Review

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Remote HbA\textsubscript{1c} testing via microsampling: fit for purpose?

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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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{1c} analysis in the lab.

Keywords: dried blood microsampling; HbA\textsubscript{1c}; liquid blood microsampling; microsampling

Introduction

Hemoglobin A\textsubscript{1c} (HbA\textsubscript{1c}) is a glycated protein which is formed \textit{in vivo} by the non-enzymatic reaction of glucose with the N-terminal valine residue of beta globin chains of HbA. Determination of HbA\textsubscript{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\textsubscript{1c} is not subject to wide day-to-day and hour-to-hour fluctuations [1]. HbA\textsubscript{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\textsubscript{1c} value of $\geq 6.5\%$ or 48 mmol/mol is used as a threshold for the diagnosis of diabetes [2]. Pre-diabetes is defined by HbA\textsubscript{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\textsubscript{1c} results have a strong predictive value for the occurrence of diabetes complications. Therefore, during treatment, HbA\textsubscript{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.
HbA1c 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 HbA1c testing, leading to incorrect results and consequently inappropriate treatment decisions. For each HbA1c 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].

HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 sub-punch. 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 HbA1c-based methods.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Microsampling technique</th>
<th>Complete DBS vs. punch (P) (diameter)</th>
<th>Extraction/elution solution</th>
<th>Analytical technique</th>
<th>Application + included patients/samples</th>
<th>Sample collection</th>
<th>Time between collection and analysis</th>
<th>Specific remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egier et al. [13]</td>
<td>DBS</td>
<td>P 3/16 inch</td>
<td>1 ml hemolyzing buffer</td>
<td>Ion-exchange HPLC (Bio-Rad Variant II)</td>
<td>Comparison between lab-prepared venous DBS and whole blood from 85 clinical specimens</td>
<td>Whole blood applied on filter paper in the lab</td>
<td>Within 96 h of sample collection</td>
<td></td>
</tr>
<tr>
<td>Jeppson et al. [24]</td>
<td>DBS</td>
<td>P 3 mm</td>
<td>75 µL Na phosphate-citrate Triton X-100 buffer + cysteine-containing buffer</td>
<td>Ion-exchange HPLC (in house)</td>
<td>Comparison between capillary DBS and venous blood from 41 patients</td>
<td>Capillary collection by participants under supervision at outpatient diabetes unit</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Lacher et al. [14]</td>
<td>DBS</td>
<td>P 3.2 mm</td>
<td>Ion-exchange HPLC (Bio-Rad Variant II)</td>
<td>Population-based epidemiology study with 386 DBS samples</td>
<td>75 µL whole blood spotted on filter paper</td>
<td>Refrigerated up to 7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maleska et al. [26]</td>
<td>DBS</td>
<td>P 6 mm</td>
<td>400 µL DW buffer</td>
<td>Ion-exchange HPLC (Arkray ADAMS HA-8180V)</td>
<td>Comparison between lab-prepared venous DBS and whole blood samples from 217 participants</td>
<td>NA</td>
<td>Full analytical validation under ISO15189 guideline</td>
<td></td>
</tr>
<tr>
<td>Pollock et al. [25]</td>
<td>DBS</td>
<td>?</td>
<td>?</td>
<td>Ion-exchange HPLC (Tosoh G7)</td>
<td>NA</td>
<td>Blood spotted on filter paper</td>
<td>Up to 6 days</td>
<td>Focus on HbA1c as marker in newborn screening</td>
</tr>
<tr>
<td>Thomas et al. [15]</td>
<td>DBS</td>
<td>?</td>
<td>?</td>
<td>Ion-exchange HPLC (Bio-Rad D-10)</td>
<td>Comparison between venous DBS and venous blood from 143 subjects</td>
<td>Venous blood was spotted on filter papers both in controlled clinic settings and in field-type setting</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Crawford et al. [34]</td>
<td>DBS</td>
<td>P 91 mm²</td>
<td>2000 µL hemolyzing reagent</td>
<td>Immuno-turbidimetry (Roche Tina-quant)</td>
<td>Comparison between HbA1c, measured from dried blood fractions of collection device to venous whole blood samples from healthy donors</td>
<td>Both assisted- as self-collected capillary blood samples via finger-prick</td>
<td>NA</td>
<td>Samples were collected via a device which uses laminar-flow migration to separate cellular components from plasma. HbA1c was measured on the dried RBC portion (and cholesterol on the dried plasma).</td>
</tr>
<tr>
<td>Elliott et al. [18]</td>
<td>DBS</td>
<td>P 6 mm</td>
<td>500 µL hemolyzing reagent</td>
<td>Immuno-turbidimetry (Roche Tina-quant)</td>
<td>Comparison between capillary DBS and venous whole blood from 50 diabetes patients</td>
<td>Capillary collection under supervision and by participants at home</td>
<td>DBS included in study up to 7 days</td>
