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Proof of concept: stabilized whole blood material suitable for external quality assessment of near-patient testing devices

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Abstract

Objectives: Even though reliable glucose concentration measurements are essential in diagnosis and monitoring of diabetes mellitus, external quality assurance based on mandatory reference method values can only be conducted to a limited extent for measurements in whole blood. The reason is the lack of stabilized whole blood materials suitable for the application in glucose measurement devices used in near-patient testing.

Methods: Two patented whole blood stabilizers were tested using four commercially available near-patient testing devices and one patient self-testing device for plasma-referenced glucose measurements. Furthermore, a laboratory method for plasma-glucose measurements was included. Venous whole blood samples from 30 apparently healthy volunteers were used. Two whole blood samples (stabilizer A and B) per subject were kept at room temperature over the study period of seven days and aliquots were taken each day from the original sample for measurement on all devices. After venous puncture, left over whole blood from the collection system was used for immediate glucose measurements without stabilizer on the near-patient testing devices.

Results: Each investigated device gave stable results at least for one of the two stabilizers for a period of four days. Imprecision based on quality controls ranged between 1.7 and 4.8 % coefficient of variation for near-patient testing devices, but did not reflect observed variability in measurement results from stabilized and unstabilized whole blood in one device. In addition, a considerable deviation of 0.8 mmol/L was observed among the near-patient testing devices underlining the need for reference method values in external quality control.

Conclusions: Our study provides proof of concept that for each investigated device at least one stabilizer of glucose in whole blood shows a good performance for at least four days. Therefore, these stabilizers appear to be suitable candidate materials for external quality assessment of near-patient testing devices.

Keywords: external quality control; glucose concentration measurement; near-patient testing; patient self-testing; quality assurance; reference method

Introduction

Glucose concentration measurements are essential in the diagnosis and monitoring of diabetes mellitus [1, 2]. To ensure patient safety, glucose measurements require a high analytical quality. While internal quality controls are mainly suitable to continuously monitor random error (imprecision), external quality controls focus on identifying systematic measurement deviation (bias) [3].

In Germany, quality assurance of medical laboratory examinations for internal and external quality schemes are both regulated by the “Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations – Rili-BAEK” [4]. Rili-BAEK applies the same quality criteria independent of where the measurement takes place: in medical laboratories or near the patient. Based on Rili-BAEK, the acceptable relative deviation in external quality controls for glucose in plasma and whole blood has just recently (May 2023) been lowered from ±15 to ±8 %, with a transitional period of three years. Importantly, the target value for glucose has to be based on a reference method value (RMV) for external quality assessment (EQA).

These requirements are easily met by plasma glucose methods used on analyzers in medical laboratories. In 2021, average plasma glucose passing rates in EQA schemes

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reported by two German officially accepted EQA organizations, Reference Institute for Bioanalytics (RfB, Bonn, Germany; https://www.rfb.bio/) and INSTAND e.V. (Duesseldorf, Germany; https://www.instand-ev.de/), were 98.0 and 98.6 %, respectively. In the same year, EQA schemes for glucose near-patient testing of the given institutions showed average passing rates of 92.6 % (RfB) and 90.8 % (INSTAND). Whereas RfB did not use the RMV due to lacking suitable control material and instead used group medians (consensus value) for groups of at least four participants, INSTAND evaluated 24.6 % of the participants according to the RMV and the rest according to group medians for groups of at least eight participants. If INSTAND had evaluated all participants according to RMV, average passing rates would have been 33.8 % in 2021. Since the underlying cause, poor performance of the participants or unsuitable control material, cannot not be distinguished, the EQA institutions rely partly or completely on the consensus value for issuing certificates. Even though the Rili-BAEK demands a reference method target value for glucose measurements, using the consensus value is covered by the Rili-BAEK under certain circumstances. In part E3, the document states that if the majority of the results deviate considerably from the respective target value, e.g. due to reasons caused by the material sent out to participants, alternative methods for evaluation may be used. One prerequisite for this procedure is that efforts are made by the EQA organization to overcome problems that prevent applying the demanded target, in case of glucose the RMV [4].

