Review

Nick Verougstraete, Veronique Stove and Christophe P. Stove*

Remote HbA_{1c} testing via microsampling: fit for purpose?

https://doi.org/10.1515/cclm-2023-0228
Received March 2, 2023; accepted June 6, 2023;
published online July 10, 2023

Abstract: The collection of capillary blood microsamples via finger-prick has several advantages over traditional blood collection. It is considered convenient and more patient-centric, enabling collection of the sample by the patient at her/his home with subsequent analysis in the lab following postal shipment. Determination of the diabetes biomarker HbA_{1c} in self-collected microsamples to remotely monitor diabetes patients seems to be a very promising option which could eventually lead to better treatment adaptations and disease control. This is especially convenient/relevant for patients living in areas where venipuncture is impractical, or to support virtual consultations using telemedicine. Over the years, a substantial numbers of reports on HbA_{1c} and microsampling have been published. However, the heterogeneity of the applied study designs and data evaluation is remarkable. This review provides a general and critical overview of these papers, along with specific points of attention that should be dealt with when aiming at implementing microsampling for reliable HbA_{1c} determination. We focus on the used (dried) blood microsampling techniques, collection conditions, stability of the microsamples, sample extraction, analytical methods, method validation, correlation studies with conventional venous blood samples and patient satisfaction. Lastly, the possibility of using liquid instead of dried blood microsamples is discussed. Liquid blood microsampling is expected to have similar advantages as dried blood microsampling and several studies suggest it to be a suitable approach to collect samples remotely for subsequent HbA_{1c} analysis in the lab.

Keywords: dried blood microsampling; HbA_{1c}; liquid blood microsampling; microsampling

***Corresponding author: Professor Christophe P. Stove**, Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University; Ottergemsesteenweg 460, 9000 Gent, Belgium, E-mail: christophe.stove@ugent.be. https://orcid.org/0000-0001-7126-348X
Nick Verougstraete, Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium; and Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium
Veronique Stove, Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium; and Department of Diagnostic Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

Introduction

Hemoglobin A_{1c} (HbA_{1c}) is a glycated protein which is formed *in vivo* by the non-enzymatic reaction of glucose with the N-terminal valine residue of beta globin chains of HbA. Determination of HbA_{1c} in blood is used for the diagnosis and monitoring of type 1 and 2 diabetes mellitus as its concentration is a reflection of the average glycemic control over the past two to three months, considering an average life span of normal red blood cells of 120 days. In comparison to blood glucose determinations, HbA_{1c} is not subject to wide day-to-day and hour-to-hour fluctuations [1]. HbA_{1c} values are expressed as a proportion of total hemoglobin concentration in % (National Glycohemoglobin Standardization Program (NGSP) units) or mmol/mol (System International (SI) units used by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) network). An HbA_{1c} value of ≥6.5 % or 48 mmol/mol is used as a threshold for the diagnosis of diabetes [2]. Pre-diabetes is defined by HbA_{1c} values between 5.7 and 6.4 % (39–47 mmol/mol) [3]. Alternatively, diabetes can be diagnosed by measuring fasting plasma glucose concentrations or by performing a two-hour plasma glucose tolerance test [1]. HbA_{1c} results have a strong predictive value for the occurrence of diabetes complications. Therefore, during treatment, HbA_{1c} levels should remain below 7 % (53 mmol/mol) to prevent the development and progression of microvascular long-term complications (retinopathy, neuropathy and kidney disease). Monitoring the glycemic status is recommended at least twice per year in patients with stable glycemic control or every three months in patients not meeting glycemic treatment goals or with recent treatment changes [4]. In conditions associated with altered red blood cell turnover (for example hemolytic anemia and recent blood loss or transfusion) other parameters should be used to diagnose or monitor diabetes.

HbA_{1c} should be measured by a method standardized to the IFCC reference measurement procedure [5] and preferably certified by the NGSP [6]. The presence of hemoglobin variants in blood samples may interfere with HbA_{1c} testing, leading to incorrect results and consequently inappropriate treatment decisions. For each HbA_{1c} analytical method used, the user should be aware of the impact of the presence of a hemoglobin variant and/or elevated fetal hemoglobin (HbF) [7, 8].

HbA_{1c} levels are traditionally/usually measured in whole blood obtained via an invasive venous blood sampling procedure, performed by qualified medical personnel. However, different (dried) blood microsampling techniques can be used, which offer several advantages over traditional blood collection [9]. A blood microsample refers to a sample which is typically less than 50 µL and is mostly taken by a finger-prick using a safety lancet, representing a 'minimally invasive' sample collection. This, combined with the lower sample volume, renders this type of sampling particularly relevant for use in pediatric populations. Moreover, the simplicity of the sampling procedure implies that collection of (dried) microsamples does not require a phlebotomist in a medical center, but can be performed by the patients themselves, in their home environment. In addition, logistics for transportation and storage can be simplified by using dried blood microsamples [10]. After collection, and drying if working with dried samples, the samples can be sent via standard postal services to the clinical laboratory for analysis. It can be arranged that patients send their samples a few days before their consultation. In this way, the actual HbA_{1c} value of the patient will be available at the time of the patient's visit, allowing insight into the patient's most recent glycemic status. As a result, better adaptation of the treatment and glycemic control is to be expected [11]. Another option to have the actual HbA_{1c} result readily available at the time of the patients' clinical consultation, is the use of point of care testing (POCT) [12]. However, POCT is not within the scope of this review and will not be discussed further. In addition, microsampling is also a valuable tool in population-based epidemiology studies [13–15]. Obtaining blood samples via a finger-prick may increase the availability of HbA_{1c} testing in areas where venipuncture is impractical [16]. Moreover, mailing samples by postal service can be advantageous for patients living in rural and remote areas with limited access to phlebotomy centers [17, 18]. Lastly, drying of a sample reduces biohazard risks that are associated with liquid whole blood samples.

Despite the general health threat and associated social distancing rules, the recent COVID-19 pandemic also resulted in positive effects on the healthcare landscape, triggering and speeding up many reforms, aimed at coping with issues like patient follow-up and at improving access to regular healthcare. The shift to virtual consultation for follow-up of chronic conditions using telemedicine is one of these reforms and can now be further implemented beyond the pandemic. In support of telemedicine, remote collection of (micro)samples has shown to be a valuable and attractive alternative to traditional sampling [19]. Besides the use of dried blood spots (DBS) for SARS-CoV-2 serologic testing [20], also programs to monitor diabetes patients with HbA_{1c} testing on DBS were set up [21–23]. In this way, accessibility to testing results and accompanying optimal diabetes care were guaranteed [23]. The feasibility of remote HbA_{1c} testing via DBS to monitor the glycemic status of patients treated via telemedicine during the COVID pandemic was demonstrated by Roberts et al.: upon request of a diabetes provider, DBS kits were mailed to the patients. After collection of the DBS at home, the samples were sent back to the lab and HbA_{1c} results were provided prior to the telemedicine visit. However, only 53.4 % of the patients returned their DBS to the lab and a substantial number of these DBS samples was of insufficient quality [21], suggesting that there is room for improvement.

While the pandemic may have been a facilitator of home sampling studies, the application of dried blood microsampling strategies for measuring HbA_{1c} stems from long before the pandemic, with a substantial number of papers having been published on this topic. However, the heterogeneity of the applied study designs and data evaluation is remarkable. The goal of this review is to present a general overview of the multitude of reported methods (shown in Table 1) and to discuss/address specific points of attention that should be dealt with when aiming at implementing dried or liquid blood microsampling for reliable HbA_{1c} monitoring. Whereas virtually any methodology may be capable of delivering 'a' result, the key question that should be asked is: does application of a given methodology under real-life conditions result in a correct and reliable result? Unfortunately, this is a question that is too often not addressed.

