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Alternative Sampling Devices to Collect Dried Blood Microsamples: State-of-the-Art

Lisa Delahaye, PharmD,* Herman Veenhof, PhD,† Birgit C. P. Koch, PhD,‡ Jan-Willem C. Alffenaar, PhD,§¶|| Rafael Linden, PhD,** and Christophe Stove, PhD*

Abstract: Dried blood spots (DBS) have been used in newborn screening programs for several years. More recently, there has been growing interest in using DBS as a home sampling tool for the quantitative determination of analytes. However, this presents challenges, mainly because of the well-known hematocrit effect and other DBS-specific parameters, including spotted volume and punch site, which could add to the method uncertainty. Therefore, new microsampling devices that quantitatively collect capillary dried blood are continuously being developed. In this review, we provided an overview of devices that are commercially available or under development that allow the quantitative (volumetric) collection of dried blood (-based) microsamples and are meant to be used for home or remote sampling. Considering the field of therapeutic drug monitoring (TDM), we examined different aspects that are important for a device to be implemented in clinical practice, including ease of patient use, technical performance, and ease of integration in the workflow of a clinical laboratory. Costs related to microsampling devices are briefly discussed, because this additionally plays an important role in the decision-making process. Although the added value of home sampling for TDM and the willingness of patients to perform home sampling have been demonstrated in some studies, real clinical implementation is progressing at a slower pace. More extensive evaluation of these newly developed devices, not only analytically but also clinically, is needed to demonstrate their real-life applicability, which is a prerequisite for their use in the field of TDM.

Key Words: microsampling, TDM, DBS

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From the * Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Belgium; †University of Groningen, Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, Groningen, The Netherlands; ‡Department of Hospital Pharmacy, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; §Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia; ¶Department of Pharmacy, Westmead Hospital, Westmead, NSW, Australia; ||Marie Bashir Institute of Infectious Diseases and Biosecurity, The University of Sydney, Camperdown, NSW, Australia; and **Laboratory of Analytical Toxicology, Institute of Health Sciences, Universidade Feevale, Novo Hamburgo, RS, Brazil.

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Correspondence: Christophe Stove, PhD, Ottergemsesteenweg 460, 9000 Ghent, Belgium (e-mail: Christophe.stove@UGent.be).

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INTRODUCTION

The best-known tool for the home sampling of blood is the collection of dried blood spots (DBS) on a filter paper, potentially in the DBS card format. Undoubtedly, DBS sampling remains an excellent tool when qualitative or semiquantitative results are necessary, such as newborn screening, viral load determination, and serology testing. The past decade has witnessed an increased interest in the use of DBS for delivering accurate and precise quantitative results. This is, among others, the case for therapeutic drug monitoring (TDM), in which patient adherence or the concentrations of, for instance, drugs with a narrow therapeutic-toxic window are monitored, to tailor therapy to an individual patient's need.¹ However, in this context, an accurate concentration assessment is essential, as a deviation of the result from the true value may affect clinical decision-making. Mainly because of the well-known hematocrit (Hct) effect, the use of nonvolumetrically collected DBS for quantitative purposes has been shown to pose additional analytical challenges, hence requiring further validation when compared with conventional blood or plasma samples.^{2,3} DBS are typically analyzed by obtaining a subpunch, for example, a 3-mm diameter punch, which means a fixed area of the DBS is used for extraction. As Hct may introduce a variation in the sample volume of a DBS subpunch, the measurement uncertainty of these methods may increase, potentially even rendering these methods unsuitable for TDM purposes. This area bias is introduced because of variations in the spreading of blood with a low Hct when compared with blood presenting a high Hct. Hence, when a DBS is created from a blood drop with a certain volume, the subpunch, with a fixed diameter, will contain different volumes of blood depending on the blood Hct. One strategy to overcome this is whole-spot analysis, in which an entire, volumetrically applied spot is extracted; however, in this scenario, the Hct could, for example, impact analyte extractability. Moreover, in addition to the area bias introduced by the Hct effect, the use of subpunches necessitates the evaluation of the impact of the punch site or the amount of volume spotted. In addition to these analytical challenges, the sample quality of DBS, both home-collected and collected by assisted sampling, may be insufficient, leading to a high rejection rate.⁴

As the concept of home sampling offers numerous advantages, within the field of TDM,⁵ efforts to resolve these problems have never been more extensive than in the past few years. Among these efforts are patient training, as well as the setup of approaches that allow Hct prediction of

nonvolumetrically applied DBS, which may eventually allow to correct for the Hct effect.^{6–8} Other efforts involve the development and improvement of devices aimed at combining the collection of high-quality volumetric dried blood samples with patient-friendly sampling in the home environment. This review will focus on the latter, by providing an overview and discussion of devices that can be used as an alternative to conventional DBS sampling.

In the evaluation of devices for microsampling, 4 major aspects need to be considered: (1) the ease of sampling for the patient, (2) the cost of the device and costs related to its use, (3) the compatibility of the sample handling and analysis with the typical workflow in a clinical laboratory (where most often the analytical determination is performed) and the automatability of this process, and (4) the technical performance of the device. All aspects are equally important: technically superior devices that are difficult to use or, vice versa, easy-to-use devices that are associated with considerable variations or accompanied by other issues, might be suitable for qualitative purposes, but may be deemed unsuitable for TDM purposes.

