Fully automated HPLC hemoglobin analyzer

Hb NEXT

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Erna Lenters-Westra
W. Garry John
Emma English

A.MENARINI diagnostics
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Erna Lenters-Westra[1], W. Garry John[2], Emma English[3]

1 European Reference Laboratory for HbA1c, Isala, Clinical Chemistry Department, Zwolle, The Netherlands
2 Norfolk and Norwich University Hospital, Norwich, UK
3 Faculty of Medicine and Health, University of East Anglia, Norwich, UK

Diabetes represents a serious global epidemic: about **537 million** adults (20-79 years) are living with diabetes: 1 in 10[1]. This number is predicted to rise to **643 million** by 2030 and 784 million by 2045. Diabetes was responsible for **6.7 million** deaths in 2021 - 1 every 5 seconds.
Precise and accurate measurement of hemoglobin A1c (HbA1c) is widely used as it plays an important role in predicting the prognosis of the therapeutic effect, as well as in diabetes diagnosis and follow-up.

Source: https://diabetesatlas.org/

ABSTRACT

This is a comparison study of Menarini's Hb NEXT analyzer, the new generation HPLC for HbA1c detection. We evaluated the analytical performance in comparison with six different reference systems for HbA1c testing. The Hb NEXT passed the IFCC criteria and the overall analytical performance was very good to excellent. Hemoglobin variants are an important aspect, they can interfere with HbA1c measurements, but when detected, provide essential information on the presence of a variant. It is important for the interpretation of the HbA1c result and for genetic counselling.
We evaluated hemoglobin variants AS, AC, AE, AD, elevated A2 and HbF.
The variants did not affect the correct measurement of HbA1c. All variants are easily recognized in the chromatogram, and correctly interpreted by the instrument.

METHODS

The CLSI EP-5, EP-9, EP-15, EP-6 protocols were used to evaluate reproducibility, trueness and linearity. A 24-sample panel from IFCC was tested to compare performance of the Hb NEXT with the other major methods on the market. The evaluation was performed at the Clinical Chemistry Department in Isala in Zwolle.

The 6 IFCC and NGSP Certified Secondary Reference Measurement Procedures (SRMPs) used in comparison to Hb NEXT were:

- Roche Tina-quant Gen.3 HbA1c on Cobas c513, immunoassay, IFCC and NGSP certified (Roche Diagnostics);
- Premier Hb9210, affinity chromatography HPLC, IFCC and NGSP certified (Trinity Biotech);
- Tosoh G8, cation-exchange HPLC, IFCC certified (Tosoh Bioscience);
- Abbott Enzymatic method on Alinity, IFCC and NGSP certified (Abbott Diagnostics);
- Sebia Capillarys 3 Octa, capillary electrophoresis, IFCC and NGSP certified (Sebia);
- HA-8180V, cation-exchange HPLC, IFCC and NGSP certified (A.Menarini Diagnostics).

RESULTS

Reproducibility

Reproducibility was investigated using CLSI EP-15 and EP-5 protocols.

With protocol EP-15, 3 SRL-IFCC HbA1c calibrators and 2 Lifotronic controls were tested five times for 5 days.

With protocol EP-5, 2 Lifotronic controls and aliquots from two patient samples (stored at –80°C until analysis) were tested in duplicate twice a day for 20 days. CVs were also calculated using duplicates of the fresh patient samples in the EP-9 protocol. All CVs were well within the acceptance limits (< 3% SI units, < 2% in NGSP units).

	%CV (Mean HbA1c SI units)	%CV (Mean HbA1c NGSP units)
Patient samples	2.0 (44.1 mmol/mol)	1.3 (6.18%)
	1.6 (72.4 mmol/mol)	1.2 (8.78%)
Controls	1.7 (34.4 mmol/mol)	1.0 (5.30%)
	1.4 (84.6 mmol/mol)	1.1 (9.89%)
Duplicates EP-9	0.8	0.6

Table 1
Imprecision results based on EP-5 and from the duplicates in EP-9

All CVs were well within the acceptance limits (CV: < 3% SI units, < 2% in NGSP units). The bias was for all SRL-IFCC calibrators and control 1 ≤2.0 mmol/mol (≤0.2% NGSP) and for control 2 -2.5 mmol/mol which was within the criteria of the package insert of the controls (within the range of ± 10% of the assigned value). Total Allowable Error (TAE) for all 5 samples was ≤5.1%.

