

CAPILLARYS 3 TERA: First Evaluation of a High Throughput Capillary Electrophoresis Instrument for High Resolution HbA1c Separation

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Abstract

The efficient measurement of HbA_{1c} will become of growing importance as the prevalence of type II diabetes mellitus increases. To this end the CAPILLARYS 3 TERA Instrument (Sebia, France) for high throughput HbA_{1c} testing has been developed. In this study we evaluated the instrument with respect to trueness, precision and correlation to CAPILLARYS 2.

The trueness was assessed by measuring 6 samples calibrated to IFCC secondary reference material. The measurements were performed as triplicates and the mean was calculated. The CAPILLARYS 3 TERA measured HbA_{1c} values slightly below the target values with the bias ranging from 0% to 0.2% (0.3 to 1.7 mmol/mol). The instruments precision was tested in pooled samples stored in aliquots at -80°C. Two determinations for each of the 12 capillaries were performed during 12 days. At a mean HbA_{1c} concentration of 4.4% (24.6 mmol/mol) the coefficient of variation (CV) was 1.3%, at 6.3% (45.4 mmol/mol) the CV was 1%, at 7.5% (58.5 mmol/mol) the CV was 0.6% and at 10.5% (91.3 mmol/mol) the CV was calculated to be 0.9%.

2703 HbA_{1c} routine samples were analyzed on both the CAPILLARYS 3 and the CAPILLARYS 2 electrophoresis systems and the results were correlated. The regression line followed the equation $y=0.9959x+0.0287$ with a correlation coefficient of $r=0.994$.

The implementation of capillary electrophoresis for the routine testing of HbA_{1c} has considerably improved our work flow. Due to the high resolution of different hemoglobin species provided by capillary electrophoresis it is possible to rely on the automated data analysis to reduce the time required by laboratory technicians to supervise the analytical process. In 2014 308.498 samples from 133.140 individuals were analyzed for HbA_{1c}. A total of 280 abnormal hemoglobin profiles were detected indicating that the prevalence is in the order of 0.21%. For these patients further diagnostic testing is necessary to arrive at a correct interpretation of the HbA_{1c} result and improve clinical practice.

In summary HbA_{1c} testing by capillary electrophoresis is an accurate method improving workflow and quality of laboratory analysis. The newly developed CAPILLARYS 3 TERA is a high throughput laboratory automate processing up to 70 samples an hour that is equivalent in analytical performance to the well established CAPILLARYS 2 systems.

Results

The CAPILLARYS 3 TERA (Figure 1) instrument's trueness was assessed by measuring 6 samples whose target values were assigned by IFCC Network Laboratories for HbA_{1c} with methods calibrated to IFCC secondary reference material. The measurements were performed as triplicates and the mean was calculated (Table 1). The CAPILLARYS 3 TERA measured HbA_{1c} values slightly below the target values with an overall Bias of 0.1% (1.1 mmol/mol) HbA_{1c}. The target HbA_{1c} values were: 5.3% (34 mmol/mol), 5.7% (39 mmol/mol), 6.2% (44 mmol/mol), 6.8% (51 mmol/mol), 7.5% (58 mmol/mol), and 9.1% (76 mmol/mol). The values measured on the CAPILLARYS 3 TERA instrument were: 5.1% (33.0 mmol/mol) 5.5% (37.3 mmol/mol), 6.1% (43.0 mmol/mol), 6.7% (50.0 mmol/mol), 7.5% (57.7 mmol/mol) and 9.0% (74.3 mmol/mol) for the respective samples.

The instruments precision was tested by measuring HbA_{1c} in pooled samples stored in aliquots at -80°C over a period of twelve days (Table 1). The samples were measured in the morning and in the afternoon so that two determinations on each of the twelve capillaries were performed. At a mean concentration of 4.4% (24.6 mmol/mol) HbA_{1c} the coefficient of variation (CV) was 1.3%, at 6.3% (45.4 mmol/mol) HbA_{1c} the CV was 1%, at 7.5% (58.5 mmol/mol) HbA_{1c} the CV was 0.6% and at 10.5% (91.3 mmol/mol) HbA_{1c} the CV was calculated to be 0.9% (Table 2).

