, blood vessels, eyes, kidneys and nerves.
- Incidence
  - 422 million people worldwide
  - 1.5 million deaths per year
- Types
  - Type 1: also known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin
  - Type 2: most common type, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin



## Belgium (2014)

- Prevalence: 6.33%
- 1/3 undiagnosed

# Diagnosis

Fasting plasma glucose	$\geq 7.0$ mmol/l	6.1–6.9 mmol/l
	$\geq 126$ mg/dl	110–125 mg/dl
2 h plasma glucose after OGTT	$\geq 11.1$ mmol/l	7.8–11.0 mmol/l
	$\geq 200$ mg/dl	140–199 mg/dl
Random plasma glucose	$\geq 11.1$ mmol/l	
	$\geq 200$ mg/dl	
Glycosylated haemoglobin	$\geq 6.5\%$	

\*Adapted from World Health Organization and International Diabetes Federation.<sup>6</sup>

DM = diabetes mellitus; OGTT = oral glucose tolerance test.

World Health Organization diagnostic criteria for DM or pre-diabetes\* DM Pre-diabetes

# Diagnosis

Standard interpretation norm*			IFCC (mmol/mol)	NGSP (%)
Normal reference range			20-42	4-6
Decision limits	Monitoring therapy	Target treatment	53	7
		Limit change therapy	64	8
Diagnosis	Low risk	<40	<5.8	
	Increasing risk future diabetes	40-46	5.8-6.4	
	Diabetes	>46	>6.4	

## NGSP guidelines:

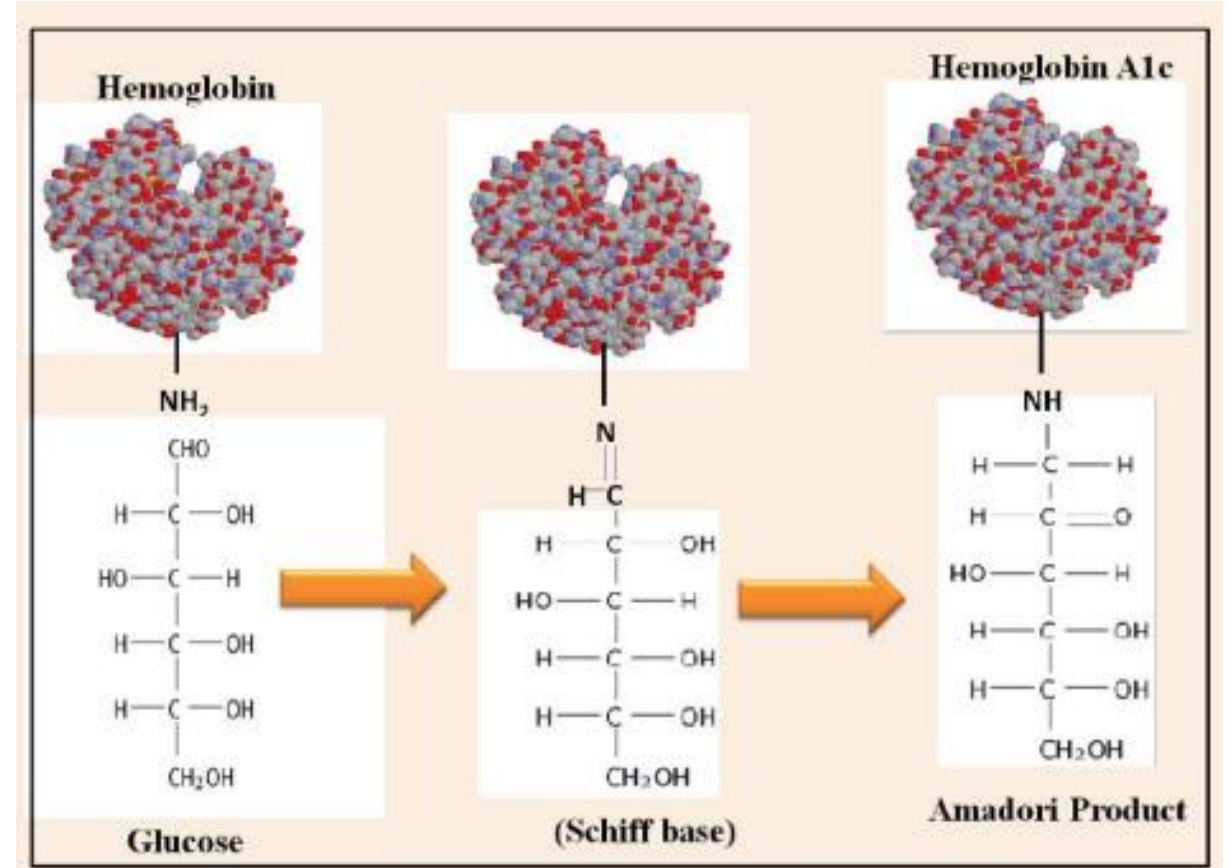
- HbA<sub>1c</sub> 2x per year for patients with stable glycemic control
- Quarterly – unstable glycemic status and change of therapy

Weykamp C. HbA1c: a review of analytical and clinical aspects. Ann Lab Med. 2013

# HbA1c

- Synonym: Glycated hemoglobin, Glycohemoglobin
- Glycated Hb at one or both N-terminal valines of beta chain
- Level of HbA1c = length of exposure to plasma glucose
- Non-enzymatic reaction