Sample	Assigned Value (mmol/mol)	Mean measured (mmol/mol)	Bias (mmol/mol)	Within Run CV (%)	Total CV (%)	TAE (%)
IFCC Cal 1	30.6	30.6	0.0	1.1	1.3	2.6
IFCC Cal 2	59.9	61.4	1.5	0.7	0.9	4.3
IFCC Cal 3	89.2	89.3	0.1	0.8	0.9	1.9
Control 1	34.4	34.8	0.4	1.1	1.3	3.8
Control 2	88.0	85.8	-2.2	1.1	1.3	5.1

Table 2
Imprecision results based on EP-15

Trueness

Trueness was investigated according to CLS EP-9 protocol. With this protocol 40 frozen patient samples (8 samples per day for 5 days in duplicate) were tested with Hb NEXT and the 6 SRMPs.
The data were used to calculate the trueness and compare the methods between Hb NEXT and the single SRMP and the mean of 6 SRMPs in SI units.

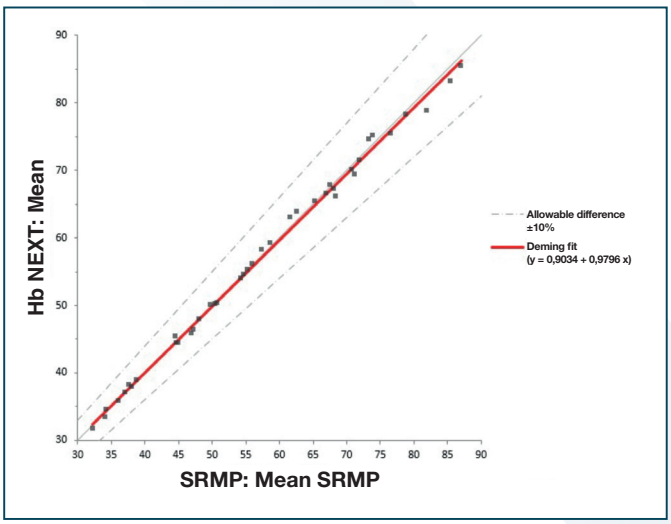

Figure 1
Mean 6 SRMP versus Hb NEXT in SI units

	HbA1c (mmol/mol) (Mean SRMP vs Hb NEXT)
EP-9 parameters	
Slope (95% CI)	0.979 (0.957 to 1.002)
Intercept (95% CI)	0.903 (-0.209 to 2.016)
Bias at different HbA1c levels	
30 mmol/mol (95% CI)	30.3 (29.81 to 30.77)
48 mmol/mol (95% CI)	47.9 (47.68 to 48.16)
75 mmol/mol (95% CI)	74.4 (73.73 to 75.01)

Table 3
Trueness of Hb NEXT as assessed by EP-9 protocol in SI units

There were no statistically significant differences for slope and intercept at 30, 48 and 75 mmol/mol between Hb NEXT and the mean of the 6 SRMPs in SI units. The data were also used to calculate the NGSP certifcation criteria for Hb NEXT. The Hb NEXT met the NGSP criteria compared with all 6 SRMPs.

Deming regression lines			Bias	Samples Out ± 5% SRMP	NGSP criteria*
Hb NEXT (Y)	vs Premier (X)	Y=0.95X + 0.31	-0.08	1	Passed
	vs Abbott (X)	Y=0.98X + 0.15	-0.01	0	Passed
	vs Tina-quant (X)	Y=0.97X + 0.18	-0.01	0	Passed
	vs Tosoh G8 (X)	Y=0.98X + 0.11	-0.02	0	Passed
	vs HA-8180V	Y=0.98X + 0.14	-0.01	0	Passed
	vs Sebia Cap 3	Y=0.99X + 0.05	0.01	0	Passed

Table 4
EP-9 results in NGSP units and results of calculations of NGSP certification criteria

*36 of the 40 samples must be within 5% relative of the SRMP

Linearity

Linearity was assessed by using the CLSI EP-6 protocol.