METHODS

A systematic search was performed using the keywords “microsampling,” “dried blood spot,” and “microsampling device” to identify relevant references from an electronic database (PubMed). The identified publications were further investigated for relevant references, and alternatively, citing articles were identified, after which the whole process was repeated until no new literature could be determined. Gray literature and manufacturer websites were accessed using the Google search engine. The discussed devices were included based on the literature search and based on the authors’ knowledge of the field. To the best of our knowledge, there is no published or publicly available information on other microsampling devices that could fall within the scope of this review.

This review provides an overview of volumetric collection devices that are currently available or under development for the collection of dried blood (-based) microsamples for TDM. However, we have not included the collection of conventional dried blood microsamples on a DBS card or filter paper or the deposition on filter paper through conventional end-to-end capillaries. Undoubtedly, conventional DBS samples also have their place in the field of TDM. For a summary on the use of conventional DBS in TDM, we refer to earlier published reviews.^{9–14}

First, we provide a short description of all microsampling devices that can be used for TDM and their application in home sampling. Next, we discuss the most important aspects for the implementation of microsampling devices for TDM (cfr. Introduction), considering input from members of the Alternative Sampling Strategies Committee of the International Association for TDM and Clinical Toxicology. Although cost is undoubtedly an important aspect of this evaluation, no in-depth information will be provided on the cost of each device. The price of these devices is not readily available for all, and a comparison of

the cost of sampling with an alternative device and a traditional venous blood sampling may also be dependent on the intended application. This review will not, or only very briefly, discuss general issues related to dried blood microsampling, including the influence of Hct and recent developments to overcome the Hct effect. For more information on this specific topic, we refer to earlier published work and to the recently published guideline.^{1,2,15} For the use and applications of dedicated instruments available for (semi-) automated dried blood-based microsample analysis, we refer to Henion et al, and Luginbühl et al.^{16,17}

DRIED BLOOD SAMPLING DEVICES

Volumetric Absorptive Microsampling

Volumetric absorptive microsampling (VAMS) is a sampling technique that entered the market in 2014, with a device called Mitra (Neoteryx, Torrance, CA). This device consists of a porous absorbent white tip attached to a plastic handler (Fig. 1A). The tip, which consists of a hydrophilic polymer, permits the collection of the desired fluid by wicking up an accurate volume by capillary action. Different sample volumes can be collected (10, 20, or 30 μ L) depending on the tip size.¹⁸ As indicated on the certificate of analysis, provided by the manufacturer, the exact volume that is absorbed may marginally differ from lot to lot (eg, 10.4 versus 10.7 μ L). This needs to be considered, for example, when setting up larger-scale studies in which devices from multiple lots are used. After filling the hydrophilic tip, which generally takes 2–4 seconds, the VAMS samples are typically dried under ambient room conditions. As recommended by the manufacturer, the samples need to be stored in zip-locked bags with a desiccant and then dispatched to the laboratory. Sample preparation can be performed manually by detaching the matrix-filled tip from the plastic handler and transferring it into a suitable recipient for extraction. Alternatively, VAMS samples can be placed in a Mitra 96-Autorack, which allows (semi-) automated analysis. In the latter method, the VAMS sample tips can be submerged in a 96 deep-well plate for extraction, after which automated processing by a liquid handling robot is possible.¹⁸ VAMS claimed to allow sampling of a fixed volume of blood, irrespective of the Hct, a claim that now has been supported by several groups.^{19–21} From an analytical perspective, the Hct may still play a role, although, Hct may affect the extractability of analytes from devices, as reported by numerous research groups.^{22–26} This requires optimization of the extraction process.

Since 2014, multiple methods for TDM have been developed using VAMS.^{24,27–48} An overview of the main drug classes suited for TDM and selected examples are presented in Table 1.

An extensive discussion of the different applications is beyond the scope of this review; the interested reader is referred to earlier reviews for this purpose.^{49,50}

Verougstraete et al, who evaluated the use of VAMS for hemoglobin A1c (HbA1c) monitoring in patients with diabetes in 2 subsequent studies, also provided study participants with a questionnaire on the use of VAMS devices in a home sampling

FIGURE 1. Mitra VAMS device (A), Capitainer qDBS device (B), hemaPEN device (C) HemaXis DB10 device (D), touch activated phlebotomy device (TAP; E). Figures are used with permission from the manufacturers.