<td>Comparison between DBS collected under supervision at the lab and unsupervised at participants’ home</td>
</tr>
<tr>
<td>Fokkema et al. [28]</td>
<td>DBS</td>
<td>P 3 mm</td>
<td>250 µL hemolyzing buffer</td>
<td>Immuno-turbidimetry (Roche Tina-quant)</td>
<td>Comparison between capillary DBS and venous whole blood from 93 diabetes patients</td>
<td>Capillary collection by laboratory personnel and by participants at home</td>
<td>Within 10 days</td>
<td>Comparison between DBS collected by laboratory personnel and by participants at home</td>
</tr>
<tr>
<td>Reference</td>
<td>Microsampling technique</td>
<td>Complete DBS vs. punch (P) (diameter)</td>
<td>Extraction/elution solution</td>
<td>Analytical technique</td>
<td>Application + included patients/samples</td>
<td>Sample collection</td>
<td>Time between collection and analysis</td>
<td>Specific remarks</td>
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<tr>
<td>Hu et al. [30]</td>
<td>DBS</td>
<td>P 3.2 mm</td>
<td>300 µL hemolyzing reagent</td>
<td>Immuno-turbidimetry (Olympus AU HbA\textsubscript{1c})</td>
<td>NA</td>
<td>?</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Jones et al. [16]</td>
<td>DBS</td>
<td>2 P 1/8 inch</td>
<td>500 µL hemolyzing reagent</td>
<td>Immuno-turbidimetry (Roche Tina-quant)</td>
<td>Comparison between (lab-prepared) venous DBS and whole blood from 73 routine HbA\textsubscript{1c} samples</td>
<td>20 µL whole blood spotted on filter paper</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Lakshmy et al. [29]</td>
<td>DBS</td>
<td>P 6 mm</td>
<td>400 µL hemolyzing reagent</td>
<td>Immuno-turbidimetry (Agappe latex method)</td>
<td>Comparison between (lab-prepared) venous DBS and whole blood samples from 30 diabetes patients</td>
<td>10 µL whole blood spotted on filter paper</td>
<td>15 days when stored at 4°C</td>
<td></td>
</tr>
<tr>
<td>Mastronardi et al. [31]</td>
<td>DBS</td>
<td>P 3.2 mm</td>
<td>Haemolysing reagent</td>
<td>Immuno-turbidimetry (Thermo Fisher HbA\textsubscript{1c})</td>
<td>Comparison between (capillary and venous) DBS and whole blood from 115 participants</td>
<td>Capillary collection by participants in a controlled research setting DBS were collected at the participants' home by health technicians or field interviewers</td>
<td>Up to 14 days, results were acceptable up to 7 days ?</td>
<td>Application of correction equations taken into account time between sample collection and analysis Performance of collecting DBS by experienced health workers vs. unexperienced interviewers was compared</td>
</tr>
<tr>
<td>Miller et al. [32]</td>
<td>DBS</td>
<td>P 3 mm</td>
<td>Deionized water</td>
<td>Immuno-turbidimetry (Kamiya Biomedical kit)</td>
<td>Comparison between capillary DBS and venous blood from 125 DBS</td>
<td>Capillary collection by patient in-clinic setting</td>
<td>3–12 days after sample collection</td>
<td>Multicenter study focused on the impact of different storage conditions</td>
</tr>
<tr>
<td>Parkes et al. [27]</td>
<td>DBS</td>
<td>?</td>
<td>?</td>
<td>Immuno-turbidimetry (Roche Tina-quant)</td>
<td>1625 DBS from 59 diabetes patients</td>
<td>Capillary collection by participants at the clinical site or by the participants at home</td>
<td>Range from 0 to 400 days</td>
<td></td>
</tr>
<tr>
<td>Scherer et al. [33]</td>
<td>DBS</td>
<td>?</td>
<td>?</td>
<td>Immuno-turbidimetry (Thermo Fisher HbA\textsubscript{1c})</td>
<td>14,243 DBS cards from 2,237 participants atrisk of developing diabetes</td>
<td>DBS either taken by a nurse at the clinical site or by the participants at home</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Henderson et al. [35]</td>
<td>DBS</td>
<td>P 3.2 mm</td>
<td>100 mM ammonium bicarbonate + trifluoroethanol</td>
<td>LC-PRM-MS (in house)</td>
<td>Comparison between venous whole blood and DBS obtained from 36 healthy volunteers</td>
<td>Capillary DBS were obtained from each volunteer by a trained phlebotomist 1 DBS and 2 VAMS were collected by the patients at their home</td>
<td>NA</td>
<td>Simultaneously measurement of HbA\textsubscript{1c}, apoA-I and apoB in DBS</td>
</tr>
<tr>
<td>Verougstraete et al. [9]</td>
<td>DBS + VAMS</td>
<td>P 3 mm</td>
<td>DBS: 250 µL deionized water; VAMS: 400 µL deionized water</td>
<td>Ion-exchange HPLC (Tosoh G8)</td>
<td>Capillary DBS and VAMS were compared to liquid blood results from 86 diabetes patients (40 adults and 44 children)</td>
<td>1 DBS and 2 VAMS were collected by the patients at their home</td>
<td>Up to 6 days</td>
<td>Collection of two types of dried blood microsamples: DBS and VAMS</td>
</tr>
<tr>
<td>Verougstraete et al. [38]</td>
<td>Wet VAMS</td>
<td>NA</td>
<td>800 µL deionized water</td>
<td>Ion-exchange HPLC (Tosoh G8)</td>
<td>Comparison between wet VAMS and liquid capillary</td>
<td>2 VAMS samples were collected by the patients</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Verougstraete et al.: HbA\textsubscript{1c} testing via microsampling
### Table 1: (continued)