For this protocol, patient samples with a low HbA1c value and a high HbA1c value were mixed in incremental amounts to generate a series of samples (n=11) over a broad HbA1c concentration range.

The samples were made fresh and then frozen at -80 °C until analysis. Eleven samples were analyzed in duplicate on one day. The difference between the fitted values of the linear line or the best polynomial line and the regression line for the 11 samples was compared. CLSI states for EP-6 that goals for linearity should be derived from goals for bias, and should be less than or equal to these goals.

The IFCC Task Force on Implementation of HbA1c Standardization has set a TAE of 10% at an HbA1c concentration of 50 mmol/mol. Taking into account the whole clinical relevant range, we set a TAE of 6 mmol/mol with a nonlinearity budget of 50% (=3 mmol/mol).

If the deviation exceeds allowable nonlinearity (3 mmol/mol) the data is considered nonlinear. The Hb NEXT passed the set criteria not exceeding nonlinearity of 3 mmol/mol. The Hb NEXT was linear throughout the assessed range; as there was an observed nonlinearity of 0 mmol/mol.

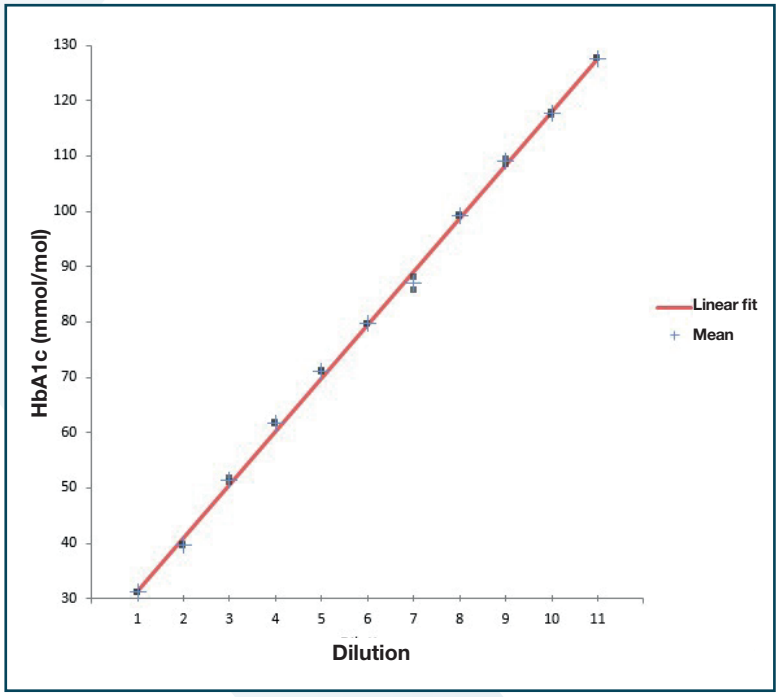

Figure 2
Results linearity study with Hb NEXT.
Hb NEXT was linear from 31 mmol/mol up to at least 127 mmol/mol

Interferences

We investigated three different interferences: Schiff base, different hemoglobin concentrations, and bilirubin.

Schiff base

Three samples were split into two aliquots. The first aliquot was stored in the refrigerator (sample with normal Schiff base). From the second aliquot the plasma was aspirated into a separate tube. The volume was measured and glucose was added (12, 16, 20 mg/ml respectively). After two hours of incubation, the plasma was added to the red cells again, followed by additional 4 hours of incubation at 37°C (sample with high Schiff base).
Table 5 shows the difference in HbA1c concentrations (mmol/mol) between the original sample and the same sample incubated with 12, 16 and 20 mg/ml, respectively.