For the Intra-Assay precision controls were measured for at least 11 days. The CV ranged from 0.3 to 1.9% (Table 3).

The HbA_{1c} measurements on the new CAPILLARYS 3 TERA was compared to the capillary electrophoresis system CAPILLARYS 2 Flex Piercing established in our laboratory. Over a period of 17 consecutive work days 2703 routine samples were analyzed on both electrophoresis systems and the results were correlated. The measured values ranged from 4.2 to 14.4 % HbA_{1c}. The regression line followed the equation $y=0.9959x+0.0287$ with a correlation coefficient of $r=0.9959$ (Figure 2).

Summary

The CAPILLARYS 3 TERA represents the next generation of analytic instruments that extends the precision of separation methods such as electrophoresis and chromatography to high sample throughput and the analytic flexibility needed in the routine laboratory nowadays. The CAPILLARYS 3 processes 70 samples per hour and can change between analytical techniques automatically. The instruments handling has been improved by providing a LCD screen to pilot maintenance procedures and give instantaneous information on the instrument status. RFID tagged reagents and buffers as well as bulk loading of samples reduce the hands on time of technicians. In our trueness study we demonstrated the CAPILLARYS 3 measures HbA_{1c} with a small bias of lower HbA_{1c} values compared to IFCC standardized methods. The instrument measures HbA_{1c} with an excellent precision as demonstrated by the determination of pooled patient samples and controls. Further the correlation of the HbA_{1c} values determined on the CAPILLARYS 3 TERA was almost identical to determinations performed on the established CAPILLARYS 2 Flex Piercing system.

Materials and Methods

For the determination of the Trueness of HbA_{1c} measurement of the CAPILLARYS 3 TERA SEBIA provided 6 samples stored at -80°C, whose target values were assigned by IFCC Network Laboratories for HbA_{1c} with methods calibrated to IFCC secondary reference material. The samples were processed in triplicate on the CAPILLARYS 3 TERA to assess the trueness. For each sample, the mean bias from the target value was calculated.

The Precision of HbA_{1c} measurement for the CAPILLARYS 3 TERA was tested on 4 pools prepared from fresh samples with different HbA_{1c} concentrations. EDTA-samples with no hemoglobin disorders were pooled. Patients were chosen to create pools with HbA_{1c} concentrations of 4.4% (24.6 mmol/mol), 6.3% (45.4 mmol/mol), 7.5% (58.5 mmol/mol) and 10.5% (91.3 mmol/mol). The pooled samples were stored in aliquots at -80°C, and thawed only once for analysis. For the further assessment of the interassay variation control samples with a target value of 5.1% and 8.5% HbA_{1c} were analyzed on each of the 12 capillaries daily for a period of 12 and 11 days respectively.

The correlation between CAPILLARYS 3 TERA and CAPILLARYS 2 Flex Piercing was studied by analyzing EDTA-samples from the daily laboratory routine on both instruments. In total 2703 samples were analyzed over a period of 17 consecutive workdays. Samples were either processed on the same day or stored overnight at 4°C. For each sample of this correlation study, in case of discrepant results 1 aliquot was kept at -80°C for possible complementary analysis. Correlations were calculated on the whole range of HbA_{1c} measurements, coefficient of correlation (r) and the line of regression was determined.