Chemical reaction in glycation of hemoglobin

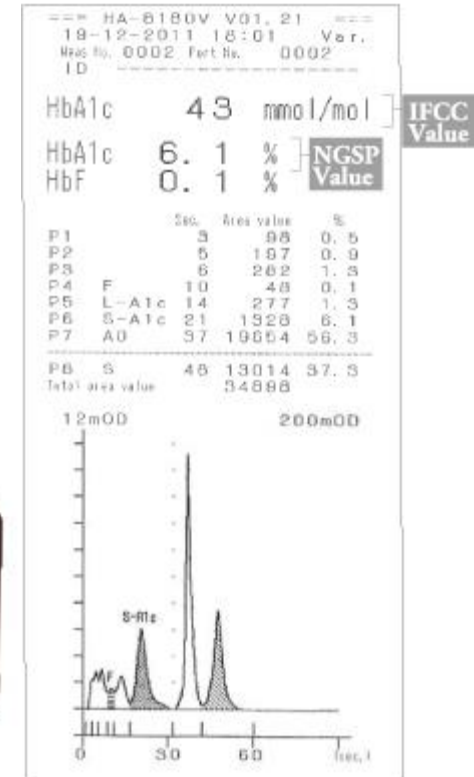


Chauhan, N. Laboratory Diagnosis of HbA1c: A Review. Journal of Nanomedicine Research 5, (2017).

# Jessa hospital

- > 10,000 HbA<sub>1c</sub> analysis per year
- Arkray ADAMS A1c HA-8180V (Arkray, Kyoto, Japan)
- Reversed phase cation exchange (HPLC) technique
- Contract: July 2014 to End of 2025
- Shift of core lab analysers from Roche module to Abbott Alinity ci-series

1. AZ Turnhout
2. Herentals
3. Heilig Hart Mol
4. Dimpna Geel
5. AZ Sint-Maarten



# Questions:

- 1) What are the methods of measurement for HbA1C quantification?
- 2) What are the current criteria for standardization of HbA1c quantification?
- 3) What are the analytical considerations in the comparison of these methods?
- 4) What is the effect of hemoglobin variants in HbA1c quantification in the four methods?

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# Methods of measurement for HbA1C

## HPLC

- Cation exchange HPLC
- Separation of different components based on ionic charge
- Tosoh HLC-723 G11
- Reduced run time from 60 seconds to 30 seconds per sample
- Hb variants in three windows
  - **H-VO: HbAD, HbAS and HbAC**
  - **P-HV3: HbAE**
  - **POO: unknown Hb variants**

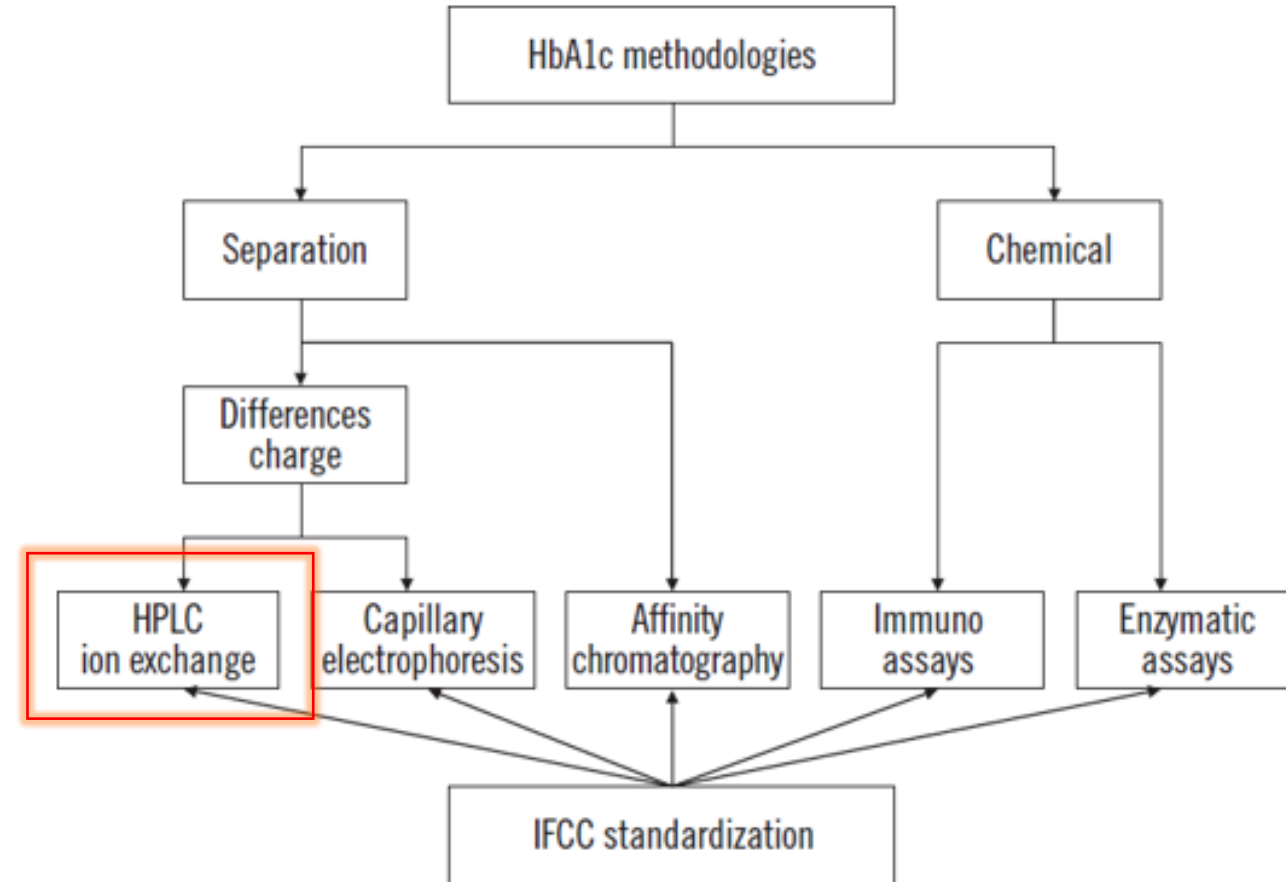


Figure 1: Methods of measurement for HbA1c and traceability to IFCC-RMP.