setting. In the first study ($n = 86$; children and adults with diabetes), VAMS home sampling was compared with DBS home sampling and traditional venous blood sampling.¹⁰ Overall, 56% of the participants indicated VAMS home sampling as the preferred sampling technique, whereas 14% preferred DBS sampling, 16% preferred traditional blood sampling, 10% preferred capillary blood sampling with no specific preference for DBS or VAMS, and 4% had no opinion. The results of a questionnaire in a follow-up study by Verougstraete et al⁴⁷ ($n = 76$, diabetic children) revealed that an increased number of participants (49%) preferred VAMS home sampling over capillary microsampling in the hospital (39%), whereas 12% had no preferred blood collection method. An unpublished but noteworthy comment was that children reportedly liked the aspect of the tip turning red on filling with blood. This aspect can be evaluated in laboratory settings; visual inspection of the tips can be performed as first quality control, to evaluate whether the tips are entirely red and not overfilled. The latter may occur on allowing the blood to flow onto the tip, rather than wicking up the blood. However, a slight “undersampling,” caused by an incomplete, but visually undetectable underfilling of the VAMS tip, may not be fully excluded. This potential underfilling was hypothesized to be a possible reason for some observed deviating results between sample replicates in a study by Veenhof et al.⁵¹

Furthermore, a VAMS-based study conducted in the context of TDM of immunosuppressants in transplant patients questioned the participants ($n = 100$) regarding their experience with VAMS devices.⁴³ After instruction on capillary sampling, the participants were able to choose whether they wanted to perform the finger stick themselves or with assistance from the study team. The filling of the VAMS devices was performed by the patients in all instances. The questionnaire, to be filled out after capillary sample collection, revealed that in the future, 81% of all participants would prefer self-collection of capillary blood samples over venous sample collection, if possible. Of the participants who preferred assisted finger stick ($n = 52$), 90% indicated that they would perform the finger stick themselves in the future after

seeing it performed on themselves. In conclusion, this study demonstrated that most patients preferred capillary microsampling by self-sampling over a venous sample collection. Another study, by Qu et al,⁴⁴ reported on the collection of VAMS and nonvolumetric DBS samples by patients in a home sampling setting. The authors noted a markedly higher number of accurately collected VAMS samples when compared with DBS samples. These results seem to be in contrast with those obtained in a study by Veenhof et al, who compared the sample quality and results of conventional DBS and VAMS samples with results of corresponding venous blood samples for the determination of tacrolimus. The samples ($n = 130$) were obtained from 107 adult kidney transplant patients during their regular follow-up hospital visit and were collected by a trained phlebotomist. Two replicates were collected for both capillary blood sample types. For the VAMS samples, only 47.7% of the samples presented 2 sufficient quality tips, and 20% of the samples had one sufficient quality tip. By contrast, for DBS, 76.2% of the samples presented 2 sufficient quality spots, and 17.7% had 1 sufficient quality spot. Hence, 32.3% and 6.2% of the samples were rejected for the VAMS and DBS samples, respectively.⁵¹ An explanation for this high rejection rate, according to the authors, might be the high number ($n = 75$) of phlebotomists who collected the samples, who were reportedly more experienced in collecting DBS samples than VAMS samples. This finding again indicates that adequate training is crucial to guarantee the sample quality.

Another study, in which both VAMS and DBS samples were collected, was performed by Velghe et al.⁵² Here, VAMS and DBS were collected for the determination of first-generation antiepileptic drugs in children with epilepsy and Nodding syndrome ($n = 263$). The samples were collected with assistance from a trained healthcare worker. In this study, the variability within VAMS on the one hand and DBS on the other hand were investigated by incurred sample reanalysis. The results revealed a significant and relevant higher variability within VAMS replicates, compared with DBS, for all antiepileptics in the study.

TABLE 1. Overview of Main Drug Classes Suitable for Therapeutic Drug Monitoring, With Selected Examples of Literature Reports Using Volumetric Absorptive Microsampling (VAMS)

Drug Class	Analytes	
Analgasic ²⁷	Oxycodone	
Bronchodilator ²⁸	Salbutamol	
Antibiotic ^{29–32}	Cefepime	Meropenem
	Ceftazidime	Piperacillin/tazobactam
	Fosfomycin	Vancomycin
	Linezolid	
	Sitagliptin	
Antidiabetic ²⁴	10-monohydroxycarbamazepine	Oxcarbazepine
Anticonvulsant ^{33–35}	Brivaracetam	Perampanel
	Carbamazepine	Phenobarbital
	Carbamazepine-10,11-epoxide	Phenytoin
	Dihydroxy carbamazepine	Pregabalin
	Ethosuximide	Primidone
	Felbamate	Rufinamide
	Gabapentine	Topiramate
	Lacosamide	Valproic acid
	Lamotrigine	Zonisamide
	Levetiracetam	
	Miltefosine	
	Asenapine	
	Cyclosporin A	Sirolimus
	Everolimus	Tacrolimus
	Mycophenolic acid	
Antirheumatic ⁴⁴	Hydroxychloroquine	
Cytotoxic ^{45,46}	Mitotane	Hydroxyurea
Endogenous compounds ^{47,48}	HbA1c	Gamma-hydroxybutyric acid