The crucial difference between centralized (medical laboratories) and de-centralized glucose concentration measurements (near-patient testing) is the material used: centralized glucose is mostly measured in plasma, de-centralized methods mostly rely on whole blood. Whole blood though is difficult to stabilize and, in addition, stabilizing agents interfere with some of the measurement systems [5]. This long lasting lack of suitable whole blood control material limits the ability to identify bias, the main purpose of EQA schemes [6]. To meet the needs of glucose EQA schemes for methods relying on whole blood, suitable control materials have to:

- remain stable at approximately room temperature over a period of up to four days (to allow aliquoting large sample numbers and postal delivery) and
- technically and analytically comply with all or at least the majority of the devices for whole blood glucose.

Already in 2003, Kleesiek pointed out the urgent clinical need for suitable control materials in near-patient testing [7]. To overcome this challenge about 20 years later, our study aims to evaluate two stabilized whole blood materials for EQA schemes of near-patient testing devices.

Materials and methods

Two whole blood stabilizers, United States Patent (US 4,675,185 A) and United States Patent (US 7,306,950 B2), named stabilizer A [8] and B [9], respectively, were tested using four commercially available near-patient immediate testing devices for plasma-referenced glucose measurements: 1.) Accu-Chek Inform II (Roche Diagnostics GmbH, Mannheim, Germany), 2.) FreeStyle Precision Pro (Abbott GmbH, Wiesbaden, Germany), 3.) HemoCue Glucose 201+ Analyzer (HITADO GmbH, Moehnesee, Germany) and 4.) StatStrip (Nova Biomedical GmbH, Moerfelden-Walldorf, Germany). Furthermore, one device for patient self-testing was included: Accu-Chek Aviva (Roche Diagnostics GmbH, Mannheim, Germany). Both whole blood stabilizers were also used for measurements with a medical laboratory method on the Dimension Vista (Siemens Healthineers, Erlangen, Germany). An overview of the underlying detection method and required sample volume for each device is given in Table 1.

The study was approved by the Ethical Committee (BB 100/15) and conducted at the University Medicine Greifswald in 2016. Informed consent was obtained from each healthy volunteer prior to sampling. Whole blood from 30 apparently healthy subjects was drawn by venous puncture using two tube types with different stabilizers: A and B. Tubes were gently and thoroughly mixed immediately after sampling. In addition, whole blood drops from the venous blood collection system were used for direct measurements on all near-patient testing devices immediately after venous puncture. Furthermore, glucose concentration in plasma was measured using the medical laboratory method after whole blood aliquots from the primary sample were centrifuged for 5 min at 3,280×g (ROTANTA 460 Robotic, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany).

Stabilized whole blood samples were stored at room temperature for a period of seven days and measured on the devices at the same hour each day. Prior to taken daily aliquots, primary samples were thoroughly mixed. Additionally, internal quality controls as provided by the manufacturers were measured on all devices once on each study day. Control levels with their respective target values are given in Table 2.

Statistical analysis

Internal quality controls were used to calculate imprecision expressed as coefficient of variation (%CV). Mean absolute and relative differences of glucose measurements in comparison to baseline (t_0) were calculated for each day and each combination of stabilizer and device. Furthermore, the distribution of glucose measurements at baseline (t_0) was visualized separately for each stabilizer and device combination using boxplots, including whole blood direct measurements without stabilizers.

Results

The study comprised 30 apparently healthy probands, 19 women (aged 23–60 years; median: 31 years) and 11 men

Table 1: Overview of measuring devices and their basic characteristics.