# Methods of measurement for HbA1C

## Capillary electrophoresis

- Utilizes capillaries to separate hemoglobin components
- Separation based on surface charge under high voltage alkaline buffer
- Hb variants can be detected in a specific order from the cathode to anode
  - A2, C, E, S, D,F, A0, A1c

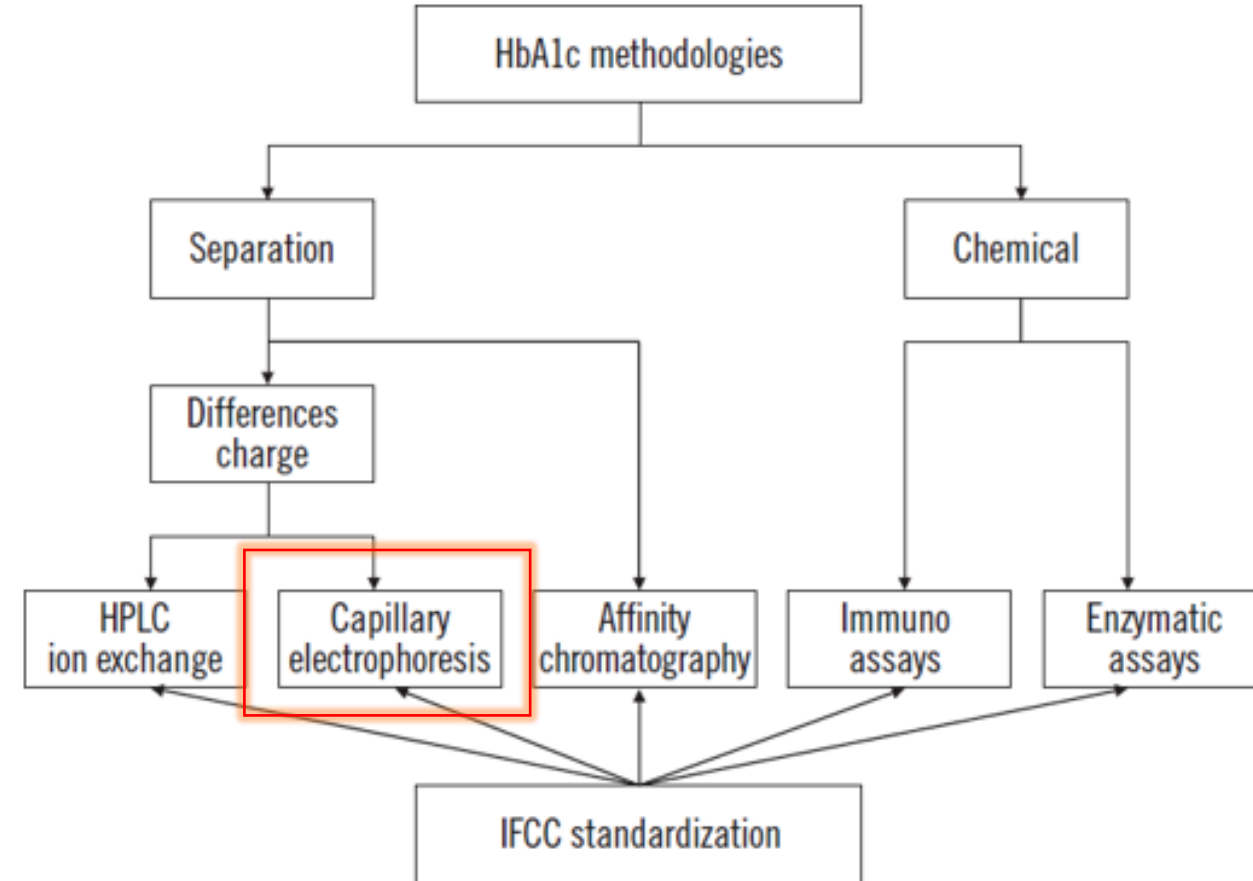


Figure 1: Methods of measurement for HbA1c and traceability to IFCC-RMP.

# Methods of measurement for HbA1C

## Affinity chromatography

- Total glycate Hb including HbA<sub>1c</sub>
- Quantification is based on the reaction between *m*-aminophenylboronic acid and the *cis*-diol group of glucose bounded to Hb
- Least interference of Hb variants