Schiff base	**HbA1c in SI units (mmol/mol)**			
Sample/glucose incubation	Hb NEXT		Mean	Diff (%)
S1 - original result	36.2	35.6	35.9	
S1 + 20 mg/mL	37.1	37.1	37.1	3.3
S2 - original result	44.5	45.5	45.0	
S2 + 16 mg/mL	48.5	49.4	49.0	8.8
S3 - original result	71.7	72.7	72.2	
S3 + 12 mg/mL	72.4	73.4	72.9	1.0

Table 5
Interference of Schiff base (labile HbA1c)

Even a large amount of Schiff base did not interfere with Hb NEXT: for the sample with 20 mg/ml glucose the relative difference is 3.3%.

Different hemoglobin concentrations

EDTA plasma was collected (20 ml), centrifuged and plasma was aspirated into a separate tube. From the packed cells, 1 ml was dispensed into 6 tubes after which the separated plasma was added in different volumes to obtain different hematocrit concentrations (as measured by Hb concentration). The samples were made fresh and then frozen at -80 °C degrees until analysis. Hemoglobin in the range of 3.3 to 16.9 mmol/L did not affect HbA1c quantification with Hb NEXT (Table 6). The maximum bias was 2.8% so well within the TAE of 10%.

Hematocrit	**HbA1c in SI units (mmol/mol)**			
Sample/Hb concentration (mmol/L)	Hb NEXT		Mean	Diff (%)
Original sample	47.0	46.3	46.7	
S1 Hb 3.3 mmol/L	46.5	46.2	46.4	-0.6
S2 Hb 5.1 mmol/L	47.0	46.9	47.0	0.6
S3 Hb 6.9 mmol/L	47.1	47.1	47.1	1.0
S4 Hb 8.4 mmol/L	47.3	47.4	47.4	1.5
S5 Hb 9.9 mmol/L	47.4	48.0	47.7	2.3
S6 Hb 12.5 mmol/L	47.6	48.3	48.0	2.8
S7 Hb 16.9 mmol/L	47.3	46.8	47.1	0.9

Table 6
Results interference of different Hb concentrations

Bilirubin

Three samples with normal bilirubin concentrations (<10 µmol/L) were split into two aliquots. For each, the first aliquot was stored in the refrigerator (sample with normal bilirubin concentration). From the second, the plasma was removed and replaced by an equal amount of plasma with high bilirubin concentrations (335, 275 and 242 µmol/L respectively for the three different samples).
As in table 7, bilirubin concentrations up to 335 µmol/L did not affect HbA1c quantification with Hb NEXT. Bias at a bilirubin concentration of 335 µmol/L was 1.2% so within the criteria of TAE of 10%.

Bilirubin	**HbA1c in SI units (mmol/mol)**			
Sample /bilirubin concentration	Hb NEXT		Mean	Diff (%)
S1 <10 µmol/L	37.6	37.7	37.7	
S1 + 335 µmol/L	38.2	38.0	38.1	1.2
S2 <10 µmol/L	52.3	51.7	52.0	
S2 + 275 µmol/L	50.8	51.4	51.1	-1.7
S3 <10 µmol/L	72.1	72.9	72.5	
S3 + 242 µmol/L	72.2	72.7	72.5	-0.1

Table 7
Results of different bilirubin concentrations

Hemoglobin variants

Six hemoglobin variants were investigated: AS, AC, AE, AD, elevated A2 and elevated HbF. For each heterozygous Hb variant 20 patient samples from our frozen whole blood bio-bank were measured on the Sebia Capillarys 3, Menarini HA-8180V and Hb NEXT. HbA1c values were assigned earlier using Trinity Biotech Premier Hb9210. The samples were measured in duplicate on Hb NEXT. Results were corrected for bias found in non- variant samples with the Premier Hb9210 in EP-9.