No clear explanation for this difference could be determined, although the potential impact of stability could not be comprehensively excluded, as the time span between the replicate VAMS analysis was longer than that for replicate DBS analysis. In a large-scale study monitoring an alcohol marker, phosphatidylethanol, 2 replicate VAMS tips were collected at 3 different time points from over 600 volunteers, by nonsupervised at-home sampling.⁵³ Several volunteers used a spare VAMS tip to collect a replicate sample ($n = 87$), and the imprecision of the entire methodology, from home sampling to analysis, could be assessed. Although the obtained imprecision of 14% was significantly higher than the imprecision (11%) of VAMS samples prepared in the laboratory and used as quality control samples (χ^2 -test, $\alpha = 0.05$), this was still quite acceptable. It can be concluded that home sampling accounts for 9.0% of the imprecision of this method. Furthermore, this study provided further evidence supporting the feasibility of home sampling by VAMS; there were only a limited number of requests for extra sampling material, with a majority (92%) of the samples passing a visual check for sample quality, and applicability, user-friendliness, and acceptability were positively assessed through a questionnaire. Notably, 80% of the respondents indicated that they would prefer this type of sampling over a conventional blood draw by trained personnel, if needed, on a monthly basis. In most other studies, no evaluation of the device in a home sampling setting was included.

In conclusion, for a wide variety of analytes, VAMS has been demonstrated as a potentially suitable alternative for conventional DBS sampling. A point of attention that remains is a Hct-independent, robust extraction. Several studies involving nonsupervised home sampling indicate that VAMS, if performed adequately, has the potential to serve as a valuable tool to collect dried blood microsamples at home. This opens the possibility of integrating VAMS into a TDM workflow, although this will likely require adaptation of existing procedures in a clinical laboratory. As applicable for any dried blood-based determination, the interpretation of a concentration from a VAMS sample requires the setup of reference intervals in blood or the conversion of blood to serum concentrations.¹ The latter ideally involves knowledge of the Hct, which may be deduced, for example through determination of the K^+ concentration in the VAMS extract.^{54,55}

Capitainer qDBS

Capitainer qDBS (formerly Capitainer B; Capitainer AB, Stockholm, Sweden) is a microsampling tool that enables the collection of 2 fixed volume (10 μ L) DBS (Fig. 1B). The Capitainer qDBS device contains 2 microchannels, each with an inlet port, designed for the application of a hanging blood drop of at least 18 μ L. When the microchannel is filled, any excess blood will dissolve the thin film at the inlet, and the

excess blood will be collected onto a filter paper (Ahlstrom 270). Subsequently, the film at the capillary outlet dissolves, after which the capillaries empty on the prepunched Ahlstrom grade 222 filter paper discs by capillary forces. The sample quality can be verified by an evaluation of the exit area of the device, which should turn dark on successful sampling. The discs are dried at room temperature for a minimum of 2 hours. To analyze the DBS, the discs need to be carefully removed with tweezers and placed into the desired recipient for analysis.⁵⁶

To date, no studies in which the Capitainer qDBS device was used for home sampling have been published. One study evaluated the applicability of the device by collecting finger-prick samples in a controlled setting.⁵⁷ In this study, using prototype devices, Lenk et al reported on the collection of capillary blood samples, in which sampling was executed by a nurse not specifically trained in collecting capillary blood and who had no experience with the device. Sample collection was successful for most participants (34/44). For 7 samples, the blood volume applied to the device was too low, and hence, the device channels were not completely filled. Three devices were sufficiently filled, but the blood in the micro-channel was not transferred to the filter paper because of insufficient membrane film-filter paper bonds. Other evaluations of the device (albeit in a somewhat different format than the currently marketed format) focused on its analytical performance.^{58–61} These evaluations demonstrated the Hct-independence of the sampling system and confirmed that an accurate and precise volume is deposited onto the filter paper, independent of blood sample volume and lipid content. There are currently no reports on the performance of the device in a home sampling context.

hemaPEN

hemaPEN (Trajan Scientific and Medical, Victoria, Australia) is a sampling system intended for the volumetric application of capillary blood on prepunched filter paper discs (Fig. 1C). Four 2.74 μL EDTA-coated capillaries integrated into the device can be filled by touching the surface of a blood drop of minimally 20 μL . Sampling takes approximately 10 seconds until all the capillaries are filled. Subsequently, the hemaPEN was clicked into a plastic base and turned upside down, allowing the capillaries to transfer the blood onto 4 integrated prepunched filter paper discs.^{62,63} The device is available with both Whatman 903 and Perkin Elmer 226 discs, 2 frequently used paper types for the collection of DBS. As the DBS are stored in the device, this implies that to collect the DBS, the device needs to be opened by cutting it with a supplementary opening tool. Then, DBS can be extracted by placing them in the desired recipient, for example, in a 96 well plate.⁶⁴