Device (manufacturer)	Sample	Method (as given in the manufacturers' description)	Sample volume, µL
Near-patient testing			
Accu-Chek Inform II (Roche Diagnostics GmbH)	Whole blood	Glucose dehydrogenase (Mut Q-GDH): Glucose + GDH-PQQ → Gluconolactone + GDH-$PQQH_2$ GDH-$PQQH_2$+ 2Fe(III) → GDH-PQQ+ 2Fe(II) + $2H^+$ Fe(II) → Fe(III) + e^-	0.6
FreeStyle Precision Pro (Abbott GmbH)	Whole blood	Glucose dehydrogenase: α-D-Glucose is converted to β-D-glucose by the enzyme mutarotase. β-D-Glucose + NAD → Gluconolactone + NADH NADH+ PQ(ox) → NAD+ + PQ(red) PQ(red) → PQ(ox) + e^-	0.6
HemoCue Glucose 201+ (HITADO GmbH)	Whole blood	α-D-Glucose (mutarotase) → zu β-D-Glucose β-D Glucose + NAD^+ → D-Gluconolactone + NADH MTT+ NADH (Diaphorase) → MATTH+ NAD The chemistry method utilized by the HemoCue Glucose 201 Microcuvette is a modified glucose dehydrogenase method described by Banauch et al. A chromogen compound is added to the reagents According to the principle outlined by Bergmeyer with saponin used for hemolyzing the erythrocytes. The absorbance is measured at two wavelengths (667 and 840 nm) to compensate for turbidity [11].	<4.0
StatStrip (Nova Biomedical GmbH)	Whole blood	Glucose oxidase: Glucose + NAD+ → gluconic acid + NADH NADH+ $Fe(CN)_6^{3-}$ → NAD^+ + $Fe(CN)_6^{4-}$ Fe $(CN)_6^{4-}$ → Fe $(CN)_6^{3-}$ + e^-	1.2
Patient self-testing device			
Accu-Chek Aviva (Roche Diagnostics GmbH)	Whole blood	Glucose dehydrogenase (Mut Q-GDH): Glucose + GDH-PQQ → Gluconolactone + GDH-$PQQH_2$ GDH-$PQQH_2$+ 2Fe(III) → GDH-PQQ+ 2Fe(II) + $2H^+$ Fe(II) → Fe(III) + e^-	0.6
Medical laboratory device			
Dimension Vista (Siemens Healthineers)	Plasma	Hexocinase: Glucose + ATP → Glucose-6-phosphate + ADP; glucose-6-phosphate-dehydrogenase: Glucose-6-phosphate + NAD^+ → 6-Phosphateqluconate + NADH + H^+ NADH at 340 and 383 nm	3 (minimum volume in cup: 50)

(aged 23–35 years; median: 27 years) in four runs. All measurements were performed according to the manufacturers' recommendations and with at least one previous valid internal quality control per study day. Imprecision based on internal quality controls is given in Table 2. Imprecision at a glucose concentration of approximately 5–6 mmol/L ranged between 1.7 and 4.8 %CV for near-patient testing devices and the patient self-testing device. Imprecision for the medical laboratory device was obtained at QC target values of approximately 2–3 mmol/L and ranged between 1.7 and 3.3 %CV.

All devices gave results for glucose measurements using tubes with stabilizer A, all but one (StatStrip) also gave results for stabilizer B. Due to the study design, it was not always possible to obtain results for all devices in all participants. Most missing values occurred on Accu-Chek Aviva (16 missing subjects). Especially the direct sampling of whole blood from the collection system was difficult and resulted in many missing values. For comparisons of glucose measurements at baseline (t_0) using whole blood as well as stabilized material, we concentrated on the measurement values of 18 subjects for whom results were available for all near-patient testing devices. The measurement results from the patient self-testing device Accu-Chek Aviva were excluded in these evaluations due to the large number of missing values.

Figure 1 displays the mean absolute and relative differences compared to initial plasma-referenced glucose concentrations at baseline for each day and each combination of

Table 2: Quality controls and thereof calculated CV% for the investigated devices.

Device	QC level 1		QC level 2		QC level 3	
	Target value, mmol/L	CV%	Target value, mmol/L	CV%	Target value, mmol/L	CV%
Accu-Chek Inform II			6.50	1.71	17.00	1.64
FreeStyle Precision Pro	2.70	3.09	5.40	4.20	16.50	4.12
FreeStyle Precision Pro	2.50	5.42	5.25	4.58	16.90	2.16
HemoCue Glucose 201+ Analyzer	2.00	2.98	6.30	2.33	10.60	2.17
StatStrip	3.30	5.16	6.10	4.81	16.70	4.99
Accu-Chek Aviva			6.50	2.69		
Dimension Vista	3.15	2.84			19.60	1.69

stabilizer and device. Absolute (relative) mean differences of glucose concentration between t_0 and day one (t_1) ranged between −0.05 mmol/L (0.7 %) (stabilizer A/HemoCue Glucose 201+ Analyzer) and 0.79 mmol/L (16.6 %) (stabilizer B/HemoCue Glucose 201+ Analyzer). When comparing the estimated means together with its 95 %-confidence interval, our results demonstrate that for each investigated device at least one of the two stabilizers produced stable results for four days. FreeStyle Precision Pro and Dimension Vista showed decreasing glucose concentrations for both stabilizers starting at day 5 (t_5), with a more pronounced decrease in stabilizer B. In contrast, stabilizer B/HemoCue Glucose 201+ Analyzer gave higher and increasing measurement results until day 4 (t_4), whereas stabilizer A/HemoCue Glucose 201+ Analyzer produced strongly decreasing glucose concentrations starting on day 2 (t_2).