Used microsampling technique and conditions of sampling

To date, most of the papers on dried blood microsampling have used DBS sampling [9, 13–16, 18, 24–35]. After pricking the finger, multiple blood drops are collected on a dedicated piece of filter paper (typically Whatman 903 or PE226). Subsequently, the samples are dried for 2–3 h, after which they are stored in sealed plastic bags together with a desiccant. In a typical DBS workflow, a 3–6 mm diameter disc is punched from the DBS, followed by extraction of this subpunch. Already in 1982 Goldstein et al. reported on the measurement of glycated protein in whole blood deposited

Table 1: Overview of published dried blood microsample HbA_{1c}-based methods.

Reference	Microsampling technique	Complete DBS vs. punch (P) (diameter)	Extraction/elution solution	Analytical technique	Application + included patients/samples	Sample collection	Time between collection and analysis	Specific remarks
Egier et al. [13]	DBS	P 3/16 inch	1 mL hemolyzing buffer	Ion-exchange HPLC (Bio-Rad Variant II)	Comparison between (lab-prepared) venous DBS and whole blood from 85 clinical specimens	Whole blood applied on filter paper in the lab	Within 96 h of sample collection	
Jeppson et al. [24]	DBS	P 3 mm	75 µL Na phosphate-citrate Triton X-100 buffer + cysteine-containing buffer	Ion-exchange HPLC (in house)	Comparison between capillary DBS and venous blood from 41 patients	Capillary collection by participants under supervision at outpatient diabetes unit	NA	
Lacher et al. [14]	DBS	P 3.2 mm	Buffer	Ion-exchange HPLC (Bio-Rad Variant II)	Population-based epidemiology study with 386 DBS samples	Capillary collection by experienced phlebotomists in controlled environment	Refrigerated up to 7 days	
Maleska et al. [26]	DBS	P 6 mm	400 µL DW buffer	Ion-exchange HPLC (Arkray ADAMS HA-8180V)	Comparison between (lab-prepared) venous DBS and whole blood samples from 217 participants	75 µL whole blood spotted on filter paper	NA	Full analytical validation under ISO15189 guideline
Pollock et al. [25]	DBS	?	?	Ion-exchange HPLC (Tosoh G7)	NA	Blood spotted on filter paper	Up to 6 days	Focus on HbA_{1c} as marker in newborn screening
Thomas et al. [15]	DBS	?	?	Ion-exchange HPLC (Bio-Rad D-10)	Comparison between venous DBS and venous blood from 143 subjects	Venous blood was spotted on filter papers both in controlled clinic settings and in field-type setting	?	Evaluation of the influence of environmental conditions during DBS preparation
Crawford et al. [34]	DBS	P 91 mm^2	2000 µL hemolyzing reagent	Immuno-turbidimetry (Roche Tina-quant)	Comparison between HbA_{1c} measured from dried blood fractions of collection device to venous whole blood samples from healthy donors	Both assisted- as self-collected capillary blood samples via finger-prick	NA	Samples were collected via a device which uses laminar-flow migration to separate cellular components from plasma. HbA_{1c} was measured on the dried RBC portion (and cholesterol on the dried plasma).
Elliott et al. [18]	DBS	P 6 mm	500 µL hemolyzing reagent	Immuno-turbidimetry (Roche Tina-quant)	Comparison between capillary DBS and venous whole blood from 50 diabetes patients	Capillary collection under supervision and by participants at home	DBS included in study up to 7 days	Comparison between DBS collected under supervision at the lab and unsupervised at participants' home
Fokkema et al. [28]	DBS	P 3 mm	250 µL hemolyzing buffer	Immuno-turbidimetry (Roche Tina-quant)	Comparison between capillary DBS and venous whole blood from 93 diabetes patients	Capillary collection by laboratory personnel and by participants at home	Within 10 days	Comparison between DBS collected by laboratory personnel and by participants at home

Table 1: (continued)

Reference	Microsampling technique	Complete DBS vs. punch (P) (diameter)	Extraction/elution solution	Analytical technique	Application + included patients/samples	Sample collection	Time between collection and analysis	Specific remarks
Hu et al. [30]	DBS	P 3.2 mm	300 µL hemolyzing reagent	Immuno-turbidimetry (Olympus AU HbA_{1c})	NA	?	NA	
Jones et al. [16]	DBS	2 P 1/8 inch	500 µL hemolyzing reagent	Immuno-turbidimetry (Roche Tina-quant)	Comparison between (lab-prepared) venous DBS and whole blood from 73 routine HbA_{1c} samples	20 µL whole blood spotted on filter paper	NA	
Lakshmy et al. [29]	DBS	P 6 mm	400 µL hemolysis reagent	Immuno-turbidimetry (Agappe latex method)	Comparison between (lab-prepared) venous DBS and whole blood samples from 30 diabetes patients	10 µL whole blood spotted on filter paper	15 days when stored at 4 °C	
Mastronardi et al. [31]	DBS	P 3.2 mm	Haemolysing reagent	Immuno-turbidimetry (Thermo Fisher HbA_{1c})	Comparison between (capillary and venous) DBS and whole blood from 115 participants	Capillary collection by participants in a controlled research setting	Up to 14 days, results were acceptable up to 7 days	Application of correction equations taken into account time between sample collection and analysis
Miller et al. [32]	DBS	P 3 mm	Deionized water	Immuno-turbidimetry (Kamiya Biomedical kit)	Comparison between capillary DBS and venous blood from 125 DBS	DBS were collected at the participants' home by health technicians or field interviewers	?	Performance of collecting DBS by experienced health workers vs. unexperienced interviewers was compared
Parkes et al. [27]	DBS	?	?	Immuno-turbidimetry (Roche Tina-quant)	1625 DBS from 59 diabetes patients	Capillary collection by patient in-clinic setting	3–12 days after sample collection	
Scherer et al. [33]	DBS	?	?	Immuno-turbidimetry (Thermo Fisher HbA_{1c})	14,243 DBS cards from 2,237 participants at risk of developing diabetes	DBS either taken by a nurse at the clinical site or by the participants at home	Range from 0 to 400 days	Multicenter study focused on the impact of different storage conditions
Henderson et al. [35]	DBS	P 3.2 mm	100 mM ammonium bicarbonate + trifluoroethanol	LC-PRM-MS (in house)	Comparison between venous whole blood and DBS obtained from 36 healthy volunteers	Capillary DBS were obtained from each volunteer by a trained phlebotomist	NA	Simultaneously measurement of HbA_{1c}, apoA-I and apoB in DBS
Verougstraete et al. [9]	DBS + VAMS	P 3 mm	DBS: 250 µL deionized water; VAMS: 400 µL deionized water	Ion-exchange HPLC (Tosoh G8)	Capillary DBS and VAMS were compared to liquid blood results from 86 diabetes patients (40 adults and 44 children)	1 DBS and 2 VAMS were collected by the patients at their home	Up to 6 days	Collection of two types of dried blood microsamples: DBS and VAMS
Verougstraete et al. [38]	*Wet* VAMS	NA	800 µL deionized water	Ion-exchange HPLC (Tosoh G8)	Comparison between *wet* VAMS and liquid capillary	2 VAMS samples were collected by the patients		

Table 1: (continued)

Reference	Microsampling technique	Complete DBS vs. punch (P) (diameter)	Extraction/elution solution	Analytical technique	Application + included patients/samples	Sample collection	Time between collection and analysis	Specific remarks
					blood from 76 pediatric diabetes patients	at home and tips were brought into water within 30min after collection	Exclusion criteria: up to 6 days	Evaluation of adapted sampling protocol by collecting VAMS tip in water
Lima et al. [39]	VAMS	NA	20 mM ammonium bicarbonate + deoxycholic acid	LC-MS/MS (in house)	NA	Capillary VAMS were collected via fingerprick from volunteers	NA	
Hall et al. [37]	HemaSpot™	HemaSpot™ filter blade	One blade in 1 mL hemolysis/wash solution	Ion-exchange HPLC (Tosoh G8)	Comparison between capillary DBS and capillary liquid blood from 40 insulin requiring adult patients	Capillary collection by a study researcher	Up to 5 days	Evaluation of the impact of time between HemaSpot™ DBS preparation and HbA_{1c} analysis
Hall et al. [11]	HemaSpot™	HemaSpot™ filter blade	One blade in 1 mL hemolysis/wash solution	Ion-exchange HPLC (Tosoh G8)	Comparison between venous blood and capillary DBS collected at clinic and at home from 128 diabetes patients	Capillary collection by participants under guidance of study nurse and subsequently unsupervised at home	Within 4 days of preparation	Comparison between HemaSpot™ DBS collected at home and supervised at the clinic. Conclusions are not sufficiently supported by data.
Acar et al. [40]	Bloodstains	NA	Dissolved in water	Ion-exchange HPLC (Tosoh G8)	NA	50 µL whole blood pipetted on glass and fabric	NA	Forensic investigation