Currently, there are no published applications that have evaluated the use of hemaPEN in a home sampling setting. An initial evaluation of the precision and accuracy of the capillaries, as well as blood deposition on the filter paper discs, were performed by Neto et al.⁶⁵ Next, Deprez et al⁶³ performed an extensive evaluation of the device with respect to different parameters, possibly affecting its technical performance, such as the Hct and blood sample volume. The results

of this evaluation revealed that the performance of the device is not influenced by blood sample volume, device lot, and analytical operator. A marginal Hct-based bias was detected when patient caffeine and paraxanthine concentrations were compared with corresponding venous liquid blood concentrations over a Hct range of 0.20–0.50. However, this bias was markedly lower than that observed with subpunch DBS samples for the same analytes and is likely to be of minor, if any, relevance in practice. The technical performance of the device in the case of an extremely high Hct or markedly high blood volume ($>50 \mu\text{L}$) should also be further investigated. Ion et al⁶² performed a limited evaluation of the precision of the device for the determination of iohexol in blood, reporting intradevice repeatability below 10%, based on the analysis of 4 replicate spots per hemaPEN. Protti et al⁶⁶ evaluated the hemaPEN device for the quantitative determination of fluoxetine and sertraline, and their metabolites, norfluoxetine, and N-desmethylertraline. Their report demonstrated a precise determination of these compounds (RSD $<12\%$) and high recovery rates ($>81\%$). In addition, the influence of Hct and the effect of contact time between the blood drop and the capillaries were evaluated. It was concluded that the analysis was Hct-independent and that a contact time of less than 8 seconds led to a significant decrease in the number of filled capillaries. However, this did not affect the analytical response of the correctly generated samples. Training is required to use the device. Independent studies are still needed to evaluate whether patients who receive training can successfully and independently use the device and generate DBS that meets predefined quality criteria.

HemaXis DB 10

HemaXis DB 10 (DBS System SA, Gland, Switzerland) is a DBS collection device, the prototype of which was introduced in 2016, where a conventional DBS card (eg, Whatman 903 or Perkin Elmer 226) is combined with volumetric sampling.⁶⁷ The device consists of 4 capillary channels and a DBS card in a cartridge (Fig. 1D). Contact is made between a blood drop and the inlet of the capillary, which induces capillary action. An accurate volume (10 μL) of blood was collected per channel. When filling of a channel is completed, blood can be observed at the outlet of the channel. This serves as an indicator that a sufficient amount of blood was applied. After filling the capillary channel, the device was manually closed, allowing the outlet of the capillary to be in contact with the card, resulting in the transfer of a defined volume of blood (10 μL) to the DBS card, which takes about 5 seconds.^{68–70} After completion of the transfer, the capillary channel should be completely empty, which can be visually verified. The filling of the capillary channel and deposition of blood on the DBS card can then be individually repeated for the remaining channels. Next, the sample was transported to the laboratory. Here, the DBS card is removed from the holding element and processed like a classical DBS card. The extraction can be performed after manual or automated punching of the entire DBS, or fully automated, by dedicated instrumentation.⁷¹

Bosilkovska et al⁶⁹ used this device in a clinical trial and did not report any issues with sample collection. In a

study by Zwart et al,⁷² TDM was performed for immunosuppressants tacrolimus and mycophenolic acid using the HemaXis DB 10 device in 65 patients. A nurse supported the patients for the collection of the first spot, whereas the collection of other spots was executed by the patients themselves. Difficulties related to the device capillary were reported by 23.1% of patients; however, details regarding the encountered difficulties were not specified. One patient needed to excessively squeeze the finger to obtain an adequate amount of blood. Furthermore, 4.6% of patients had difficulties in seeing the capillary because of impaired vision. Because of these difficulties, 8 spots needed to be discarded because they were of insufficient quality for analysis. It should be mentioned that patients had only received a brief instruction on the use of the device and sampling methodology, which could explain the encountered issues. Currently, a clinical trial is ongoing, in which the HemaXis DB 10 device is being evaluated for the collection of capillary blood samples (NCT02590185).

HemaSpot HF

HemaSpot HF (Spot on Sciences, San Francisco, CA) is a fan-shaped device consisting of 8 identical blades in a protective plastic cassette.⁷³ First, 2–3 drops of blood have to be applied to the center of the device. The device setup claims to ensure an equal distribution of the blood over the blades, which takes approximately 1 minute. Each blade can hold 1/8th of the total sample volume, reportedly approximating 9–10 μL of blood. The exact amount of blood per blade is dependent on the initial volume of blood applied to the device. The device can be closed after filling and is then left to dry, which is facilitated by the integrated desiccant, after which it can be sent to a laboratory in a plastic bag. To analyze the sample, one or more blades can be obtained by slightly twisting the fan. Subsequently, the blade(s) can be transferred into a 96 well plate for sample extraction.

The feasibility of using the HemaSpot HF device in a home sampling setting was investigated by Hirshfield et al.⁷⁴ Participants received a HemaSpot kit, which consisted of the HemaSpot HF device, collection materials such as the lancet and disinfectant pads, and an instruction card. Of the 554 enrolled participants, who were all men, 79.2% collected blood and 75% effectively mailed the kit to the laboratory. Of the 75%, 89.5% felt comfortable collecting their own blood. The kit was classified as “very easy” or “easy” to use by 83.6% of participants who mailed the kit to the laboratory. In total, 98.1% were willing to use an at-home DBS kit in the future. Of the 413 kits received by the laboratory, 337 kits were effectively analyzed. Overall, 65/413 (15.7%) samples were of unsatisfactory quality, that is, the device was not completely filled with blood. The most common issues were difficulties with the lancet and some complications when positioning the blood into the center of the device. In a study on the determination of HbA1c in HemaSpot HF samples, collected both at the clinic and at home, the study participants ($n = 128$) were also questioned regarding sampling.⁷⁵ The results revealed that 60.7% preferred to use this system at home over conventional blood sampling at the general practitioner. Concerning the use of the device itself, applying the