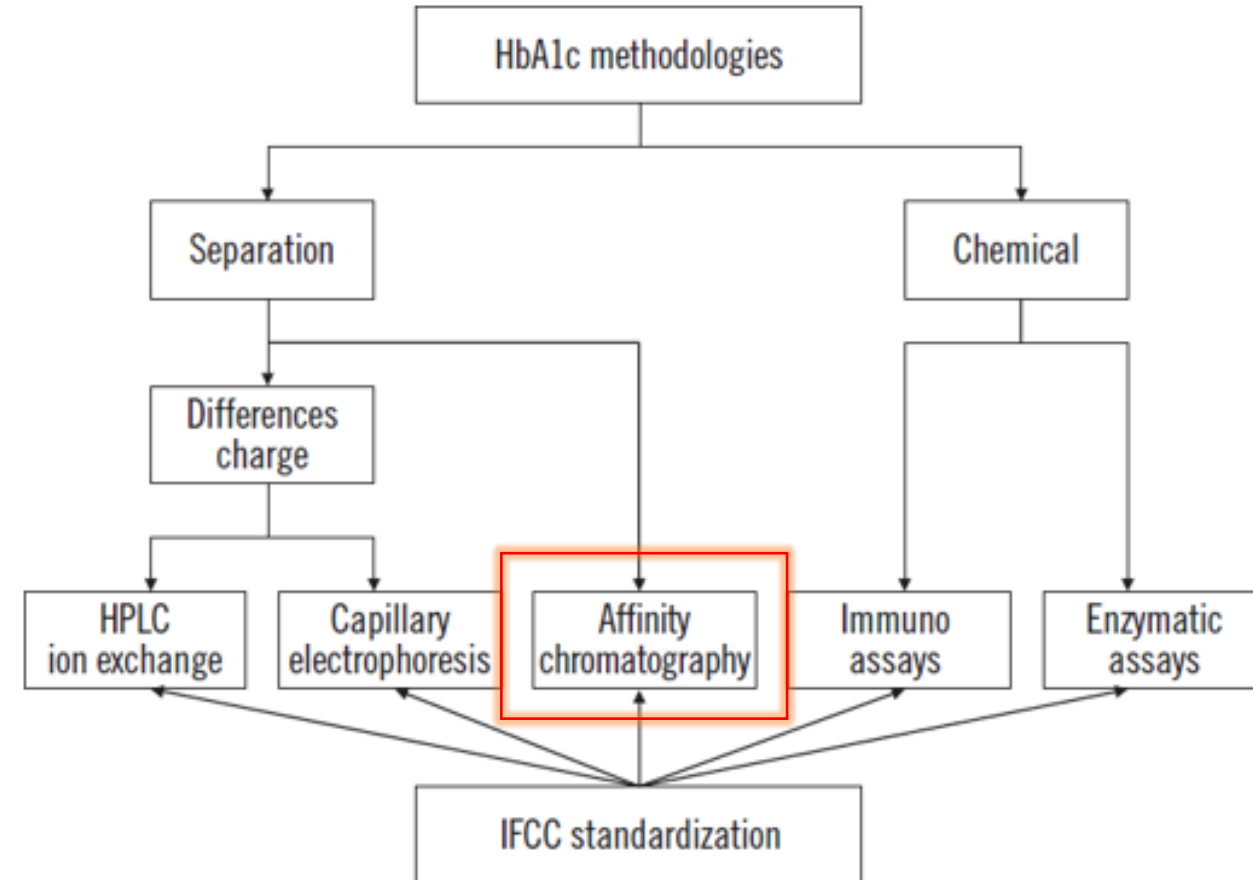


Figure 1: Methods of measurement for HbA1c and traceability to IFCC-RMP.

# Methods of measurement for HbA1C

## Immunoassay

- Routine chemical analyser
- Turbidimetric inhibition technique

## Enzymatic assay

- Routine chemical analyser
- Photometric, potentiometric or turbidimetric method

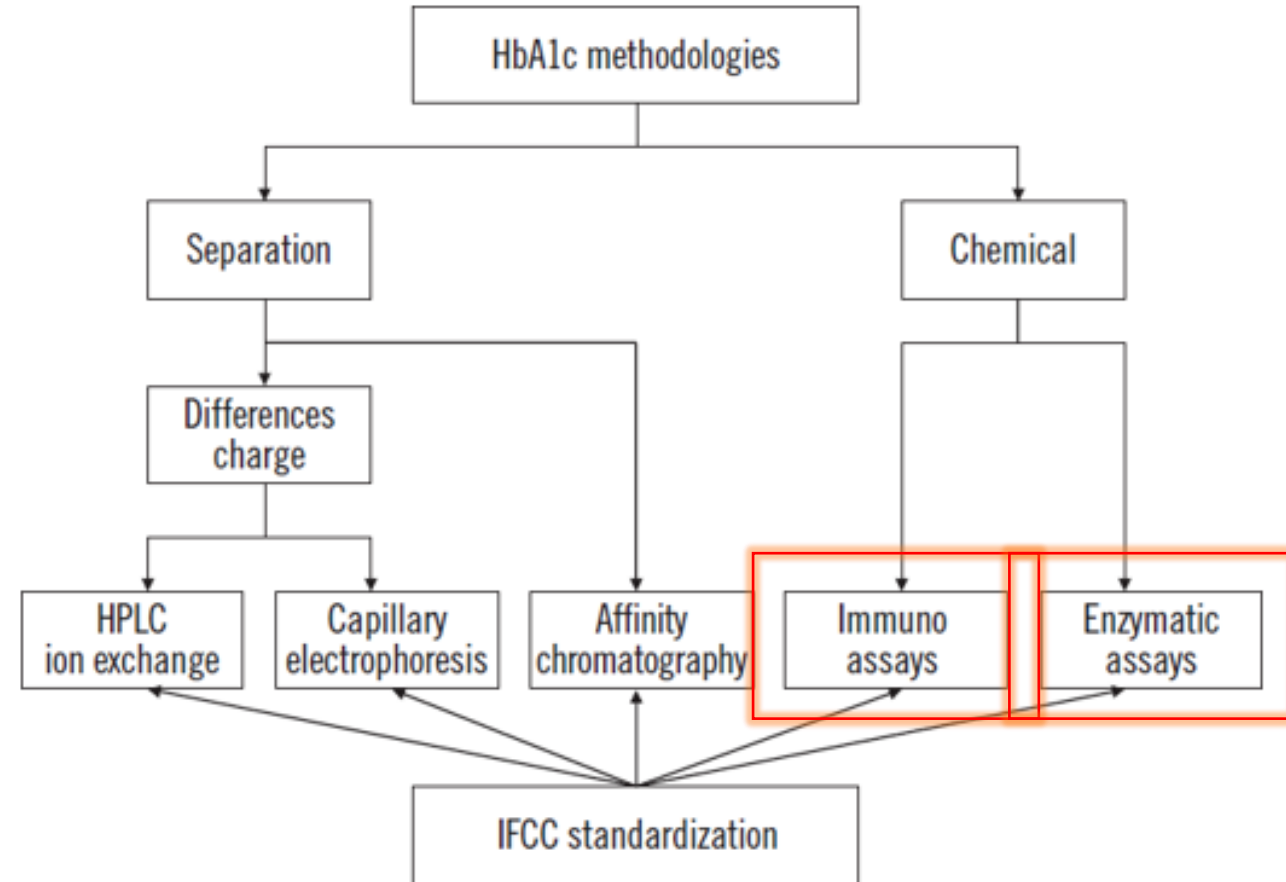


Figure 1: Methods of measurement for HbA1c and traceability to IFCC-RMP.

