The efficient measurement of HbA1c will become of growing importance as the prevalence of type II diabetes mellitus increases. To this end the CAPILLARY 3 TERA system (Siemens, France) for high throughput HbA1c testing has been continuously improved. In this study we evaluated the instrument with respect to trueness, precision and correlation to CAPILLARY 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 CAPILLARY 3 TERA measured HbA1c values slightly below the target values with the bias ranging from 0% to 0.2% (0.9 to 1.7 mmol/mol). The instruments precision was tested in pooled samples stored in aliquots at -80°C. Two determinations each of the 12 capillaries were performed during 12 days. At a mean HbA1c concentration of 4.4% (24.6 mmol/mol) the coefficient of variation (CV) was 1.3%, at 5% (25.3 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 HbA1c routine samples were analyzed on both the CAPILLARY 3 and the CAPILLARY 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 HbA1c has considered to be a 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 HbA1c. 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 HbA1c result and improve clinical practice.

In summary HbA1c testing by capillary electrophoresis is an accurate method improving workflow and quality of laboratory analysis. The newly developed CAPILLARY 3 TERA is a high throughput laboratory automatic processing up to 70 samples an hour that is equivalent in analytical performance to the well established CAPILLARY 2 systems.

Results

The CAPILLARY 3 TERA (Figure 1) instrument’s trueness was assessed by measuring 6 samples whose target values were assigned by IFCC Network Laboratories for HbA1c, with methods calibrated to IFCC secondary reference material. The measurements were performed as triplicates and the mean was calculated (Table 1). The CAPILLARY 3 TERA measured HbA1c values slightly below the target values with an overall Bias of 0.1% (1.1 mmol/mol) HbA1c. The target HbA1c values were: 5.2% (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 CAPILLARY 3 TERA instrument were: 5.1% (33 mmol/mol) 5.5% (37 mmol/mol), 6.1% (40 mmol/mol), 6.7% (50 mmol/mol), 7.5% (57 mmol/mol) and 9.0% (74 mmol/mol) for the respective samples.

The instruments precision was tested by measuring HbA1c in pooled samples stored in aliquots at -80°C over a period of twelve days (Table 2). 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 24 mmol/mol, the coefficient of variation (CV) was 1.3%, at 6.3% (45.4 mmol/mol) HbA1c, the CV was 1%, at 7.5% (58.5 mmol/mol) HbA1c, the CV was 0.6% and at 10.5% (91.3 mmol/mol) HbA1c, the CV was calculated to be 0.9% (Table 2).

For the Intra-Assay precision controls were calculated for at least 11 days. The CV ranged from 0.3% to 1.9% (Table 3). The HbA1c measurements on the new CAPILLARY 3 TERA was compared to the capillary electrophoresis system CAPILLARY 2 Flex Piercing established in our laboratory. Over a period of 17 consecutive working 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% HbA1c. The regression line followed the equation y = 0.9959x + 0.0287 with a correlation coefficient of r = 0.9959 (Figure 2).

Summary

The CAPILLARY 3 TERA represents the next generation of analytic instruments that extend 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 CAPILLARY 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 for technicians. Our trueness study measured HbA1c values compared to IFCC standardized methods. The instrument measures HbA1c with an excellent precision as demonstrated by the determination of pooled patient samples and controls. Further the correlation of the HbA1c values determined on the CAPILLARY 3 TERA was almost identical to determinations performed on the established CAPILLARY 2 Flex Piercing system.

Materials and Methods

For the determination of the Trueness of HbA1c measurement of the CAPILLARY 3 TERA SEBIA provided 6 samples stored at -80°C, whose target values were assigned by IFCC Network Laboratories for HbA1c, with methods calibrated to IFCC secondary reference material. The samples were processed in triplicate on the CAPILLARY 3 TERA to assess the trueness. For each sample, the mean bias from the target value was calculated.

The Precision of HbA1c measurement for the CAPILLARY 3 TERA was tested on 4 pools prepared from fresh samples with different HbA1c concentrations. EDTA-samples with no hemoglobin disorders were pooled. Patients were chosen to create pools with HbA1c 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 inter assay variation control samples with a target value of 5.1% and 8.5% HbA1c were analyzed on each of the 12 capillaries daily for a period of 12 and 11 days respectively.

The correlation between CAPILLARY 3 TERA and CAPILLARY 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 working days. 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 HbA1c measurements, coefficient of correlation (r) and the line of regression was determined.