blood to the device was found “difficult” or “very difficult” by 33.3% of the participants, whereas 44.5% found this “easy” to “very easy,” and 22.2% found it neither difficult nor easy. Furthermore, several participants reported difficulties in collecting sufficient blood to completely fill the device and decide when the amount of blood that was applied was sufficient. In addition, Yamamoto et al and Lingani et al used the HemaSpot HF device for finger-prick sampling in their studies but did not provide any details regarding sampling performed by the participants.^{76,77} Other studies concerning the HemaSpot HF device did not imply finger-prick sampling.^{78–81}

DRIED PLASMA DEVICES

Noviplex Plasma Prep Cards

Noviplex (Novilytic, West Lafayette, IN) is a micro-sampling device that enables the collection of dried plasma based on membrane filtration. Two versions of the device are available: Noviplex UNO, for the collection of one 2.5 μL dried plasma spot (DPS), and Noviplex DUO, for the collection of 2 3.8 μL DPS. To generate the DPS, blood is applied to the top layer of the device. This layer allows the blood to spread. If a sufficient amount of blood is applied to the test area (25 μL of blood for Noviplex UNO and 60 μL of blood for Noviplex DUO), an indicator control spot on the top layer changes color. Under the top layer is a separation membrane, which is capable of retaining red blood cells (RBCs) through size filtration, whereas plasma can still flow through.⁸² The plasma collection disc(s) are situated underneath the separation membrane, attached to a base card.⁸³ After approximately 3 minutes, the top layer should be peeled off and discarded.⁸² The DPS then needs to dry for an additional 15 minutes, after which the sample can be transported to the laboratory. For analysis of the DPS, the spots can be removed from the base layer using tweezers.^{83–85}

Currently, there is only one published report in which the Noviplex plasma prep cards were used for the collection of DPS through finger-prick sampling.⁸² In this study, sampling was performed with the assistance of a trained phlebotomist. In other reports concerning Noviplex plasma prep cards, samples were prepared from venous blood by pipetting.^{83–87} Based on these reports, it can be concluded that the device is capable of producing plasma spots with low volume variations (coefficient of variation less than 2%) over a wide Hct range. The concordance between analyte concentrations found in venous plasma and plasma generated by this device should be further investigated because only extremely limited data are available on this matter.

DEVICES UNDER DEVELOPMENT

HemaSpot SE

HemaSpot SE (Spot on Sciences, San Francisco, CA) is a microsampling device with a spiral-shaped design.⁸⁸ The purpose of this device is to separate larger whole blood components such as RBCs, platelets, and leukocytes from serum and serum soluble components. After adding 3 or 4 blood

drops to the center, the spiral-shaped membrane allows the separation of the different blood components through lateral flow. The serum and serum components flow freely over the membrane, whereas the larger components are retained near the center of the spiral. After drying for 2 minutes, the sample cartridge was closed and sent to the laboratory for analysis. Depending on the desired matrix, a subpunch can be made in different parts of the spiral. The suitability of this device for TDM purposes has not been demonstrated. Like regular DBS, one can envisage issues related to chromatographic effects exerted by the filter paper.

Book-Type Dried Plasma Spot Card

A book-type microsampling device (Q2 Solutions, Ithaca), designed to produce up to 4 DPS per card, is currently being developed. This device consists of 2 different layers that filter RBCs from the blood to produce plasma, similar to the above-mentioned Noviplex plasma prep cards. Blood was applied to the book-type card in a closed configuration, and after approximately 3 minutes, the card can be opened to collect the DPS by removing the DPS paper substrate (Ahlstrom°601). The volume of the DPS depends on the applied blood volume, with approximately 0.303 μL filtered plasma per μL of applied blood.⁸⁹ Although the initial report states a method with good accuracy and precision, with a high extraction recovery developed and validated using these devices, no follow-up reports on the applicability of TDM are available.

HemaXis DX

HemaXis DX (DBS System SA) is a device that produces plasma or serum, based on passive separation through sedimentation.^{90,91} Because of the difference in viscosity between the cell and noncell components of blood, plasma, or serum is obtained. The amount of noncell fluid that is formed (plasma/serum) depends on the amount of applied blood, but a sample ejection mechanism allows the collection of a 2 μL sample aliquot from the metering area. The biological fluid can be stored in a dried or liquid form. Currently, prototypes of the device are being evaluated, but no published reports are available.