Figure 2 illustrates the distribution of measured glucose concentrations separately for the investigated stabilizer and device combinations at baseline (t_0) together with the distribution of glucose measurements from the initial whole blood measurement without stabilizers at the different devices. The figure is based on the measurement results for 18 subjects with valid measurements in all combinations of stabilizer and devices. Because of missing values, the results for stabilizer B/StatStrip and Accu-Chek Aviva were not displayed in Figure 2. Median glucose concentrations in whole blood without stabilizer ranged from 4.75 to 5.55 mmol/L. Median glucose concentration measured in tubes using one of the two stabilizers were on average lower. In stabilizer A and B median glucose concentrations ranged from 3.90 to 4.99 mmol/L and 4.30–5.15 mmol/L, respectively.

Discussion

When diagnosing and monitoring Diabetes mellitus, high quality glucose concentration measurements are inevitable [1, 2]. External quality controls are essential in quality assurance and for glucose measurements in near-patient testing, suitable stabilized whole blood materials are needed [7]. In this proof-of-concept study, we investigated glucose concentration stability over seven days in two stabilizers for whole blood EQA sample materials on five near-patient testing devices and one medical laboratory device.

Each investigated device gave stable results at least for one of the two stabilizers for a period of four days. For packing and distributing external quality control samples to the participants, this period can be sufficient if the measurement is conducted immediately on the day of arrival. Usually, postal mail will be delivered within two days, allowing two days' time for preparing, aliquoting and packaging samples per survey. Still, as illustrated by comparing medians of the different stabilizer-device combinations at t_0 to direct measurements without stabilizers, there are detectable differences between the measured median glucose concentrations in whole blood and stabilized material. Since no reference method values were available in this study, further studies are needed to elucidate which stabilizer-device combination gives the lowest bias. The need for RMV is underlined by the finding that the measured median glucose concentration in unstabilized and fresh whole blood samples on the different devices deviated up to 0.8 mmol/L (lowest median 4.75 mmol/L, highest median 5.55 mmol/L), i.e. about 16 % (Figure 2). The Rili-BAEK currently allows a maximum deviation of 15 % (8 % in three years) in external quality controls and 11 % (5 % in three years) in internal quality controls, the latter including bias and imprecision, allowing for even less bias since imprecision will not be nil [4]. The identified measurement differences between the devices were similarly observed for both investigated stabilizers, only at a slightly lower concentration level. If stabilizers are to be used in external quality control, reference method values, of course, should be determined in the given stabilizer-whole blood combination.

The distribution width of glucose measurements at baseline differs considerably, with the HemoCue Glucose 201+ Analyzer showing the widest and Accu-Chek Inform II the smallest spread of glucose concentration (Figure 2). The wider spread of measurement results indicates higher imprecision. For the HemoCue Glucose 201+Analyzer this poor imprecision is not reflected by the imprecision data based on QC material provided by the manufacturer, which is with approximately 3 % CV fairly good. Therefore, the