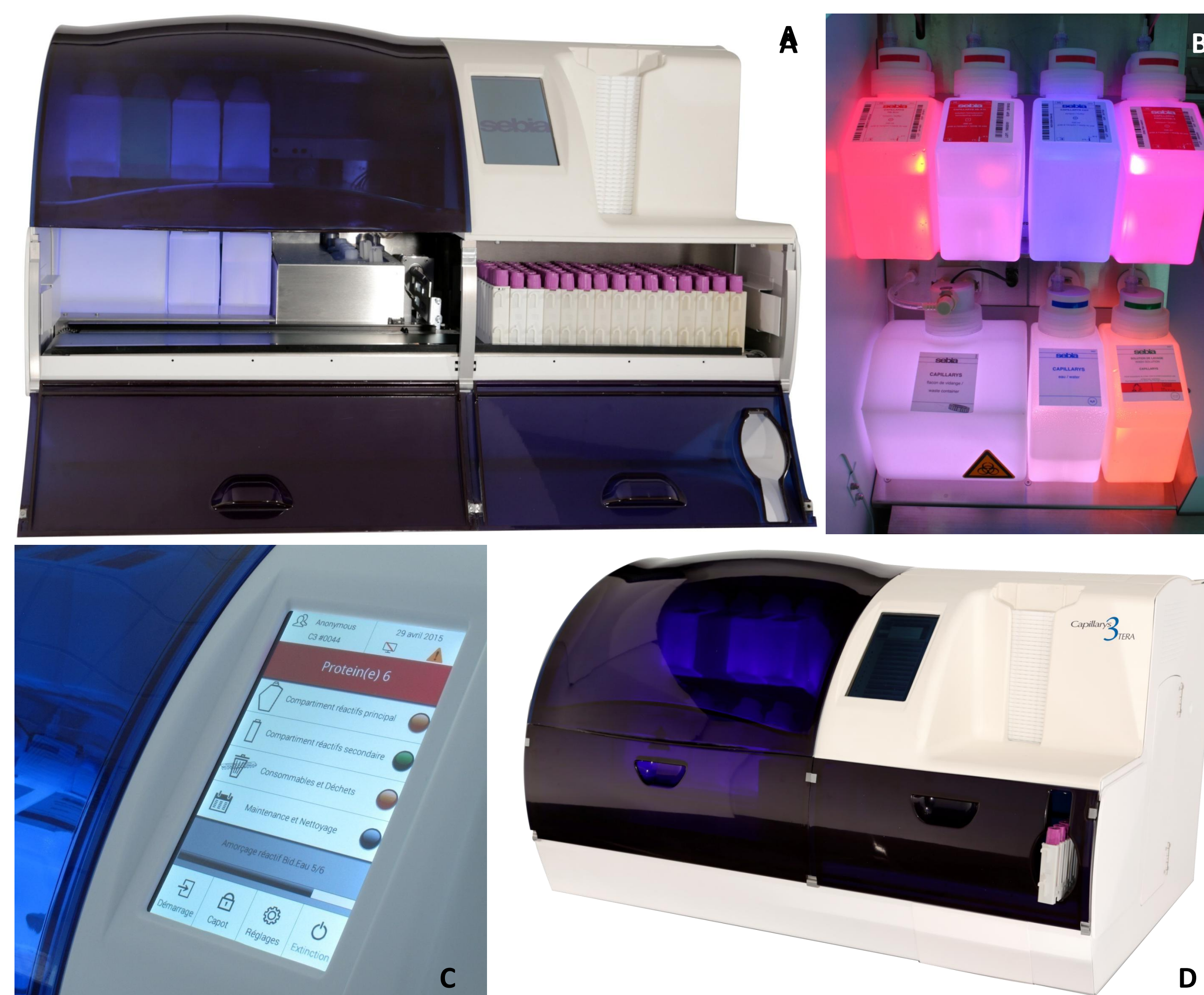


Figure 1:

The CAPILLARYS 3 TERA is new high throughput capillary electrophoresis analyzer. A: opened instrument with the sample loading area for up to 120 samples on right hand side and the empty unloading area on the left. B: the reagent compartment with full reagent traceability due to RFID tagged reagents. C: LCD touch screen as a user interface for the management of the instrument status, reagent status, maintenance status and change of techniques. D: the CAPILLARYS 3 TERA in a side view with closed sample and reagent hatches.