# Most used analyzers

Table 1. 15 Most used analyzers for HbA1c quantification

1	Abbott Architect c Enzymatic	Abbott Park, Illinois, U.S.A.
2	Alere Afinion	Abbott Park, Illinois, U.S.A.
3	Arkray ADAMS A1c HA-8180V (Menarini)	Adams Arkray, Kyoto, Japan
4	Beckman HbA1c Advanced B00389 Manual Application on DxC 700 AU AU system	Beckman Coulter Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA
5	Beckman HbA1c Advanced B93009 Online Application on DxC 700 AU	Beckman Coulter Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA
6	Beckman Synchron System Unicel DxC	Beckman Coulter Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA
7	Bio-Rad D-100 (A1c program)	Bio-Rad Laboratories N.V.3, Wippinglaan 9140 Temse, Belgium
8	Bio-Rad Variant II Turbo 2.0	Bio-Rad Laboratories, Milan, Italy
9	Ortho-Clinical Vitros	1 International Business Park The Synergy #01-12 Singapore 609917
10	Roche Cobas c513	Roche Diagnostics, Germany
11	Sebia Capillarys 2 Flex Piercing	Capillarys 2FP, Sebia Lisses, France
12	Siemens DCA Vantage	1717 Deerfield Road Deerfield, Illinois, USA
13	Siemens Atellica	1717 Deerfield Road Deerfield, Illinois, USA
14	Siemens Dimension	1717 Deerfield Road Deerfield, Illinois, USA
15	Tosoh G8 ver. 5.24, 5.28	Tosoh Bioscience, Tokyo, Japan

NGSP: Certified Methods and Laboratories update 06/17/2022

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# Standardization of HbA<sub>1c</sub>

## NGSP

- Unit: %
- Directly linked to clinical outcomes
- Criteria:
  - Bias and variability from the NGSP target within  $\pm 2SD$
  - Absolute mean bias between 0 to 0.37%

## IFCC

- Unit: mmol/mol
- Traceability to a higher order reference method
- Based on the sigma metrics
- Criteria:
  - Total error not  $> 5\text{mmol/mol}$  (0.46%) at a  $50\text{mmol/mol}$  (6.7%)

# Evolution of NGSP criteria for certification

**Figure 2.** NGSP certification criteria (16):

Certification type	Certification criteria 1996–1998	Certification criteria 1999–2012	Certification criteria 2013–2018	Certification criteria 2019	Monitoring protocol
Manufacturer	EP-5 precision ( $\leq 5\%$ ) EP-9: 95%CI for predicted bias must overlap $\pm 5\%$ of SRL at 6% and 9% HbA1c	EP-5 precision ( $\leq 5\%$ to $\leq 4\%$ in 2002, dropped in 2007) Bland/Altman assessment of agreement: 95% CI of differences within $\pm 1.0\%$ HbA1c in 1999 to $\pm 0.75\%$ in 2010	37 of 40 results within $\pm 7\%$ in 2013 to $\pm 6\%$ in 2014	36 of 40 results within $\pm 5\%$	None
Level II Lab					
Level I Lab	EP-5 precision ( $\leq 3\%$ ) EP-9: 95%CI for predicted bias must overlap $\pm 3\%$ of SRL at 6% and 9% HbA1c	EP-5 precision ( $\leq 3\%$ , dropped in 2007) 95% CI of differences within $\pm 0.75\%$ HbA1c in 1999 to $\pm 0.70\%$ in 2010	38 of 40 results within $\pm 7\%$ in 2013 to $\pm 6\%$ in 2014	37 of 40 results within $\pm 5\%$	10 samples quarterly

SRL: secondary reference laboratories



# In Belgium: EKE 2022

Figure 3: Sciensano criteria for EKE evaluation in IFCC unit (18)

Parameter	Excellent	Goed	Acceptabel	Slecht	Onacceptabel
Afwijking Doelwaarde	<2 mmol/mol	2 – 2.9 mmol/mol	3 – 3.9 mmol/mol	4 – 4.9 mmol/mol	≥ 5 mmol/mol
Reproduceerbaarheid (CV)*	<2%	2.0 – 2.99%	3.0 – 3.99%	4.0 – 4.99%	≥ 5%
Lineariteit (r)	>0.9970	0.9950-0.9970	0.9900- 0.9949	0.9800- 0.9899	<0.9800

Sciensano criteria for EKE evaluation of accuracy, CV and linearity

Figure 4: Sciensano criteria for EKE evaluation in NGSP unit (18)

Parameter	Excellent	Goed	Acceptabel	Slecht	Onacceptabel
Afwijking Doelwaarde	<0.2%	0.2 - 0.29%	0.30 - 0.39%	0.40 - 0.49%	≥ 0.50%
Reproduceerbaarheid (CV)*	<1.4%	1.4 – 1.99%	2.0 – 2.99%	3.0 – 3.99%	≥ 4%
Lineariteit (r)	>0.9970	0.9950-0.9970	0.9900- 0.9949	0.9800-0.9899	<0.9800

Sciensano criteria for EKE evaluation of accuracy, CV and linearity

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# Pre-analytical considerations