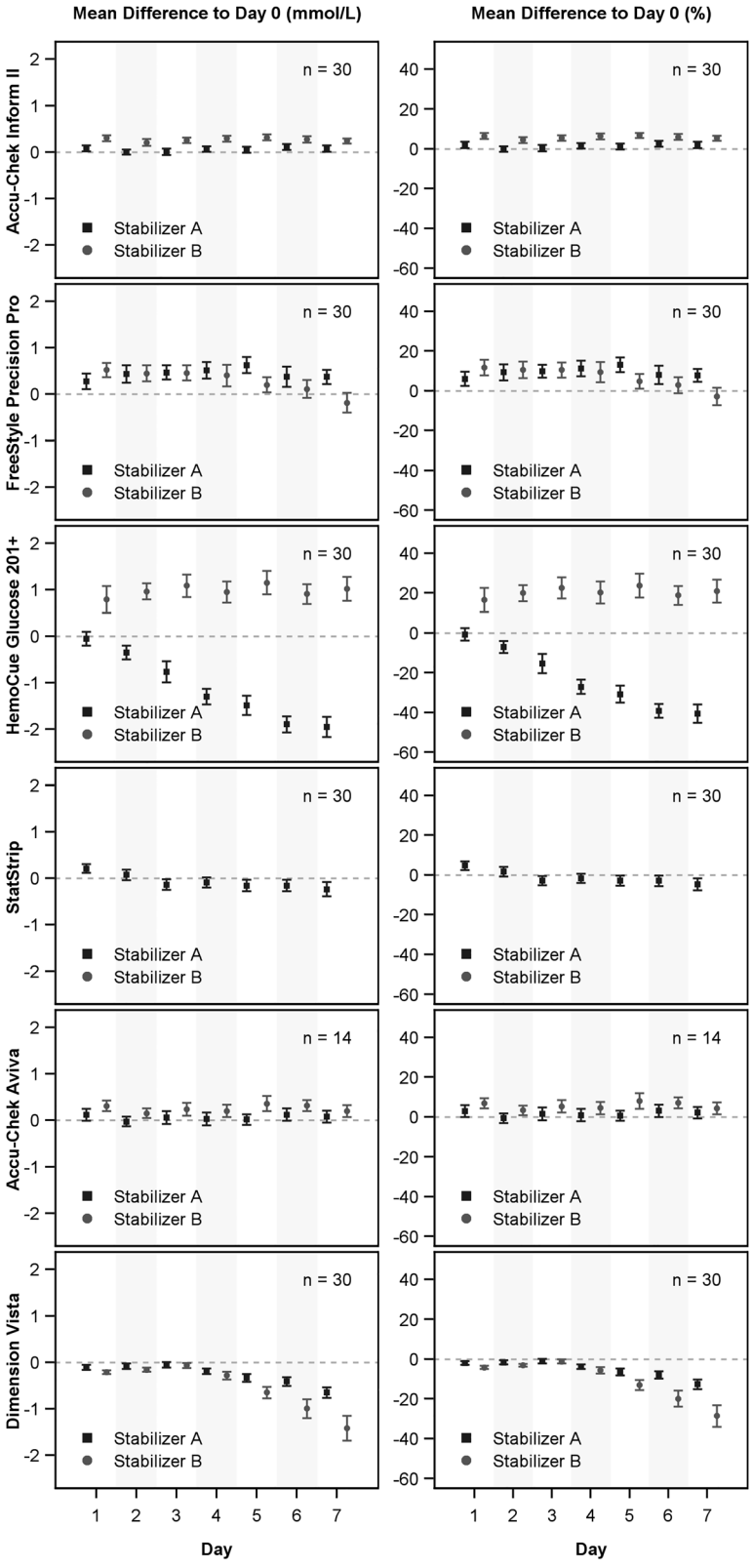

Figure 1: Absolute and relative mean differences of glucose measurements at each study day compared to initial plasma-referenced glucose concentrations at baseline, separately for each investigated device and stabilizer A and B. Displayed are the absolute (left) and relative (right) mean differences of glucose measurements at each study day compared to baseline measurements (t_0) together with its 95 %-confidence interval (mean $\pm$ 1.96 $\times$ standard error), separately for the investigated devices and stabilizers. The number of considered subjects is shown in each part of the Figure.