CAPILLARYS 3 TERA HbA _{1c}		IFCC Secondary Reference Method IFCC		Bias	
mean (%A1c)	mean (mmol/mol)	IFCC Target (%)	Target (mmol/mol)	Bias (%)	Bias (mmol/mol)
5,1	33,0	5,3	34	0,2	1,0
5,5	37,3	5,7	39	0,2	1,7
6,1	43,0	6,2	44	0,1	1,0
6,7	50,0	6,8	51	0,1	1,0
7,5	57,7	7,5	58	0,0	0,3
9,0	74,3	9,1	76	0,1	1,7
			Mean	0,1	1,1

Table 1:

For the determination of the instruments trueness samples with target values assigned by an IFCC secondary reference method were run in triplicates. The CAPILLARYS 3 measured HbA_{1c} with a bias of 0.15 (1.1mmol/mol) lower than assigned reference values.

Control Level 1 run on the 12 capillaries each day					Control Level 2 run on the 12 capillaries each day				
Day	min	mean	max	CV	Day	min	mean	max	CV
Day 1	5,1	5,1	5,2	0,61	Day 1	8,4	8,5	8,6	0,66
Day 2	5,1	5,2	5,2	0,76	Day 2	8,4	8,5	8,6	0,87
Day 3	5,1	5,1	5,2	0,76	Day 3	8,3	8,4	8,5	0,49
Day 4	5	5,1	5,3	1,68	Day 4	8,3	8,4	8,5	0,52
Day 5	5,1	5,2	5,2	0,76	Day 5	8,2	8,3	8,5	0,82
Day 6	5,1	5,2	5,2	0,72	Day 6	8,3	8,4	8,5	0,63
Day 7	5,1	5,2	5,4	1,9	Day 7	8,4	8,4	8,5	0,38
Day 8	5	5,1	5,2	1,05	Day 8	8,4	8,4	8,5	0,38
Day 9	5,1	5,2	5,2	1,01	Day 9	8,4	8,4	8,5	0,33
Day 10	5,1	5,2	5,3	1,32	Day 10	8,3	8,4	8,5	0,6
Day 11	5,1	5,2	5,2	0,85	Day 11	8,4	8,4	8,5	0,52
Day 12	5,1	5,2	5,2	0,57	Day 12	-	-	-	-

Table 3: Control materials were run on each of the 12 capillaries for at least 11 days. The coefficient of variation was calculated.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	CV	Mean		
	run 1	run 2	run 1	run 2	run 1	run 2	run 1	run 2	run 1	run 2	run 1	run 2				
Pool 1	4,4	4,4	4,5	4,3	4,4	4,5	4,5	4,4	4,5	4,4	4,4	4,4	4,4	4,5	1,32%	4,4
Pool 2	6,3	6,3	6,3	6,2	6,3	6,3	6,3	6,2	6,3	6,3	6,3	6,2	6,3	6,3	1,00%	6,3
Pool 3	7,4	7,4	7,5	7,5	7,5	7,5	7,4	7,5	7,5	7,5	7,5	7,5	7,5	7,5	0,59%	7,5
Pool 4	10,3	10,5	10,5	10,4	10,6	10,5	10,6	10,5	10,6	10,5	10,6	10,6	10,6	10,6	0,88%	10,5

Table 2:

Pooled samples at different HbA_{1c} concentrations were prepared from patient EDTA-samples and stored at -80°C until analyzed. Each sample was analyzed in duplicates on each of the 12 capillaries. The coefficient of variation was calculated for each of the 4 pool samples.