Papers are ordered per microsampling technique, per analytical technique and lastly, in alphabetical order of the first author. Papers of special interest/relevance have been highlighted in bold.

on filter papers [36]. Also variations on the conventional filter paper cards have been used. In two reports by Hall and colleagues, DBS were collected using HemaSpot™ devices. These devices comprise a plastic cover enclosing eight wedge-shaped filter papers (or "blades") with a built-in desiccant, to promote drying of the samples. The blood flows through a central opening in the device to fill all the blades simultaneously [11, 37]. Also the determination of HbA_{1c} in samples collected via volumetric absorptive microsampling (VAMS) has been reported [9, 38, 39]. VAMS is a dried blood based sampling technique in which a fixed volume of blood is collected by an absorbent tip attached to a plastic handler. Collection of VAMS samples has been reported to be more straightforward compared to DBS collection [9]. Moreover, HbA_{1c} has also been determined in dried red blood cell (RBC) fractions obtained via a collection device using laminar-flow migration [34] and in bloodstains, both on cotton fabric and glass surfaces [40].

Besides the used microsampling technique itself, it is also important to look at the conditions in which the samples were collected in the different studies, as this can have an important influence on the quality of the dried blood sample and subsequently on the obtained HbA_{1c} result. Not only the context of the sampling (clinical setting vs. home environment), but also whether the samples were collected by an experienced phlebotomist or by the patient her/himself should be taken into account. Prior to routine implementation, the clinical acceptability of the dried blood method should be evaluated. To this end, it is important that during method validation study samples are collected in a context reflecting the future routine use. For example, when aiming at home-sampling, HbA_{1c} results of samples collected at home by study participants must be compared with the conventional results. It is not sufficient to perform an evaluation on DBS that were artificially generated in a lab setting by spotting venous whole blood onto filter paper [13, 15, 16, 25, 26, 29, 30], as the latter approach does not cover issues that may be encountered during e.g. transport of samples at ambient temperature. In some studies, capillary DBS were collected by trained phlebotomists instead of by the patients [14, 35, 37] or participants collected capillary samples supervised in a controlled research setting [24, 31].

Several studies comparing HbA_{1c} results obtained from capillary DBS collected by experienced phlebotomists or supervised in a controlled environment with those obtained from DBS collected by the patients themselves at their home did not find a bias between both sample types [11, 18, 28]. However, using a similar set-up, others did report significant differences, although without apparent cause (time between sample preparation and analysis was excluded as a potential source) [11]. In a study conducted by Miller and colleagues, the performance of collecting DBS samples by experienced health technicians and unexperienced field interviewers at the participant's home was compared. DBS samples, collected by both groups, were of similar good quality and the obtained HbA_{1c} DBS results were not significantly different, indicating that DBS can be collected by unexperienced non-phlebotomists in a non-clinical environment [32]. In a substantial number of studies the dried blood microsamples were collected unsupervised by the participants at their home [9, 11, 18, 28, 33, 38]. This requires a thorough explanation concerning the sampling technique. To this end, apart from the instructions given during inclusion, a sampling brochure could be added to the home sampling kit [9, 38] or a link to a brief instruction video could be provided (see for example a video which was used in a large-scale alcohol biomarker study [41]).

Stability, transport and storage

As dried blood microsamples are intended to be collected by patients in their home environment, the samples need to be transported to the analyzing lab after collection. This implies that it should be evaluated whether the time between collection and analysis and the shipment conditions may have an influence on the HbA_{1c} result. In the following section the stability of HbA_{1c} in dried blood microsamples will be discussed. The impact of time between collection and analysis is also further implemented in the discussion on correlation experiments.

In general, drying of a blood sample is associated with increased stability of the analyte. For measuring HbA_{1c} in routine settings the added value of this potentially increased stability is somewhat limited, given the stability of HbA_{1c} in conventional liquid whole blood samples. Liquid whole blood for HbA_{1c} analyses should be stored in the refrigerator (4 °C) no longer than 7 days and for long-term storage, samples should be stored at −70 °C or lower [42, 43]. However, when considering sample transportation and storage, working with dried blood microsamples does offer logistical advantages. E.g., dried microsamples can be transported via regular mail (under ambient conditions) and stored for prolonged periods at room temperature. This is especially relevant in low-resource settings [44]. Transport and storage of microsamples also requires less space [14]. Although most studies do not report issues regarding instability during transport, the time between collection and analysis does seem to have an impact (see part on correlation studies further). For long-term storage, DBS should be stored at −70 °C or less, similar as for liquid whole blood samples [13, 24, 42].

If specific weather conditions are to be expected during sample transport, this should be evaluated during method

validation. Samples mailed via standard postal services are typically prone to temperature and humidity variations. For example, elevated temperatures are to be expected in a sun-exposed mailbox during summer.

The results from stability experiments performed in various studies are summarized in Table 2, with quite striking differences in outcome. Several explanations have been proposed for the apparent discrepancies between studies. It is hypothesized that HbA_{1c} may be formed by *ex vivo* glycation of hemoglobin due to the contact between hemoglobin and glucose, i.e. the ongoing Amadori arrangement of the labile A_{1c} form. This reaction is believed to be time- and temperature-dependent, so varying temperatures during shipment of the samples would add more variability to the dried blood HbA_{1c} results [11, 18, 28, 33, 39]. A protocol was introduced in which filter papers for generating DBS were pretreated with a citrate-based solution in order to prevent possible *ex vivo* glycation of hemoglobin in the blood spot. However, pretreatment of filter papers did not improve assay results. This finding should be interpreted with caution as the authors did not present data/results for their experiment [30].

Lastly, several studies provided insight into the average time interval between sample collection and arrival in the lab: samples were transported via standard postal services and arrived on average between 3 and 7 days after the collection [9, 16, 18, 38, 45, 46]. Such information is relevant, as the stability of the sample should be guaranteed during this time period.

Table 2: Results from stability experiments of HbA_{1c} in different microsamples.

Reference	Microsample	Storage condition	Observed effect on HbA_{1c} values
Acar et al. [40]	Bloodstains	18–22 °C and relative humidity of 50 %	Values decreased over time in both fabric (after 28 days) and glass surface (after 56 days)
Fokkema et al. [28]	DBS	RT	Increase of values from 0 to 10 days (irrespective whether exposed to air or stored in sealed plastic bag)
Jones et al. [16]	DBS	RT; 4 °C; 25 °C and 40 °C	Stable up to 44 days storage at RT and up to 9 days at 4, 25 and 40 °C
Lakshmy et al. [29]	DBS	4 °C	No significant differences between results after 10 and 15 days compared to day 0 values
Maleska et al. [26]	DBS	RT	Significant decrease after 7 days of storage
Parkes et al. [27]	DBS	35–65 °C	Stable for 12 h at 55 °C; values decreased at higher temperatures with largest decrease for DBS kept at 65 °C for 24 h
Thomas et al. [15]	DBS	RT and 4–6 °C (in darkness)	Stable for 10 days in all conditions
Verougstraete et al. [38]	*Wet* VAMS	Tips in water (within 30min after collection) at ambient temperature	Stable for 6 days

Papers are displayed in alphabetical order of the first author. RT, room temperature.