Autonomous Microfluidic DPS Device

Another DPS-generating device is currently being developed by Capitainer AB (Stockholm, Sweden). This device allows the collection of a fixed volume of DPS in less than 6 minutes. The driving force is a combination of filtration and capillary forces. This device consists of a filtration membrane, a capillary metering channel, absorbent paper, and a drainage valve to remove excess plasma. The filtration membrane is positioned in a wedge configuration, inducing capillary action. A blood drop (40–80 μL) is applied to the filtration membrane, and the filtered plasma fills the capillary metering channel. The DPS paper absorbs the volume of plasma that is present in the filled channel (11.6 μL). The excess plasma is removed through the drainage valve.^{92,93} Some proof-of-principle measurements demonstrated the capability of the device to generate DPS of a fixed volume, within a limited Hct range (39%–45%) and a blood

input volume range of 40–80 μL . This initial evaluation also revealed that, compared with plasma obtained through centrifugation, 73% $\pm$ 8% of the plasma proteins were recovered after filtration of venous and capillary blood.^{92,93}

Touch-activated phlebotomy

The touch-activated phlebotomy (TAP) device (Seventh Sense Biosystems, Medford, MA) can collect blood through vacuum pressure through microneedles in a virtually painless manner.^{94,95} This device sticks onto the skin, usually, the upper arm. After the push of a button on the device, a ring of 30 small microneedles projects out of the device and generates micropunctures in the skin (Fig. 1E). Next, the needles were immediately withdrawn, creating a vacuum and inducing the collection of 100 μL capillary blood. The blood flows through microfluidic channels into a storage space, prefilled with lithium heparin anticoagulant. An indicator window turns red when the reservoir is full, indicating that the sampling process is complete, which takes approximately 2–3 minutes. Then, the entire TAP device can be sent to the laboratory, where the blood can be collected from the device. In its current form, only liquid microsamples can be collected. However, in the future, additional accessories might be added to the device, allowing the generation of DBS from whole blood. Therefore, a discussion regarding this device is readily included here. A first evaluation of the device was performed by comparing HbA1c measurements from blood collected with the TAP device and venous blood ($n = 144$).⁹⁵ Good concordance was observed between the 2 blood samples, demonstrating that capillary blood samples collected with the TAP device are also suitable for HbA1c determinations. Furthermore, participants were asked to report pain scores for both sampling techniques (TAP device and venipuncture) using the Wonk-Bakes FACES scale, where 0 corresponds to “no hurt” and 10 corresponds to “hurts worst.” The results indicated a significantly lower pain score for capillary sampling with the TAP device (range of pain score 0–4) when compared with venipuncture (range of pain score 0–10). In a recent report, the device was evaluated for use in clinical trials and analysis of protein biomarkers.⁹⁶ In total, 72 TAP capillary samples were collected from 16 healthy volunteers. For 8/72 samples, the fill indicator did not turn red, resulting in the rejection of these samples. Possible reasons for rejection included improper seal of the device against the skin surface, poor blood flow, and device malfunction. In addition, hemolysis of the capillary samples was evaluated, both in freshly collected samples and capillary samples only processed 72 hours after collection and compared with a freshly collected venous blood sample. Hb concentrations were markedly higher in both capillary sample types (13.71–38.64 mg/dL) than in venous blood samples (0.72–2.96 mg/dL). Shear stress caused by the frictional force of blood against the microcapillary channels in the device could contribute to hemolysis because there is increased hemolysis observed in samples, even after direct processing.

TASSO OnDemand

The TASSO OnDemand device (Tasso Inc, Seattle, WA) consists of a TASSO Button and Sample pod and is

meant to be placed on the upper arm. Collection of whole blood can be started by pressing the button, which results in a lancet puncturing the skin, leading to blood withdrawal from the capillaries in the skin under vacuum. The blood is collected in the sample pod, which exists in different formats. For the TASSO-SST device, the sample pod collects liquid whole blood, which can be spun into serum when the sample arrives at the laboratory. The TASSO-M20 and TASSO-Sport devices both collect four 20 μL dried whole blood samples. The sample pod can be removed from the button and sent to the laboratory. The samples can be further processed, either by centrifugation of the sample pod (TASSO-SST) to obtain serum or by removal of the dried blood samples from the sample pod (TASSO-M20 and TASSO-SPORT).

Roadcap et al evaluated the use of the TASSO OnDemand device for the determination of gefapixant in a pharmacokinetic study. The capillary blood, collected by the TASSO Button, was absorbed onto 4 10 μL Mitra VAMS tips contained in the sample pod. The evaluation included a conventional analytical method validation, including incurred sample reanalysis of 15 patient samples, which fulfilled all criteria. Next, a bridging study was performed in 12 participants, with a total of 240 sampling timepoints. In 10/240 samplings, the device failed to puncture the skin on pushing the button, and a second device was needed to collect the sample. The clinicians who collected samples did not report any complaints regarding pain during sample collection.⁹⁷

DISCUSSION

Overall, 6 commercially available devices, as well as 6 under development, were evaluated with respect to ease of sampling, clinical laboratory compatibility, and technical

performance. The most important positive and negative points, or points of attention associated with each commercially available device, are summarized in Table 2 and are discussed extensively below.