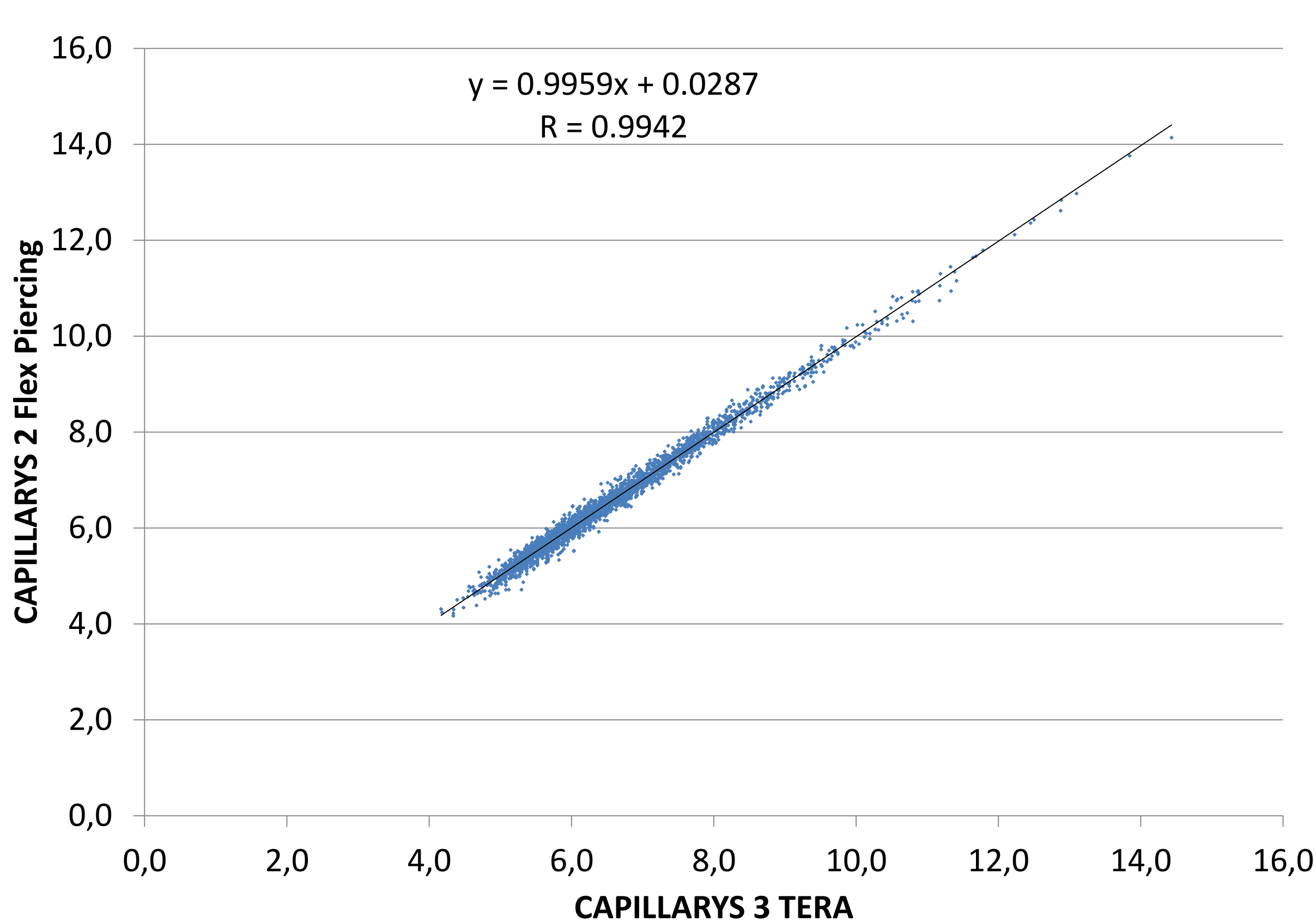


Figure 2:

2703 patient samples from our daily laboratory routine were analyzed for HbA_{1c} on both the CAPILLARYS 3 TERA and the capillary electrophoresis system CAPILLARYS 2 Flex Piercing.