Dried blood sample extraction

Compared to sample preparation protocols of dried blood microsamples, developed for application in therapeutic drug monitoring (TDM), extraction protocols for measurement of HbA_{1c} are more straightforward and require less lab time. For most of the published dried blood based methods, a simple one-step extraction of the sample was performed by adding an amount of a buffer [14, 26], a commercial hemolysing reagent [13, 16, 18, 28–31, 34], a hemolysis/wash solution [11, 37] or deionized water [9, 32, 38] to the sample, with a subsequent incubation and/or shaking step. The reported elution times ranged from 30 s [9] to 3 h [16]. The elution conditions (volume, time and temperature) were not always reported. Jeppsson et al. used a more elaborate two-step elution protocol by adding a sodium phosphate-citrate buffer containing Triton X-100 as detergent to the DBS punch, followed by the addition of an equal volume of a cysteine containing buffer to eliminate the interfering Hb glutathione adduct [24]. One should be aware that since 2021 the use of Triton X-100 detergent in laboratories is restricted in the European Union.

Analytical methods

The same methods used for measurement of HbA_{1c} in conventional liquid blood samples can be applied on extracted dried blood microsamples. Most of the published dried blood based methods used an automated ion-exchange HPLC analyser: Bio-Rad analyser [13–15], Arkray ADAMS analyser [26] or Tosoh G series [9, 11, 25, 37, 38, 40], in which HbA_{1c} is

separated from other hemoglobin fractions and variants based on different ionic interactions with the stationary phase. Automated HPLC analysers allow rapid and reproducible analysis of samples in a clinical lab, comparable with typical immunoassay workflows. In an older paper, a non-automated HPLC ion-exchange was used [24]. DBS extracts have also been analyzed with turbidimetric immunoassays, in which an anti-HbA_{1c} antibody is used, which reacts with HbA_{1c} to form a complex. Whole blood assay protocols were applied on DBS using different commercially available immunoassays: direct turbidimetric inhibition immunoassay (Thermo Fisher Scientific) [31,33], Tina-quant HbA_{1c} assay (Roche Diagnostics) [16,18,27,28,34], immunoturbidimetric latex method (Agappe Diagnostics) [29], Kamiya Biomedical HbA_{1c} kit [32] and the Olympus HbA_{1c} kit [30]. Two (older) studies reported on the measurement of HbA_{1c} in DBS using affinity chromatography [47,48] and, to the best of our knowledge, none with capillary electrophoresis.

Two mass spectrometry-based HbA_{1c} methods in dried blood matrices were published. In a proof-of-concept study, HbA_{1c} was quantified (and Hb variants identified) in VAMS samples using a data-independent acquisition (DIA) liquid chromatography-mass spectrometry (LC-MS) approach, a technique commonly used in proteomics [39]. A similar method for HbA_{1c} quantification in DBS, together with apoB and apoA-I, using nanoflow LC-parallel reaction monitoring (PRM)-MS was also described [35].

In a few papers, the precision of measuring HbA_{1c} in dried blood microsamples was evaluated. Overall, the obtained coefficients of variation (CVs) were reasonable [16, 26, 32], even though the imprecision of dried blood based methods compared to conventional liquid blood is expected to be higher due to an increased variation in pre-analytical conditions, sample volume and elution efficiency [14]. Hall et al. reported comparable CVs for DBS and fresh blood samples, suggesting a negligible error introduced by DBS collection and elution of the spot [11]. A CV of 5.80 % was obtained for HbA_{1c} duplicate measurements performed on dried VAMS [9] and 2.84 % on *wet* VAMS samples [38], indicating reproducibility of both sampling techniques (based on results expressed in IFCC units).

Method validation

During method development of a dried blood based analytical method the effect of deviating hematocrits, volume of blood spotted on the DBS card and spot homogeneity (for DBS) on obtained quantitative results should be evaluated [49]. However, since HbA_{1c} values are expressed as a relative ratio (mmol/mol or percentage), these parameters are unlikely to affect the measured HbA_{1c} result. Furthermore, as the standard matrix for HbA_{1c} analysis is whole blood, HbA_{1c} results obtained from dried blood microsamples are directly comparable to liquid blood results. Hence, when compared to for example the application of dried blood microsampling for TDM [10], the validation of HbA_{1c} measurements in dried blood microsamples should be more straightforward.

As dried blood microsamples are typically collected following a finger-prick, capillary blood is collected instead of venous blood. Several studies have demonstrated a good agreement between venous and capillary blood HbA_{1c} results, indicating that capillary and venous blood can be used interchangeably [31, 45, 46, 50–53]. However, one should be aware that slight differences might be present in patients at the intensive care unit due to oxidative stress [54].

Correlation studies with conventional samples

Most of the studies covered by this review included a method comparison between HbA_{1c} results in the alternative dried blood microsample vs. conventional liquid blood. However, there was a marked heterogeneity in study design, and too often critical evaluation of the data was missing and/or the conclusions were not supported by the presented data. It should also be noted that in a substantial number of these studies DBS were actually not collected via capillary finger-prick, but were artificially laboratory-generated samples, obtained by spotting venous whole blood onto filter paper (see part on sampling conditions above) [13, 15, 16, 25, 26, 29, 30]. Furthermore, in some study designs DBS and whole blood samples were measured in the same run quite shortly after sample collection, which does not mimic a real-life home-sampling situation. Table 1 provides details of the performed correlation studies.

To evaluate the agreement -rather than the correlation- between HbA_{1c} results obtained from dried blood microsamples and venous liquid blood, objective acceptance criteria based on clinical perspectives should be taken into account [55]. The applied acceptance criteria for HbA_{1c} in (dried) blood microsampling methods are summarized in Table 3. For example, in the studies from Verougstraete [9, 38] and Hall [37], Royal College of Pathologists of Australasia (RCPA) quality requirements for total allowable error were used. Alternatively, a maximum acceptable difference of 5 mmol/mol was suggested by Groenendijk et al., as this is the smallest difference in consecutive HbA_{1c} results that guides clinicians to change therapy [56].

Most studies reported a high correlation between HbA_{1c} results obtained from DBS and venous liquid whole blood

Table 3: Applied acceptance criteria for evaluating the agreement of HbA_{1c} results obtained in (dried) blood microsamples vs. conventional samples.

Acceptance criterion	Criterion based on	Applied in
Total allowable error: ±4 mmol/mol (IFCC) or ±0.4 % (NGSP)	RCPA based on biological variation [63]	Verougstraete et al. [9, 38] + Hall et al. [37]
Total allowable relative error of ±6 % in at least 92.5 % of all samples	Little RR [64]	Elliott et al. [18]
±6 % evaluation limit	CAP evaluation limits for proficiency testing	Miller et al. [32]
Total allowable relative error of ±5 % in ≥90 % of all samples	NGSP [65]	Beck et al. [45]
Maximum acceptable difference of ±5 mmol/mol (IFCC) or ±0.5 % (NGSP).	Smallest difference in HbA_{1c} values of consecutive tests that guide to change in therapy	Groenendijk et al. [56]
95 % confidence interval of the differences must be within ±0.75 % (NGSP) or ±8.2 mmol/mol (IFCC) HbA_{1c}	NGSP [66]	Jones et al. [16]
Total allowable error: ±5 mmol/mol (IFCC) or ±0.5 % (NGSP)	Weykamp et al. [67]	Verougstraete et al. [9] + clinical laboratory of UZ Ghent
Total allowable relative error of ±4.6 %	Biological variation of HbA_{1c} [68]	Crawford et al. [34]

If absolute HbA_{1c} values were used, the values are shown in both IFCC (mmol/mol) and NGSP (%) units. CAP, College of American Pathologists; IFCC, International Federation of Clinical Chemistry; NGSP, National Glycohemoglobin Standardization Program; RCPA, Royal College of Pathologists of Australasia.