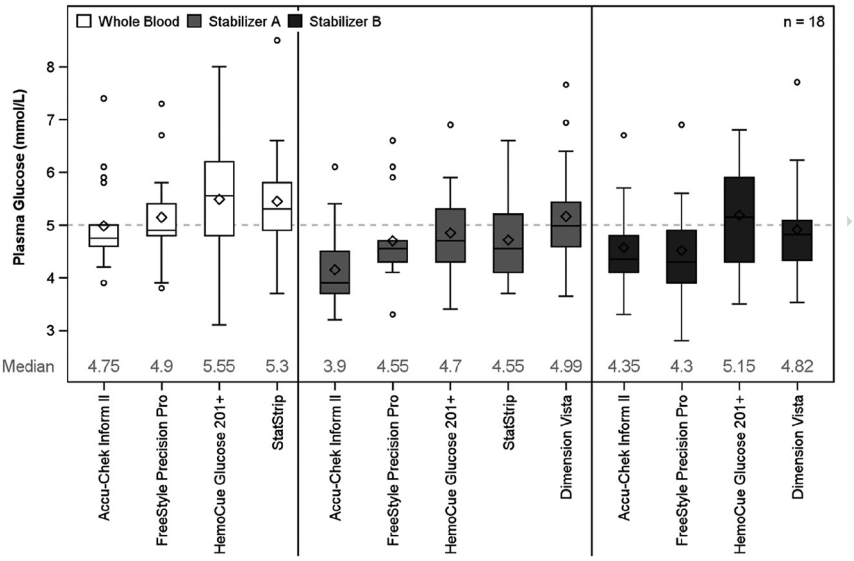

Figure 2: Boxplots of plasma glucose concentration at baseline (t_0), separately by device, whole blood and stabilizer. The measurement values of 18 subjects for which results of all near-patient testing devices could be obtained, were used for comparisons of glucose measurements at baseline (t_0). From left to right: whole blood from direct measurement using blood drops from blood sampling system (white boxplots), whole blood with stabilizer A (light grey boxplots) and B (dark grey box plots). The median concentration for each combination of device and stabilizer is displayed at the bottom of the Figure.

results indicate that imprecision obtained through QC material may not be appropriate to reflect whole blood patient material measured on HemoCue Glucose 201+ Analyzer. For the other investigated devices, QC material provided by the manufacturer seems to be more suitable to reflect imprecision of whole blood samples with a slightly smaller imprecision and narrower distribution observed in measured values at the Accu-Chek Inform II than in those measured at the remaining two devices (FreeStyle Precision Pro, StatStrip).

Interestingly, glucose concentrations developed differently over time depending on the stabilizer-device combinations: decreasing considerably in a few cases (e.g. HemoCue Glucose 201+ Analyzer/stabilizer A and Dimension Vista with both stabilizers), while most device-stabilizer combinations showed a comparably stable glucose concentration (Figure 1). It has to be pointed out, that each day only the amount of sample was taken from the original tube that was needed for that specific study day. Therefore, the glucose concentration should be the same in all aliquots of a study day. Furthermore, we observed that some stabilizer-device combinations (Accu-Chek Aviva and Inform II for both stabilizers and to a lesser extent also stabilizer A on StatStrip) gave stable results over the complete study period, indicating that the glucose concentration remains constant in the original stabilizer samples.

Methods relying on the measurement on an electric current based on iron oxidation show a stable performance with at least one stabilizer (Accu-Chek Aviva, Accu-Chek Inform II and StatStrip). If Glucose Dehydrogenase (Mut Q-GDH, both Roche systems) is used, both stabilizers give stable glucose concentration results over a period of seven days. When aliquots were centrifuged for plasma measurements on the Dimension Vista, it was observed that haemolysis in the samples increased towards the end of the study period. This may lead to an increase of compounds in plasma, which might interfere with some of the investigated methods, e.g. HemoCue Glucose 201+ Analyzer/stabilizer A. For example, phosphate is known to increase in samples that have been haemolysed or stored for a long time [10]. Furthermore, changes in pH might occur due to the stabilizers used. Further studies are needed to investigate these and evaluate the influence of these factors. Although not all investigated combinations of stabilizers and devices yielded results, this proof of concept approach demonstrates that glucose concentrations in whole blood samples can be maintained stable for at least four days with at least one of the investigated stabilizers for each used device. This in turn is a prerequisite for the successful use in EQA schemes for devices relying on whole blood. As a first step pilot-EQA employing the investigated whole blood materials could be allocated according to the device and run in parallel to plasma samples to collect more data.

Strengths and limitations

Our results demonstrate that whole blood stabilizers may be used in external quality control of glucose concentration measurements. Venous whole blood is not explicitly stated in the intended use of the investigated devices. So far, only five near-patient testing devices could be investigated using samples from a limited number of 30 participants. In addition only whole blood from apparently healthy participants was used. Further studies should include more subjects, also those with pathological glucose levels, as well as all devices participating in the current EQA schemes and two or more

test strip lots per device. A limitation of this study is the missing RMV. This method should be included in future study designs as well.

Conclusions

Our study provides proof of concept that for each considered device at least one of the investigated stabilizers of glucose in whole blood shows a good performance for at least four days. Therefore, these stabilizers appear suitable candidate materials for external quality assessment of near-patient testing devices.

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Research ethics: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Ethical Committee (BB 100/15).

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Emmanuel Bissé is owner of one of the patented materials investigated in this manuscript (Patent US 7306950 B2), but neither received money nor have been paid in connection with this manuscript. Astrid Petersmann and Matthias Nauck are EQA scheme advisor of EQA 800 Glucose for INSTAND e.V.

Research funding: The manufacturers mentioned in the article provided reagents.

Data availability: The raw data can be obtained on request from the corresponding author.

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