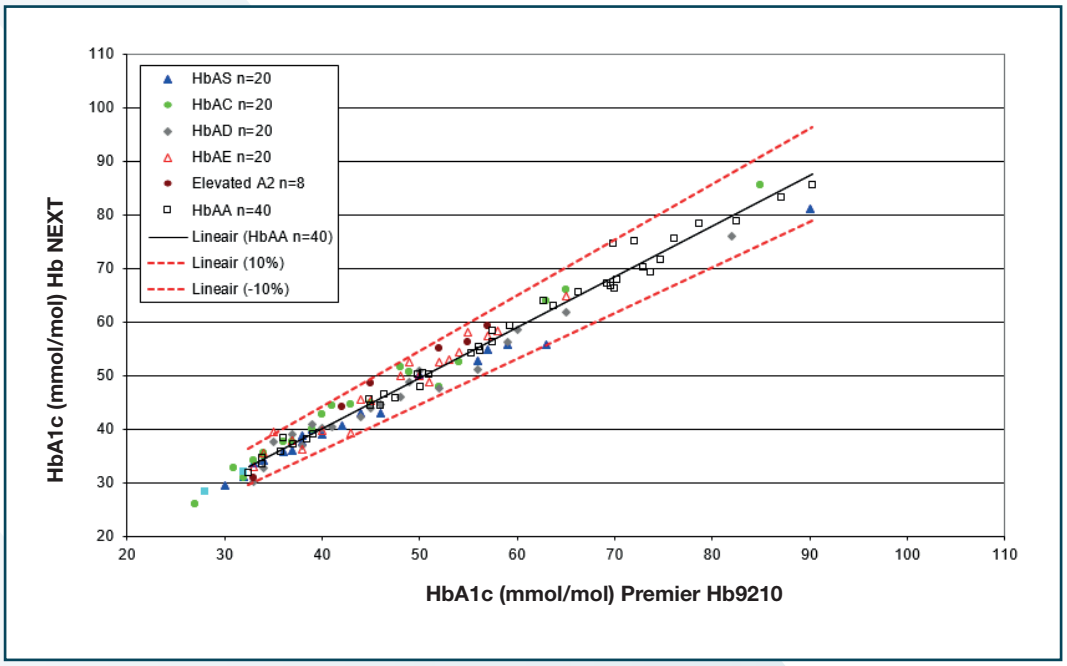

Figure 3
Interference of the HbA1c result by different Hb-variants: Hb NEXT mean vs Premier Hb9210

Table 8 shows the mean relative difference (%) of the common Hb-variants compared to the assigned value and corrected for bias in non-variant samples with the number of samples tested shown in brackets.

	HbAS (n=20)	HbAC (n=20)	HbAD (n=20)	HbAE (n=20)
Hb NEXT	-2.1	3.2	-1.1	-2.6

Table 8
Mean relative difference (%) of common Hb variants vs the assigned values

Hb NEXT had no interference of HbAC, HbAD, HbAE, elevated A2 and HbF <19% and HbAS when HbA1c was <59 mmol/mol.

PERFORMANCE IN EQA PROGRAM

IFCC Certification Program for manufacturers

This program is designed for manufacturers to be able to demonstrate their degree of traceability to the IFCC Reference method. The program consists of 24 blind, frozen, whole blood samples.
From the results the bias is calculated at low (30 mmol/mol), medium (50 mmol/mol) and high (70 mmol/mol) HbA1c levels. The medium level is used to calculate Total Error (TE = |B| + 2I). Reproducibility (CV) is calculated from deviations from the regression line. Linearity (R) iscalculated from the linear relation with target values.
Table 9 summarizes the results. The performance criterion is a Total Error Allowable (TEa) of 5 mmol/mol and the risk of failure is set at 2 sigma. In addition, grades are given: TEa < 3.3 = Bronze, <2.2 = Silver, <1.1 = Gold. As can be seen, the grading was silver for the IFCC certification.
To get an idea of how the results from EP-5, EP-9, and EQA relate to each other, results were presented in one sigma metrix graph. Figure 4 shows the analysis of the performance of HbNext using sigma metrix statistics.