[13, 14, 16, 24, 28–30, 32]. However, a critical evaluation of the obtained data, using one of the formal acceptance criteria in Table 3, was lacking in several instances. Several studies applied a conversion formula to adjust DBS results to yield a better correlation (or agreement) with traditional venous blood levels [11, 15, 18, 26, 31, 33]. However, when working with such experimentally determined conversion factor or formula, it is important that the utility of such a conversion is evaluated on an independent cohort, and not only on the cohort which was used for the set-up of the factor or formula [49]. This can also be achieved by randomizing samples into two distinct pools: one to set up a calibration model, and the other for evaluation of the model or correction factor [55]. For example, Scherer et al. measured 14,243 DBS cards to set up a regression analysis to correct for storage time, and validated the predictive performance of this model using 4,272 distinct samples from the same participants [33]. Crawford et al. set up an initial comparison study with assisted finger-prick sampling to establish a calibration factor to convert finger-prick HbA_{1c} results to venous values using a "training dataset" of more than 100 samples. Subsequently, the obtained assay-specific calibration factor was independently verified on a smaller "testing dataset" including participants' self-collected microsamples [34].

The time elapsed between sample collection and analysis does seem to be a key factor impacting HbA_{1c} DBS results. Mastronardi et al. observed that the correlation between DBS and venous blood HbA_{1c} values became weaker over time: whereas results were still acceptable up to 7 days after collection (storage conditions were not mentioned), the obtained differences were clinically unacceptable after 14 days. Moreover, the (analytical) variability increased in older samples. Multiple correction equations were set-up, for each particular day of sample analysis after collection, and applied to predict HbA_{1c} whole blood values from DBS measurements [31]. Similarly, Elliott et al. observed that the obtained biases on HbA_{1c} measured in DBS samples increased with time between collection and analysis. DBS samples received more than 7 days after collection were excluded because of the expectation of significant and unpredictable errors [18]. Hall et al. evaluated the impact of time between HemaSpot DBS preparation and HbA_{1c} analysis. DBS HbA_{1c} results, collected with HemaSpot™ devices, analyzed up to five days after collection (stored at ambient temperature) were compared with capillary liquid blood HbA_{1c} results. After applying a day-dependent correction formula, home-prepared DBS HbA_{1c} results were stated to be equivalent to the liquid blood results [37]. Remarkably, in their previous study the authors stated that the time between HemaSpot™ DBS preparation and analysis (up to 4 days) did not affect the HbA_{1c} results and the regression model. However, the validity of that statement can be questioned as over 80 % of the samples were analyzed within 2 days and only 2 (out of 104) samples were analyzed 4 days after collection [11]. Obviously, application of a correction equation yielded better results in all the above-mentioned studies. However, an important limitation is that in none of these studies the equations were verified on independent datasets. This is also illustrated in the Hall study [37], where the predicted HbA_{1c} values, based on the day-dependent regression equations, correlated better than the ones obtained using a more generic, previously published formula [11]. This stresses once more the need for critical data evaluation.

In a recent multicenter study the impact of different DBS storage conditions and analysis delay on HbA_{1c} measurement validity was evaluated. Delay in analysis ranged from 0 to 400 days; with 50.4 % of the samples being analyzed within the first week after collection and approximately 90 % within 4 weeks. Overall, HbA_{1c} levels increased with increasing analysis delay. A correction method, taking into account the delay (in days) and a center-specific variable,

reflecting different storage conditions per participating center, was set up. Application of this method on an independent set of DBS led to a significant improvement of the correlation of the DBS HbA_{1c} results with the corresponding whole blood values [33].

In a recent study, Verougstraete et al. compared HbA_{1c} results in conventional liquid blood samples to results obtained in DBS and VAMS samples collected at home by the patients. Poor agreements were found between DBS/VAMS HbA_{1c} results and the liquid blood results and too many samples did not meet the RCPA quality requirements. Interestingly, higher agreements with liquid blood samples were obtained for DBS and VAMS samples that were analyzed 1–3 days after collection compared to those that were analyzed 4–6 days after collection. A possible explanation for this was that by ageing of the dried samples the hemoglobin components were converted to oxidized and degraded hemoglobin which had an unpredictable effect on the HbA_{1c} result, making it impossible to set-up a bias correction equation [9]. In another study it was also observed that aged liquid whole blood samples often yielded unacceptable chromatograms by the appearance of unidentified extra peaks, possibly corresponding to hemoglobin degradation products [42]. Another study, analyzing HbA_{1c} with LC-MS in samples collected by VAMS, found that upon storage at room temperature, HbA_{1c} values increased. This was a specific limitation of glycated peptides, since the non-glycated evaluated peptides showed stability up to 3 or 10 days at room temperature [39]. Besides the overall good performance obtained during validation of another LC-MS method on DBS, the developed method was considered not clinically useful as the authors observed sample-specific disagreement compared to the conventional validated HbA_{1c} assay on venous samples [35]. Degradation and conversion of hemoglobin products during drying, transport and storage of dried samples has also been described in other reports. In particular ion-exchange chromatography-based methods may struggle with these conversions, which yield abnormal elution patterns, as described above. Moreover, degradation products have the potential to co-elute or may be incompletely separated from the HbA1c peak, both potentially leading to erroneous HbA_{1c} results [9, 13, 26]. This was nicely exemplified by a study conducted by Thomas et al. These authors obtained DBS HbA_{1c} values that were substantially and significantly higher compared to the corresponding liquid blood values using ion-exchange HPLC. This upward bias was considered clinically relevant, with potential overestimation of diabetes prevalence rates. Interestingly, it was observed by the authors that their HPLC ion-exchange technique failed to capture a part of the total Hb fraction that should be included in the denominator of the $\%HbA_{1c}$ calculation. This was due to the presence of an extra unexpected peak to the right of A_0, probably misclassified by the software as a Hb variant. The origin of this peak could not be found. After recalculation by including chromatographic areas that were initially ignored, HbA_{1c} values from DBS closely agreed with the corresponding venous values [15]. Whereas the formed hemoglobin degradation products cause interfering peaks on ion-exchange methods because of their altered ionic charges, it is expected that boronate affinity HPLC methods would not be affected, as the cis-diol group on the glycated hemoglobin is not changed [43]. To the best of our knowledge, only two (older) filter paper-based methods were published using this methodology [47, 48] and measuring HbA_{1c} with boronate affinity analysers is not widespread in clinical labs because of the limited availability of automated analysers. Another possibility is using turbidimetric immunoassays for measuring dried blood microsamples as these might be more robust towards the formed hemoglobin conversions, however, also these assays yielded deviating DBS HbA_{1c} values [18, 28, 31, 34].

In their study, Verougstaete et al. also evaluated the effect of analyzing VAMS samples immediately after collection, with the samples being capped with a shell to delay the drying process. The obtained HbA_{1c} results on these VAMS samples showed an excellent agreement with the corresponding liquid capillary blood sample and all samples fulfilled the RCPA requirements. This observation showed the intrinsic potential of using VAMS as a sampling technique for obtaining clinically acceptable HbA_{1c} results [9]. Based on the latter finding, a follow-up study was set-up in diabetic children who used VAMS for home-sampling. However, instead of drying the samples, the VAMS tips were deposited in 800 µL sterile water shortly after collection (within 30 min). The samples were also stored and sent to the lab in water at ambient temperature. Overall, this approach showed a substantial improvement over the dried VAMS approach and the apparent hemoglobin conversion taking place during drying, and impacting the HPLC ion-exchange analysis, was possibly prevented [38].

Patient satisfaction

In several studies the patient satisfaction was evaluated by taking questionnaires. Overall, patients were satisfied with the alternative sampling techniques and indicated that the sample collection was easy to perform and that they preferred home sampling over traditional venous sampling at a medical center [11, 18, 28]. A recurrent concern from subjects was the difficulty of ensuring that there was enough blood on the DBS paper. To obtain high quality samples collected by the patients themselves, proper instruction (preferably with written instructions containing pictures)

prior to collection is essential. In a study which combined DBS and VAMS sampling, participants experienced both sampling techniques as very convenient, with VAMS standing out as the preferred sampling technique (especially in the pediatric population) [9, 38].