## Patient variables

- Age, gender, ethnicity, physical activity and season
- Erythrocyte lifespan → HbA1c
  - Falsely decreased: hemolytic anemia, hemoglobinopathies etc
  - Falsely increased: IDA, splenectomy
- Biologic variation:
  - With-in subject: 1.9%
  - Between subject: 5.7%

## Sample

- No special collection condition
- No fasting
- Whole blood or hemolysate sample
  - Cool temp: 1 week
  - Freezer: 1 year

# Abbott Architect c

Reproducibility

0.73% – 1.37% IFCC

Accuracy

<3 mmol/mol

Linearity

0.995 – 0.999

- Routine chemical analyser
- Enzymatic method of quantification
  - Glycated dipeptide
  - Total hemoglobin
- 800 analysis per hour
- Run time: 10 min per sample
- Reagent: 350 tests per cartridge
- Stability: 50 days
- Calibration: every 50 days



<https://www.corelaboratory.abbott/int/en/offerings/brands/architect/architect-c4000.html>

Lenters-Westra E, English E. Evaluating new HbA1c methods for adoption by the IFCC and NGSP reference networks using international quality targets. Clin Chem Lab Med. 2017 Aug 28;55(9):1426-1434. doi: 10.1515/cclm-2017-0109. PMID: 28432844.

Tesija Kuna A, Dukic K, Nikolac Gabaj N, Miler M, Vukasovic I, Langer S, Simundic AM, Vrkic N. Comparison of Enzymatic Assay for HBA1C Measurement (Abbott Architect) With Capillary Electrophoresis (Sebia Minicap Flex Piercing Analyser). Lab Med. 2018 Jul 5;49(3):231-238. doi: 10.1093/labmed/lmx090. PMID: 29528429.

# Roche Cobas c513

Reproducibility

1.4% – 2.1% IFCC

Accuracy

<3 mmol/mol

Linearity

0.995

- Routine chemical analyser
- Analysis: turbidimetric immunoassay
- 400 tests per hour
- Run time: 12 minutes
- Reagent kit: 500 tests per cartridge
- Stability: 4 weeks
- Calibration: every 28 days

**cobas c 513 analyzer**



# ADAMS A1c HA-8180V

Reproducibility

0.46% – 0.52% IFCC

Accuracy

0.03 – 0.8 mmol/mol

Linearity

0.996

- Reversed phase cation exchange HPLC
- Run time:
  - 90 seconds per sample: Variant mode
  - 48 seconds per sample: Fast mode
- 40 samples per hour
- Reagent: >400 test per pack
- Stability: until expiration
- Calibration: not routinely done



# Capillarys 2 Flex Piercing

Reproducibility

1.67% – <3.4% IFCC

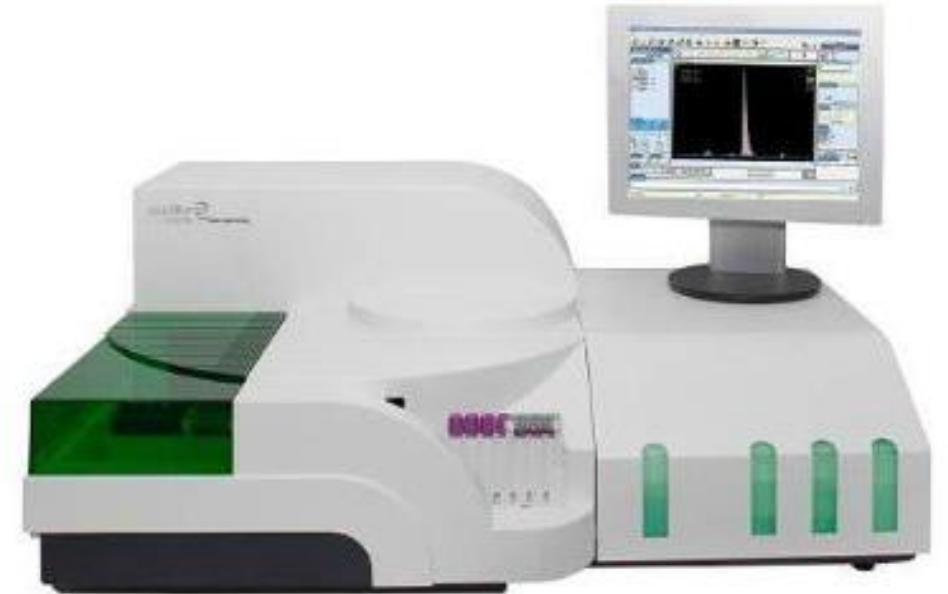
Accuracy

<3 mmol/mol

Linearity

0.996 – 0.999

- Capillary electrophoresis
- Separate Hb variants – surface charge
- Hb variants detected in the following order:
  - HbA2>C>E>S>D>F>AO>A>A1c
- 38 tests per hour (batch of 8)
- Reagent stability:
  - Until expiration – cool temp
  - 20 days – room temperature
- Calibration: every 2 months



# Analytical performance

Table 2. Bias, CV and Linearity per analyzer

	Bias	CV	Linearity (r)
<b>Abbott Architect c</b>	<3 mmol/mol	0.73% – 1.37% IFCC	0.995 – 0.999
<b>Roche Cobas c513</b>	<3 mmol/mol	1.4 – 2.1% IFCC	0.995
<b>ADAMS A1c HA-8180V</b>	0.03 – 0.8 mmol/mol	0.46% - 0.52% IFCC	0.996
<b>Sebia Capillarys 2</b>	< 3mmol/mol	1.67% - <3.4% IFCC	0.996 – 0.999