	IFCC certification HbA1c (mmol/mol)
Regression parameters	
Slope	0.99
Intercept	0.40
EQA result	
Deviation from target 30 mmol/mol 50 mmol/mol 70 mmol/mol	 0.0 -0.3 -0.4
Total Error	1.7
Bias	0.3
CV (%)	1.4
R	0.9983
Pass/Fail	Silver/Passed

Table 9
Performance in EQAS.
Performance of Hb NEXT in the IFCC Certification Program (24 frozen whole blood samples)

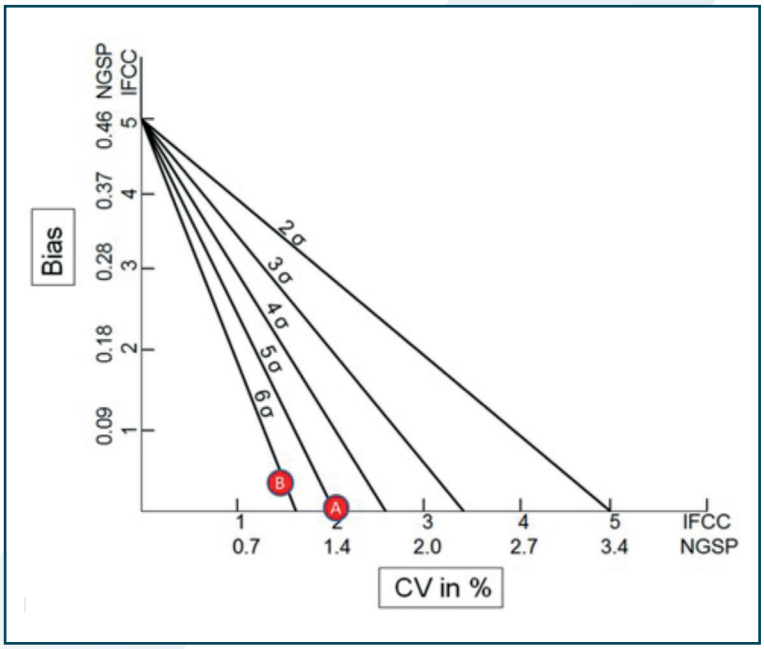

Figure 4
Analytical performance in sigma metrix for Hb NEXT using results of EP-5 and EP-9 (A:CV=2.0%, bias at 50 mmol/mol= -0.1, σ=4.9), frozen IFCC certification samples (B:CV=1.4%, bias at 50 mmol/mol=-0.30, σ=6.7)

TAKE HOME MESSAGE

Results of the evaluation of Hb NEXT, the A.Menarini Diagnostics HPLC analyzer for HbA1c detection

Analytical performance

Hb NEXT passed the IFCC criteria and the analytical performance was in general very good to excellent.

Hemoglobin variants (AS, AC, AE, AD, elevated A2 and HbF)

The variants did not affect the correct measurement of HbA1c. All variants are easily recognized in the chromatogram, and correctly interpreted by the instrument.

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A.MENARINI diagnostics

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Fully automated HPLC hemoglobin analyzer

For professional users only

List of products and codes

55607	Hb NEXT Analyzer	1 pcs
55604	Hb NEXT Column	1 pcs
55599	Hb NEXT Eluent A	1x800 mL
55600	Hb NEXT Eluent B	1x800 mL
55601	Hb NEXT Eluent C	1x800 mL
55603	Hb NEXT Haemolysis solution H	1x2500 mL
55602	Hb NEXT Eluent D	1x800 mL
55606	Hb NEXT HbA1c Calibrator	2x0,1 mL
55605	Hb NEXT HbA1c Control	2x0,1 mL
56149	ß-THALASSAEMIA & HbA1c Calibrator	2x0,1 mL
56150	ß-THALASSAEMIA & HbA1c Control Material	2x0,1 mL

Hb NEXT is available in the following A.Menarini Diagnostics countries:

Austria: https://www.menarinidiagnostics.at
Benelux: https://www.menarinidiagnostics.be
France: https://www.menarinidiagnostics.fr
Greece: https://www.menarinidiagnostics.gr
Italy: https://www.menarinidiagnostics.it
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