Liquid blood microsampling

As an alternative to dried blood microsampling, also liquid whole blood microsampling seems to be a promising option. The same advantages as with dried blood microsamples are to be expected: samples can be collected by patients via a minimally invasive finger-prick, after which they can be sent to the lab via regular post. Shipping and storage is still considered more convenient compared to conventional sampling because of the low sample volumes. However, one should be aware that liquid samples have an intrinsic higher biohazard risk and inherently carry the potential of leaking during transport, requiring suitable (leakproof) containers. Liquid capillary blood specimens were already remotely collected and sent to the lab for measurement of HbA_{1c} used as assistance for virtual consultations during the COVID pandemic [45, 56, 57]. Capillary blood was collected into suitable calibrated/precision (heparinized) capillaries [45, 46, 50] or small EDTA anticoagulated MiniCollect™ [56], Microtainer™ [57] or Hem-Col™ [58] tubes. After collection, the capillary tubes were stored in a stabilizing or hemolyzing solution and transported as such to the lab [45, 50, 59]. Comparable to dried samples, also for the measurement of HbA_{1c} in liquid microsamples different analytical techniques were used: ion-exchange HPLC [45, 46, 50, 58], capillary electrophoresis [56] and a point of care (POC) immunoassay-based analyser [57]. Compared to dried blood microsamples, no stability issues have been reported, suggesting that this may be a suitable approach. Table 4 gives an overview of the published methods using liquid blood microsamples.

An excellent agreement between capillary blood and venous blood specimens from 47 pediatric diabetes patients was obtained. After collection, calibrated capillary tubes were placed into vials containing a stabilizing solution of EDTA and KCl [50]. The same researchers also demonstrated the feasibility of mailing such capillary blood samples to the lab at ambient temperatures [60]. Similar results were reported by Cross et al.: diabetes patients collected 50 µL capillary blood (5 drops) in Microtainer™ tubes at home and sent these to the lab via postal services. Clinically acceptable results compared to capillary samples taken at the diabetes

Table 4: Overview of published liquid blood microsample HbA_{1c}-based methods.

Reference	Analytical technique	Application + included patients/samples	Sample collection	Time between collection and analysis	Specific remarks
Beck et al. [45]	Ion-exchange HPLC (Tosoh G8)	Comparison between liquid capillary and venous blood from 240 diabetes patients	5–15 µL capillary blood collected by the participants (or their parents) in the clinic unsupervised	Up to 10 days	Extensive study on the use of liquid blood micro-samples as alternative for venous specimens
Huijskens et al. [58]	Ion-exchange HPLC (Tosoh G8)	Comparison between liquid capillary and venous blood from random patients requiring blood collection	200 µL capillary (≈4–5 drops) blood collected by experienced phlebotomists	Up to 5 days	
Nathan et al. [46]	Ion-exchange HPLC (Tosoh)	Comparison between liquid capillary and venous blood from 122 diabetes patients	Capillary blood collected by the participants in the clinic	Up to 22 days	
Voss et al. [50]	Ion-exchange HPLC (Bio-Rad Diamat)	Comparison between venous blood and liquid capillary blood from 47 pediatric diabetes patients	5 µL capillary blood at the hospital	Up to 22 days	
Groenendijk et al. [56]	Capillary electrophoresis (Sebia Capillarys 3 Tera)	Comparison between liquid capillary and venous blood from 41 diabetes patients	20 µL capillary blood collected by the participants remotely	?	
Cross et al. [57]	POC analyser (Siemens DCA Vantage)	Comparison between 286 capillary blood samples collected at the diabetes center and at home by diabetes patients	50–100 µL capillary blood (≈5–10 drops) collected at the participant's home	Up to 12 days	Evaluation of at-home blood sampling method for measuring HbA_{1c} for virtual consultations

Papers are ordered per analytical technique and in alphabetical order of the first author. Papers of special interest/relevance have been highlighted in bold.

center were obtained [57]. In another study, almost identical HbA_{1c} values were observed in 41 paired capillary (collected at home by the participants in MiniCollect™ tubes and subsequently mailed to the lab) and venous (collected during clinic appointment) samples: 40/41 of the capillary results were within the total allowable error of ±5 mmol/mol [56].

Commercial kits for collection of these capillary samples at home already exist. For example the Bio-Rad Hemoglobin Capillary Collection System [61] consists of a 5 µL plastic capillary which should be filled with capillary blood from a fingertip. Thereafter, the vial should be transferred to a sample preparation vial, containing an aqueous solution of EDTA and potassium cyanide (0.25 mmol/L). After capping, the vial should be shaken to rinse the blood completely from the capillary into the solution. After that, the sample is ready for analysis, transport or storage. According to the insert, samples are stable for two weeks at room temperature, four weeks at 2–8 °C and four days at 42 °C [61]. These collection kits were used in two recent studies and were found suitable for HbA_{1c} monitoring at home with shipping of the samples to a central laboratory. In the first study, liquid capillary blood samples were compared with corresponding venous blood samples obtained from 240 diabetes patients (adult and pediatric). The Bio-Rad kit was compared to another kit containing a microfuge tube filled with a stabilizing solution of EDTA and other undisclosed preservatives. Capillary results from both collection kits agreed with the conventional results as more than 95 % of the results were within ±5 % of the venous HbA_{1c} value. Moreover, no differences in HbA_{1c} results were observed between samples shipped with or without a cold pack by USPS, or shipped on dry ice by overnight carrier. HbA_{1c} results were also not affected by temperatures during the shipment exceeding 37.8 °C and by shipping times up to 10 days [45]. Nathan and colleagues confirmed the ease and possibility to obtain clinically acceptable HbA_{1c} results from capillary finger-prick samples collected using the Bio-Rad collection kit [46].

Liquid capillary microsamples were found to be stable for up to 7 days when stored at 4 °C [56] and up to 12 days when stored at room temperature [57], allowing postal delays. Using Hem-Col™ devices (microtubes containing EDTA dissolved in preservation fluid), HbA_{1c} was found to be stable for at least 5 days at room temperature, 4 °C and 37 °C [58]. Liquid capillary blood remote collections were also positively evaluated by the users and were perceived as easy to use [45, 56]. At our hospital, collecting capillary blood in a heparinized capillary tube is currently still the standard procedure for measuring HbA_{1c} in pediatric population [9].

In a recent published systematic review it was concluded that for self-collected and posted capillary blood microsamples, HbA_{1c} results in microtubes/capillary tubes agreed better with conventional venous values than dried blood microsamples. Moreover, only the liquid microsample based methods met IFCC quality targets (i.e. ±5 mmol/mol), and the limits of agreement, when compared to venous liquid blood, were smaller than those observed when using dried blood microsamples [62].

Conclusions

Over the past four decades, a plethora of papers has been published on the measurement of HbA_{1c} in blood microsamples. However, the published data on study designs, results and conclusions are considerably heterogeneous. Moreover, many of the included studies lacked details on collection, storage and shipment conditions of the dried samples. As a consequence, different conclusions from apparently similar results were drawn. To promote implementation of liquid or dried blood microsampling based approaches in routine clinical care – which is to our knowledge still sparse – the entire procedure (from collection to analysis) should be 'under control'. To achieve successful implementation in the clinical laboratory, a few issues should be encountered during method validation. First, it is important that the expected conditions of the sample collection of the future intended method are evaluated. If for example the intended goal is home-sampling, microsamples actually collected by the participants in their home environment must be compared to the conventional blood samples. Expected conditions encountered during (postal) transportation of the microsamples to the lab should also be mimicked in the validation experiments. The study population should also resemble real-life patients. Moreover, when evaluating the agreement between HbA_{1c} results obtained from microsamples and traditional venous liquid blood, objective acceptance criteria should be used. Lastly, if a conversion factor or equation is used to correct the obtained microsample results, this should be evaluated on an independent cohort and not on the dataset which was used to generate the factor or equation.