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

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\textsuperscript{TM} 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\textsubscript{c} 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\textsubscript{c} 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\textsubscript{c} 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\textsubscript{c} 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\textsubscript{c} 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\textsubscript{c} 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\textsubscript{c} result. In the following section the stability of HbA\textsubscript{c} 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\textsubscript{c} in routine settings the added value of this potentially increased stability is somewhat limited, given the stability of HbA\textsubscript{c} in conventional liquid whole blood samples. Liquid whole blood for HbA\textsubscript{c} 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 HbA1c 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 A1c 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 HbA1c 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.

### 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 HbA1c 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 hemolyzing 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 in order to prevent possible 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 HbA1c 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 HbA1c 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-HbA1c antibody is used, which reacts with HbA1c 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 HbA1c assay (Roche Diagnostics) [16,18,27,28,34], immunoturbidimetric latex method (Agappe Diagnostics) [29], Kamiya Biomedical HbA1c kit [32] and the Olympus HbA1c kit [30]. Two (older) studies reported comparable CVs for DBS and fresh blood samples, suggesting a negligible error introduced by DBS method.

Two mass spectrometry-based HbA1c methods in dried blood matrices were published. In a proof-of-concept study, HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c values are expressed as a relative ratio (mmol/mol or percentage), these parameters are unlikely to affect the measured HbA1c result. Furthermore, as the standard matrix for HbA1c analysis is whole blood, HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c results that guides clinicians to change therapy [56].

Most studies reported a high correlation between HbA1c results obtained from DBS and venous liquid whole blood
Table 3: Applied acceptance criteria for evaluating the agreement of HbA1c results obtained in (dried) blood microsamples vs. conventional samples.

<table>
<thead>
<tr>
<th>Acceptance criterion</th>
<th>Criterion based on</th>
<th>Applied in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total allowable error: ±4 mmol/mol (IFCC) or ±0.4 % (NGSP)</td>
<td>RCPA based on biological variation [63]</td>
<td>Verougustraete et al. [9, 38] + Hall et al. [37]</td>
</tr>
<tr>
<td>Total allowable relative error of ±6 % in at least 92.5 % of all samples</td>
<td>Little RR [64]</td>
<td>Elliott et al. [18]</td>
</tr>
<tr>
<td>±6 % evaluation limit</td>
<td>CAP evaluation limits for proficiency testing NGSP [65]</td>
<td>Miller et al. [32]</td>
</tr>
<tr>
<td>Total allowable relative error of ±5 % in ≥90 % of all samples</td>
<td></td>
<td>Beck et al. [45]</td>
</tr>
<tr>
<td>Maximum acceptable difference of ±5 mmol/mol (IFCC) or ±0.5 % (NGSP).</td>
<td>Smallest difference in HbA1c values of consecutive tests that guide to change in therapy NGSP [66]</td>
<td>Jones et al. [16]</td>
</tr>
<tr>
<td>95 % confidence interval of the differences must be within ±0.75 % (NGSP) or ±8.2 mmol/mol (IFCC) HbA1c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total allowable error: ±5 mmol/mol (IFCC) or ±0.5 % (NGSP)</td>
<td>Weykamp et al. [67]</td>
<td>Verougustraete et al. [9] + clinical laboratory of UZ Ghent</td>
</tr>
<tr>
<td>Total allowable relative error of ±4.6 %</td>
<td>Biological variation of HbA1c</td>
<td>Crawford et al. [34]</td>
</tr>
</tbody>
</table>