Table 3. Interpretation

	Bias	CV	Linearity (r)
<b>Abbott Architect c</b>	Good - Excellent	Excellent	Good - Excellent
<b>Roche Cobas c513</b>	Good - Excellent	Good - Excellent	Good
<b>ADAMS A1c HA-8180V</b>	Excellent	Excellent	Good
<b>Sebia Capillarys 2</b>	Good	Acceptable - Excellent	Good - Excellent

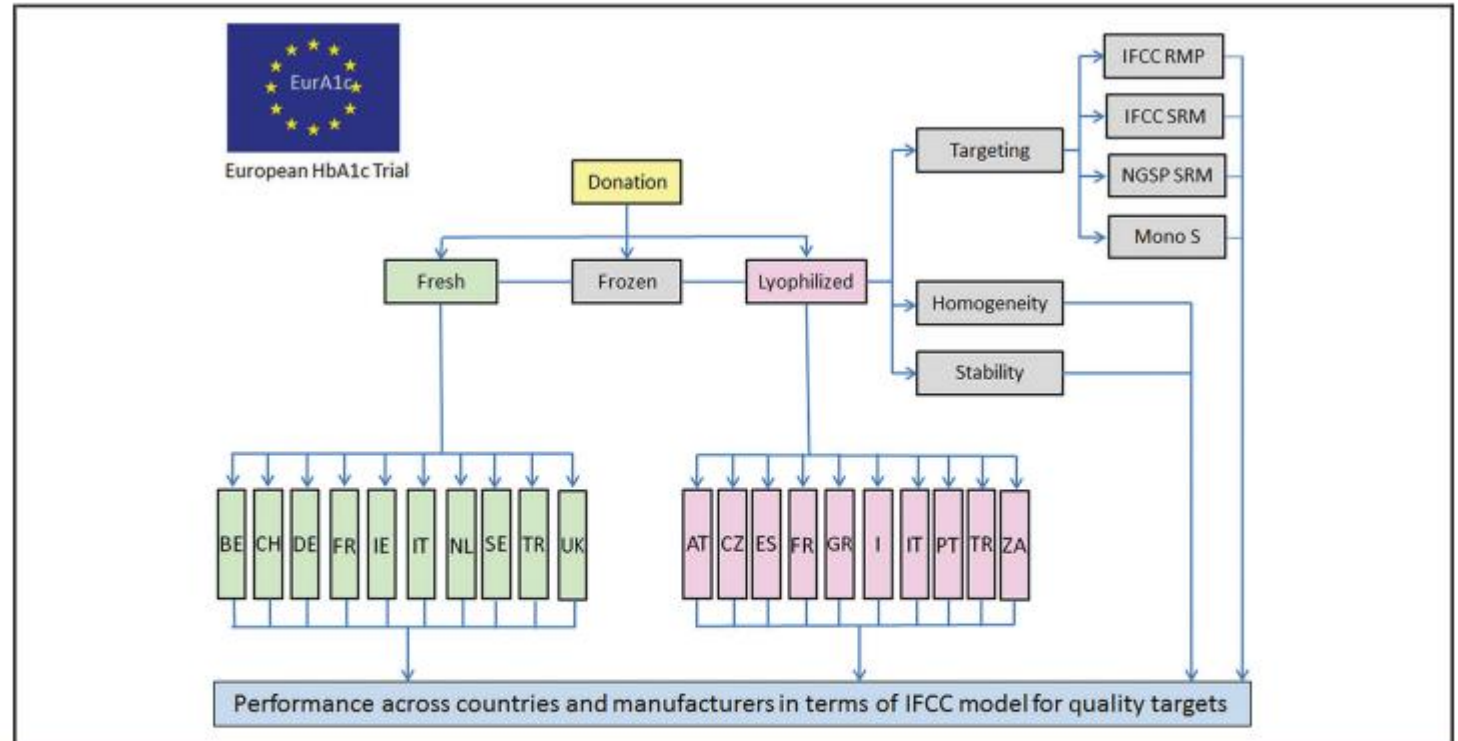


# EurA1c Trial

- 17 EQA
- 17 EU countries
- 24 manufacturers
- 2166 laboratories

- Whole blood
- Lyophilized hemolysate
- Tested 4 fold

- 42.3mmol/mol (6.02%)
- 57.9mmol/mol (7.45%)



**Fig. 1. Design of the European HbA1c Trial.**

Donation (yellow) from which fresh WB (green) and LH (pink) samples are prepared and used in the respective countries (blue); supporting tests (gray). Countries: Austria (AT), Belgium (BE), Switzerland (CH), Czech Republic (CZ), Germany (DE), Spain (ES), France (FR), Greece (GR), group of individual laboratories in multiple countries (I), Ireland (IE), Italy (IT), the Netherlands (NL), Portugal (PT), Sweden (SE), Turkey (TR), United Kingdom (UK), South Africa (ZA).

EurA1c Trial Group. EurA1c: The European HbA1c Trial to Investigate the Performance of HbA1c Assays