Despite the heterogeneity of the findings from the studies included in this review, it is clear that there is an issue with drying and ageing of the microsamples to obtain clinically acceptable HbA_{1c} results. Instead of using dried blood microsamples, liquid microsamples may be a better alternative to remotely monitor HbA_{1c}: high agreements

with HbA_{1c} results from conventional samples have been described and no stability issues have been reported. Several commercial sampling kits are already available to collect and transport capillary liquid blood in a convenient manner.

Research funding: None declared.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Informed consent: Not applicable.
Ethical approval: Not applicable.

References

1. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: standards of medical care in diabetes-2022. Diabetes Care 2022;45:S17–38.
2. The International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327–34.
3. Zhang X, Gregg EW, Williamson DF, Barker LE, Thomas W, Bullard KM, et al. A1C level and future risk of diabetes: a systematic review. Diabetes Care 2010;33:1665–73.
4. American Diabetes Association Professional Practice Committee. Glycemic targets: standards of medical care in diabetes—2022. Diabetes Care 2021;45:S83–S96.
5. Gillery P. HbA(1c) and biomarkers of diabetes mellitus in clinical chemistry and laboratory medicine: ten years after. Clin Chem Lab Med 2022;61:861–72.
6. National Glycohemoglobin Standardization Program. Certified methods and laboratories. Available from: http://ngsp.org/certified.asp [Accessed Sept 2022].
7. Little RR, Roberts WL. A review of variant hemoglobins interfering with hemoglobin A1c measurement. J Diabetes Sci Technol 2009;3:446–51.
8. National Glycohemoglobin Standardization Program. HbA1c assay interferences. Available from: http://www.ngsp.org/interf.asp [Accessed Sept 2022].
9. Verougstraete N, Lapauw B, Van Aken S, Delanghe J, Stove C, Stove V. Volumetric absorptive microsampling at home as an alternative tool for the monitoring of HbA1c in diabetes patients. Clin Chem Lab Med 2017;55:462–9.
10. Verougstraete N, Stove V, Verstraete AG, Stove CP. Therapeutic drug monitoring of tyrosine kinase inhibitors using dried blood microsamples. Front Oncol 2022;12:821807.
11. Hall JM, Fowler CF, Barrett F, Humphry RW, Van Drimmelen M, MacRury SM. HbA(1c) determination from HemaSpot™ blood collection devices: comparison of home prepared dried blood spots with standard venous blood analysis. Diabet Med 2020;37:1463–70.
12. Schnell O, Crocker JB, Weng J. Impact of HbA1c testing at point of care on diabetes management. J Diabetes Sci Technol 2017;11:611–7.
13. Egier DA, Keys JL, Hall SK, McQueen MJ. Measurement of hemoglobin A1c from filter papers for population-based studies. Clin Chem 2011;57:577–85.
14. Lacher DA, Berman LE, Chen TC, Porter KS. Comparison of dried blood spot to venous methods for hemoglobin A1c, glucose, total cholesterol, high-density lipoprotein cholesterol, and C-reactive protein. Clin Chim Acta 2013;422:54–8.
15. Thomas D, Seeman T, Potter A, Hu P, Crimmins E, Herningtyas EH, et al. HPLC-Based measurement of glycated hemoglobin using dried blood spots collected under adverse field conditions. Biodemogr Soc Biol 2018;64:43–62.
16. Jones TG, Warber KD, Roberts BD. Analysis of hemoglobin A1c from dried blood spot samples with the Tina-quantR II immunoturbidimetric method. J Diabetes Sci Technol 2010;4:244–9.
17. Wikblad K, Smide B, Bergström A, Wahren L, Mugusi F, Jeppsson JO. Immediate assessment of HbA1c under field conditions in Tanzania. Diabetes Res Clin Pract 1998;40:123–8.
18. Elliott TG, Dooley KC, Zhang M, Campbell HSD, Thompson DJS. Comparison of glycated hemoglobin results based on at-home and in-lab dried blood spot sampling to routine venous blood sampling in-lab in adult patients with type 1 or type 2 diabetes. Can J Diabetes 2018;42:426–32.e1.
19. Protti M, Mandrioli R, Mercolini L. Quantitative microsampling for bioanalytical applications related to the SARS-CoV-2 pandemic: usefulness, benefits and pitfalls. J Pharmaceut Biomed Anal 2020;191:113597.
20. Wong MP, Meas MA, Adams C, Hernandez S, Green V, Montoya M, et al. Development and implementation of dried blood spot-based COVID-19 serological assays for epidemiologic studies. Microbiol Spectr 2022;10:e0247121.
21. Roberts AJ, Malik F, Pihoker C, Dickerson JA. Adapting to telemedicine in the COVID-19 era: feasibility of dried blood spot testing for hemoglobin A1c. Diabetes Metabol Syndr 2021;15:433–7.
22. Garnett ER, Recio B, Jung J, Tam E, Devaraj S. Pandemic-associated trends in measurement of HbA1c in children with diabetes mellitus and validation of dried blood spot as an alternative sample matrix. Ann Clin Lab Sci 2021;51:535–9.
23. Gill EL, Patel K, Dickerson JA, Dulik MC, Grant RP, Heaney DL, et al. Alternative sample matrices supporting remote sample collection during the pandemic and beyond. Clin Chem 2022;68:269–75.
24. Jeppsson JO, Jerntorp P, Almër LO, Persson R, Ekberg G, Sundkvist G. Capillary blood on filter paper for determination of HbA1c by ion exchange chromatography. Diabetes Care 1996;19:142–5.
25. Pollock AJ, Allen DB, Wiebe D, Eickhoff J, MacDonald M, Baker M. Development of filter paper hemoglobin A1c assay applicable to newborn screening. Clin Chim Acta 2016;457:24–6.
26. Maleska A, Hirtz C, Casteleyn E, Villard O, Ducos J, Avignon A, et al. Comparison of HbA1c detection in whole blood and dried blood spots using an automated ion-exchange HPLC system. Bioanalysis 2017;9:427–34.
27. Parkes J, Ray R, Kerestan S, Davis H, Ginsberg B. Prospective evaluation of accuracy, precision, and reproducibility of an at-home hemoglobin A1c sampling kit. Diabetes Technol Therapeut 1999;1:411–9.
28. Fokkema MR, Bakker AJ, de Boer F, Kooistra J, de Vries S, Wolthuis A. HbA1c measurements from dried blood spots: validation and patient satisfaction. Clin Chem Lab Med 2009;47:1259–64.
29. Lakshmy R, Gupta R. Measurement of glycated hemoglobin A1c from dried blood by turbidimetric immunoassay. J Diabetes Sci Technol 2009;3:1203–6.
30. Hu P, Edenfield M, Potter A, Kale V, Risbud A, Williams S, et al. Validation and modification of dried blood spot-based glycosylated hemoglobin assay for the longitudinal aging study in India. Am J Hum Biol 2015;27:579–81.