If absolute HbA1c 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 HbA1c 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 HbA1c DBS results. Mastronardi et al. observed that the correlation between DBS and venous blood HbA1c 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 HbA1c whole blood values from DBS measurements [31]. Similarly, Elliott et al. observed that the obtained biases on HbA1c 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 HbA1c analysis. DBS HbA1c results, collected with HemaSpot TM devices, analyzed up to five days after collection (stored at ambient temperature) were compared with capillary liquid blood HbA1c results. After applying a day-dependent correction formula, home-prepared DBS HbA1c 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 TM DBS preparation and analysis (up to 4 days) did not affect the HbA1c 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 abovementioned 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 HbA1c 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 HbA1c 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, HbA1c 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 HbA1c results with the corresponding whole blood values [33].

In a recent study, Verougstraete et al. compared HbA1c 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 HbA1c 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 HbA1c 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 HbA1c with LC-MS in samples collected by VAMS, found that upon storage at room temperature, HbA1c 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 HbA1c 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 HbA1c results [9, 13, 26]. This was nicely exemplified by a study conducted by Thomas et al. These authors obtained DBS HbA1c 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 %HbA1c calculation. This was due to the presence of an extra unexpected peak to the right of A0, 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, HbA1c 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 HbA1c 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 HbA1c values [18, 28, 31, 34].

In their study, Verougstraete 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 HbA1c 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 HbA1c 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 HbA1c 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 HbA1c 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 center.

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**Table 4:** Overview of published liquid blood microsample HbA1c-based methods.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Analytical technique</th>
<th>Application + included patients/samples</th>
<th>Sample collection</th>
<th>Time between collection and analysis</th>
<th>Specific remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beck et al. [45]</td>
<td>Ion-exchange HPLC (Tosoh G8)</td>
<td>Comparison between liquid capillary and venous blood from 240 diabetes patients</td>
<td>5–15 µL capillary blood collected by the participants (or their parents) in the clinic unsupervised</td>
<td>Up to 10 days</td>
<td>Extensive study on the use of liquid blood microsamples as alternative for venous specimens</td>
</tr>
<tr>
<td>Huiskens et al. [58]</td>
<td>Ion-exchange HPLC (Tosoh G8)</td>
<td>Comparison between liquid capillary and venous blood from random patients requiring blood collection</td>
<td>200 µL capillary blood (≈4–5 drops) blood collected by experienced phlebotomists</td>
<td>Up to 5 days</td>
<td></td>
</tr>
<tr>
<td>Nathan et al. [46]</td>
<td>Ion-exchange HPLC (Tosoh)</td>
<td>Comparison between liquid capillary and venous blood from 122 diabetes patients</td>
<td>Capillary blood collected by the participants in the clinic</td>
<td>Up to 22 days</td>
<td></td>
</tr>
<tr>
<td>Voss et al. [50]</td>
<td>Ion-exchange HPLC (Bio-Rad Diamat)</td>
<td>Comparison between venous blood and liquid capillary blood from 47 pediatric diabetes patients</td>
<td>5 µL capillary blood at the hospital</td>
<td>Up to 22 days</td>
<td></td>
</tr>
<tr>
<td>Groenendijk et al. [56]</td>
<td>Capillary electrophoresis (Sebia Capillaries 3 Tera)</td>
<td>Comparison between liquid capillary and venous blood from 41 diabetes patients</td>
<td>20 µL capillary blood collected by the participants remotely</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Cross et al. [57]</td>
<td>POC analyser (Siemens DCA Vantage)</td>
<td>Comparison between 286 capillary blood samples collected at the diabetes center and at home by diabetes patients</td>
<td>50–100 µL capillary blood (≈5–10 drops) collected at the participant’s home</td>
<td>Up to 12 days</td>
<td>Evaluation of at-home blood sampling method for measuring HbA1c for virtual consultations</td>
</tr>
</tbody>
</table>

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 HbA1c 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 HbA1c 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 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. The套装 should be transferred to a sample preparation vial, containing an aqueous 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 HbA1c value. Moreover, no differences in HbA1c results were observed between samples shipped with or without a cold pack by USPS, or shipped on dry ice by overnight carrier. HbA1c 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 HbA1c 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), HbA1c 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 HbA1c in pediatric population [9].

In a recent published systematic review it was concluded that for self-collected and posted capillary blood microsamples, HbA1c 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 HbA1c 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 HbA1c 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 HbA1c results. Instead of using dried blood microsamples, liquid microsamples may be a better alternative to remotely monitor HbA1c; high agreements
with HbA1c 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.

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Informed consent: Not applicable.

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