**Table 2. Summary per manufacturer of number of participating labs, bias, and BLCV in fresh WB and LH.**

Manufacturer	Fresh WB			LH		
	n	IFCC bias, mmol/mol (BLCV)	NGSP bias, % (BLCV)	n	IFCC bias, mmol/mol (BLCV)	NGSP bias, % (BLCV)
Abbott Architect Enzymatic	21	-0.1 (1.6%)	-0.01 (1.1%)	24	-4.0 (6.0%)	-0.37 (4.0%)
Abbott Architect Immuno	6	-1.8 (4.0%)	-0.16 (2.8%)			
Abbott Other	6	+1.9 (4.6%)	+0.18 (3.0%)			
Alere Afinion	76	-0.7 (3.4%)	-0.06 (2.2%)			
Beckman Coulter AU	26	-0.6 (5.6%)	-0.06 (3.8%)	7	+1.6 (6.5%)	+0.15 (4.4%)
Beckman Coulter UC DxC	15	-1.0 (3.5%)	-0.10 (2.4%)			
Bio-Rad D10	53	+0.8 (4.8%)	+0.07 (3.2%)	37	-1.2 (5.2%)	-0.11 (3.5%)
Bio-Rad D 100	11	-0.8 (1.8%)	-0.08 (1.2%)	16	-0.3 (1.9%)	-0.03 (1.2%)
Bio-Rad Variant	86	+0.9 (4.0%)	+0.08 (2.6%)	38	+1.3 (4.8%)	+0.12 (3.2%)
Medinor	6	-4.7 <sup>a</sup> (14.6%)	-0.43 (9.9%)			
Menarini HA-8160	91	+0.4 (3.4%)	+0.04 (2.3%)	87	-0.6 (2.9%)	-0.06 (2.0%)
Menarini HA-8180	82	+0.4 (3.0%)	+0.03 (2.0%)	72	-0.7 (3.5%)	-0.06 (2.4%)
Not Known	123	0.0 (5.3%)	0.00 (3.6%)	14	-0.8 (8.1%)	-0.07 (5.4%)
Roche	288	-0.9 (4.4%)	-0.08 (3.0%)	100	-0.1 (4.9%)	-0.01 (3.3%)
Sebia CapillaryS 2	57	-0.4 (2.6%)	-0.04 (1.8%)	45	-1.4 <sup>a</sup> (2.5%)	-0.14 (1.7%)
Sebia CapillaryS 3	8	0.0 (2.3%)	0.00 (1.6%)	9	-1.3 (2.1%)	-0.12 (1.4%)
Sebia Minicap	10	-0.8 (2.5%)	-0.08 (1.7%)			
Siemens Advia	15	+3.5 <sup>a</sup> (4.8%)	+0.32 (3.2%)			
Siemens DCA/Vantage	158	+0.6 (3.6%)	+0.06 (2.4%)	6	+4.0 (3.6%)	+0.38 (2.4%)
Siemens Dimension	47	0.0 (4.0%)	0.00 (2.7%)	17	+0.4 (4.7%)	+0.04 (3.1%)
Siemens Other	13	-0.3 (4.2%)	-0.03 (2.8%)			
Tosoh G7	27	+1.1 (5.6%)	+0.10 (3.8%)	33	-0.4 (4.7%)	-0.04 (3.2%)
Tosoh G8	234	+1.0 <sup>a</sup> (2.6%)	+0.09 (1.8%)	85	-0.7 (3.9%)	-0.07 (2.6%)
Trinity Premier Hb9210	27	+1.2 (3.8%)	+0.10 (2.5%)	16	-0.8 (3.7%)	-0.08 (2.5%)

<sup>a</sup> Significant different from target ( $P < 0.05$ ).

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# Hb derivatives and serum indices

	<u>LHb</u>	<u>cHb</u>	Bil	TAG
<b>Abbott Architect c</b>	Yes > 10.1%	Yes > 10%	453 µmol/l	11.2mmol/L
<b>Roche Cobas c513</b>	-	Yes > 10 - 15%	352 µmol/L	20.6 mmol/L
<b>ADAMS A1c HA-8180V</b>	No*	No	268 µmol/L	-
<b>Sebia Capillarys 2</b>	No**	No	280 µmol/L	26mmol/L

Bil – bilirubin; TAG – triglyceride; \* 1 mmol/mol difference from the target value;

\*\*within ±1 mmol/mol from the measured baseline HbA<sub>1c</sub>

# Common Hemoglobin variants

	<i>Method</i>	<i>HbC</i>	<i>HbS</i>	<i>HbE</i>	<i>HbD</i>	<i>HbF</i>
<b>Abbott Architect c</b>	Enzymatic	No	No	No	No	-
<b>Roche Cobas c513</b>	Immunoassay	No	No	No	No	No
<b>ADAMS A1c HA-8180V</b>	HPLC	No	No	Yes*	Yes**	-
<b>Sebia Capillars 2</b>	Electrophoresis	No	No	No	No	Yes > 15%

NGSP: Factors that interfere with HbA<sub>1c</sub> test results update 06/17/2022

\*small peak at the edge of A<sub>0</sub> – gives unreportable results;

\*\*extra peak in S/C window – reported as abnormal separation

	Abbott Architect c	Roche Cobas c513	ADAMS A1c HA-8180V	Sebia Capillarys 2
<b>Method</b>	Enzymatic assay	Immunoassay	Ion exchange chromatography	Capillary electrophoresis
<b>Sample</b>	Whole blood and hemolysate	Whole blood and hemolysate	Whole blood and hemolysate	Whole blood
<b>Reagent kit</b>	350 test stable for 50days	500 test stable for 28 days	400 test per kit stable until expiry date	38 test stable for 2 months
<b>Sampling time</b>	800 test per hour	400 test per hour	40 samples per hour	38 samples per hour in batch of 8
<b>Analytical performance</b>				
CV	Excellent	Good – Excellent	Excellent	Acceptable
Bias	Good – Excellent	Good – Excellent	Excellent	Good
Linearity (r)	Good – Excellent	Good	Good	Good - Excellent
<b>Influence of Hb variants</b>	No interference with HbC, HbS, HbE, HbD but no data over HbF	No interference with HbC, HbS, HbE, HbD and HbF	Interference with HbD and HbE	Interference with HbF >15%
<b>Effect of labile Hb and cHb</b>	Yes > 10%	Yes > 10-15 %	No	No
<b>Chromatogram</b>	No	No	Yes	Yes
<b>Bidirectional coupling with Glims</b>	Yes	Yes	Yes	Yes
<b>Price comparison</b>	+	-	++	++++

# Thank you

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# We ♥ icons