31. Mastronardi CA, Whittle B, Tunningley R, Neeman T, Paz-Filho G. The use of dried blood spot sampling for the measurement of HbA1c: a cross-sectional study. BMC Clin Pathol 2015;15:13.
32. Miller IM, Lacher DA, Chen TC, Zipf GW, Gindi RM, Galinsky AM, et al. Collection and laboratory methods for dried blood spots for hemoglobin A1c and total and high-density lipoprotein cholesterol in population-based surveys. Clin Chim Acta 2015;445:143–54.
33. Scherer N, Kurbasic A, Dings C, Mari A, Nock V, Hennige AM, et al. The impact and correction of analysis delay and variability in storage temperature on the assessment of HbA1c from dried blood spots – an IMI direct study. Int J Proteom Bioinf 2019;4:7–13.
34. Crawford ML, Collier BB, Bradley MN, Holland PL, Shuford CM, Grant RP. Empiricism in microsampling: utilizing a novel lateral flow device and intrinsic normalization to provide accurate and precise clinical analysis from a finger stick. Clin Chem 2020;66:821–31.
35. Henderson CM, Bollinger JG, Becker JO, Wallace JM, Laha TJ, MacCoss MJ, et al. Quantification by nano liquid chromatography parallel reaction monitoring mass spectrometry of human apolipoprotein A-I, apolipoprotein B, and hemoglobin A1c in dried blood spots. Proteomics. Clin Appl 2017;11. https://doi.org/10.1002/prca.201600103.
36. Goldstein DE, Wiedmeyer HM, England JD, Little RR, Parker KM. Glycosylated protein in whole blood spotted on filter paper. Clin Chem 1982;28:386–7.
37. Hall JM, Fowler CF, Pollock MA, MacRury SM. Haemoglobin A1c determination from dried blood spots prepared with HemaSpot™ blood collection devices: comparison with fresh capillary blood. Clin Chem Lab Med 2020;59:e79–82.
38. Verougstraete N, Stove V, Stove C. Wet absorptive microsampling at home for HbA1c monitoring in diabetic children. Clin Chem Lab Med 2018;56:e291–4.
39. Lima DA, Schuch RA, Salgueiro JS, Pintão MCT, Carvalho VM. Evaluation of volumetric absorptive microsampling and mass spectrometry data-independent acquisition of hemoglobin-related clinical markers. J Proteome Res 2022;21:1816–28.
40. Acar K, Kurtulus Dereli A, Avci E, Zeybek V, Kutlu E, Demir S, et al. Determination of haemoglobin A1c levels using high-performance liquid chromatography of bloodstains. Med Sci Law 2020;60:19–25.
41. Ghent University. VID 20190115 153239. Available from: https://www.youtube.com/watch?v=5nCyNUFCF3c [Accessed Dec 2022].
42. Little RR, Rohlfing CL, Tennill AL, Connolly S, Hanson S. Effects of sample storage conditions on glycated hemoglobin measurement: evaluation of five different high performance liquid chromatography methods. Diabetes Technol Therapeut 2007;9:36–42.
43. Rohlfing CL, Hanson S, Tennill AL, Little RR. Effects of whole blood storage on hemoglobin a1c measurements with five current assay methods. Diabetes Technol Therapeut 2012;14:271–5.
44. Affan ET, Praveen D, Chow CK, Neal BC. Comparability of HbA1c and lipids measured with dried blood spot versus venous samples: a systematic review and meta-analysis. BMC Clin Pathol 2014;14:21.
45. Beck RW, Bocchino LE, Lum JW, Kollman C, Barnes-Lomen V, Sulik M, et al. An evaluation of two capillary sample collection kits for laboratory measurement of HbA1c. Diabetes Technol Therapeut 2021;23:537–45.
46. Nathan DM, Krause-Steinrauf H, Braffett BH, Arends VL, Younes N, McGee P, et al. Comparison of central laboratory HbA1c measurements obtained from a capillary collection versus a standard venous whole blood collection in the GRADE and EDIC studies. PLoS One 2021;16:e0257154.
47. Little RR, McKenzie EM, Wiedmeyer HM, England JD, Goldstein DE. Collection of blood on filter paper for measurement of glycated hemoglobin by affinity chromatography. Clin Chem 1986;32:869–71.
48. Gay EC, Cruickshanks KJ, Chase HP, Klingensmith G, Hamman RF. Accuracy of a filter paper method for measuring glycosylated hemoglobin. Diabetes Care 1992;15:108–10.
49. Capiau S, Veenhof H, Koster RA, Bergqvist Y, Boettcher M, Halmingh O, et al. Official international association for therapeutic drug monitoring and clinical toxicology guideline: development and validation of dried blood spot-based methods for therapeutic drug monitoring. Ther Drug Monit 2019;41:409–30.
50. Voss EM, Cembrowski GS, Clasen BL, Spencer ML, Ainslie MB, Haig B. Evaluation of capillary collection system for HbA1c specimens. Diabetes Care 1992;15:700–1.
51. Shah AR, Challener J, Elsey TS, Maguire GA, Calvin J, Rayman G. A novel capillary collection method for obtaining current glycosylated haemoglobin levels in diabetic children. Diabet Med 1994;11:319–22.
52. Lytken Larsen M. Evaluation of a new capillary blood collection system for laboratory assay of glycated haemoglobin. Scand J Clin Lab Investig 1986;46:315–7.
53. Heylen O, Van Neyghem S, Exterbille S, Wehlou C, Gorus F, Weets I. Evaluation of the Sebia CAPILLARYS 2 flex piercing for the measurement of HbA(1c) on venous and capillary blood samples. Am J Clin Pathol 2014;141:867–77.
54. Godefroid MJ, De Buyzere ML, Delanghe JR. Interchangeability of venous and capillary HbA(1c) results is affected by oxidative stress. Clin Chem Lab Med 2013;51:e9–11.
55. Verougstraete N, Stove V, Stove C. Comment on HbA(1c) determination from HemaSpot blood collection devices: comparison of home-prepared dried blood spots with standard venous blood analysis. Diabet Med 2020;37:1613–4.
56. Groenendijk WN, Griffin TP, Islam MN, Blake L, Wall D, Bell M, et al. Remote capillary blood collection for HbA(1c) measurement during the COVID-19 pandemic: a laboratory and patient perspective. Diabet Med 2022;39:e14897.
57. Cross J, Sharma S, John WG, Rayman G. Validation and feasibility of a postal system for remote monitoring of HbA1c. BMJ Open Diabetes Res Care 2021;9. https://doi.org/10.1136/bmjdrc-2021-002527.
58. Huijskens M, Castel R, Vermeer HJ, Verheijen FM. Evaluation of Diabetes Care parameters in capillary blood collected with a novel sampling device. Practical Lab Med 2019;17:e00135.
59. Bendavid C, Peltier L, Letellier C. Capillary blood sampling kit for HbA1c versus venous puncture on Capillarys 2 flex piercing. Clin Chem Lab Med 2015;53(Suppl):S655.
60. Voss EM, Cembrowski GS, Haig B, Spencer ML. Stability of mailed and couriered capillary HbA1c samples. Diabetes Care 1993;16:665–6.
61. Bio-Rad. Hemoglobin capillary collection system. Available from: https://www.bio-rad.com/en-be/product/hemoglobin-capillary-collection-system?ID=676dfa9b-8395-42b4-9362-0a47c38401dc [Accessed Sept 2022].
62. Colley J, Dambha-Miller H, Stuart B, Bartholomew J, Benton M, Baykoca J, et al. Home monitoring of Haemoglobin A1c in diabetes: a systematic review and narrative synthesis on accuracy, reliability, and patient acceptability. Diabet Med 2023;40:e15033. e15033.
63. Royal College of Pathologists of Australasia. RCPA quality requirements. Available from: https://www.westgard.com/rcpa.htm [Accessed Sept 2022].

64. Little RR. Performance of hemoglobin A1c assay methods: good enough? Clin Chem 2014;60:1031–3.
65. National Glycohemoglobin Standardization Program. Obtaining certification – summary of NGSP criteria. Available from: http://www.ngsp.org/critsumm.asp [Accessed Sept 2022].
66. National Glycohemoglobin Standardization Program. NGSP steering committee meeting; 2010. Available from: http://www.ngsp.org/SC2010.asp [Accessed Sept 2022].
67. Weykamp CW, Mosca A, Gillery P, Panteghini M. The analytical goals for hemoglobin A(1c) measurement in IFCC units and National Glycohemoglobin Standardization Program Units are different. Clin Chem 2011;57:1204–6.
68. Westgard QC. Desirable specifications for total error, imprecision, and bias, derived from intra- and inter-individual biologic variation. Available from: https://www.westgard.com/biodatabase1.htm [Accessed Sept 2022].