As the Mitra device has been on the market for several years, analytical procedures for this device have been developed for a wide range of analytes.^{19–22,24} However, the number of studies that also include a clinical validation, demonstrating equivalence between results obtained in the alternative matrix and those obtained in the conventional matrix, as performed by Vethe et al and Veenhof et al, remains limited.^{42,51} A simple and straightforward sampling procedure, with a minimal number of steps to be performed by the patient, and the possibility of visual evaluation of sample quality by the patient, are both key assets of the device. The different tip sizes allow the collection of the necessary volume to have sufficient sensitivity, although it may be more challenging to completely fill the larger samplers with one drop of blood. Automation of the sample processing is still at an early stage but should allow a high throughput in the clinical laboratory.

HemaPEN is a more complex device than the Mitra device, and therefore, more extensive training of the patient may be necessary to collect good quality samples. The emptying of the capillaries can be visually checked by the patient, allowing evaluation if sampling was successful. In addition, as the DBS are embedded within the device, external contamination of the spots is rather unlikely. However, as mentioned above, this also implies that the device needs to be opened using a dedicated tool. Currently, both the opening of the device and the collection of the DBS still require manual handling. The DBS size is fixed and rather small (2.74 μL), but as 4 DBS samples are simultaneously collected, 2 or more

TABLE 2. Summary of the Most Important Strong Points, as Well as Weak Points or Points of Attention, in the Context of TDM Associated With Discussed Commercially Available Devices

Device	Strong Points	Weak Points/Points of Attention
Mitra device	Extensive analytical evaluation Visual evaluation of sample quality Automated analysis under development	Hct-based effect on recovery
Capitainer qDBS	Visual indicator for sample volume	Manual steps required in sample processing
hemaPEN	Four replicate DBS per blood drop Little risk of external contamination	Small sample volume (2.74 μL) Manual steps required in sample processing
HemaXis DB10	Visual evaluation of sample quality Visual indicator for sample volume DBS-card format (automation)	Higher risk of external contamination
HemaSpot HF	Eight replicate “blades” per device	Nonvolumetric sample collection Suitability for quantitative purposes not demonstrated
Noviplex plasma	Collection of dried plasma	Large blood drop volume required per spot
prep cards	Visual indicator for sample volume	Small sample volume (2.5 or 3.8 μL) Unclear to what extent the “dried plasma” is equivalent to conventional plasma

DBS from the same device could be combined if an analysis requires a higher sample volume. Conversely, the 4 DBS replicates could allow the evaluation of multiple clinical parameters from the replicate samples within one device.

Capitainer qDBS has been positively assessed by multiple research groups in terms of analytical performance.^{57–61} The use of this device by patients may require more training than the Mitra device, but should still be fairly easy. The visual indicator also allows a patient to monitor if sampling is successful. Furthermore, the removal of the DBS from the device for analysis still requires manual handling at this point.

The HemaXis DB 10 device also generates 10 μ L DBS, such as the Capitainer qDBS; however, the spots are generated on a conventional DBS card that can easily be removed from the cartridge. This card can then be processed using existing standard (automated) workflows for DBS analysis. Moreover, the patient can check if the sampling was successful by monitoring the channel emptying, as well as the DBS itself.

For the HemaSpot HF device, the application of blood onto the device itself is quite straightforward, although the relatively large volume (approximately 80 μ L) needed to fill the device may not be feasible for all subjects. It should also be noted that there are no reports available on the suitability of this device for absolute quantitation. It can be reasoned that the saturation of the blades (and, hence, the volume of blood contained in these blades) will depend on the originally applied blood volume, which is variable. Therefore, it remains unclear whether this device is suitable for TDM purposes. Furthermore, automation of sample preparation (ie, collection of separate blades) is likely not straightforward.

Currently, the Noviplex plasma prep card is the only commercially available device capable of quantitatively generating DPSs. The device is fairly simple to use, and the indicator control spot should ensure a sufficient sample volume. The sample volume itself is relatively small (2.5 μ L or two 3.8 μ L spots); hence, sufficiently sensitive instrumentation should be used. In addition, clinical validation of newly developed methods remains necessary to demonstrate concordance between capillary DPS and venous plasma concentrations. Furthermore, automation of sample processing could be considerably challenging.

The TAP and TASSO-SST devices both collect liquid capillary blood. This allows the generation of liquid plasma by centrifugation in the laboratory. The major advantage of these devices is that the standard matrices for clinical analysis, namely, plasma and serum, are obtained. Hence, the use of these devices could overcome several challenges associated with blood microsampling. Nevertheless, the collection of liquid samples poses some additional challenges, including the influence of hemolysis, analyte stability, and sample shipment. Similar to DPSs, concordance between capillary and evaluated through venous concentrations should be clinical validation. The sample collection itself is relatively straightforward for both devices. In the future, it should be possible to collect dried capillary blood samples by both devices, by adding a feature to the device, to generate DBS or other dried

microsamples instead of collecting liquid samples.^{94,98} This should facilitate sample transportation and stability. An evaluation was previously performed for the TASSO OnDemand device, in which four 10 μ L dried capillary blood samples were collected.⁹⁷ In this case, Mitra VAMS tips were used to collect the dried blood; hence, it can be expected that the performance of the device in terms of accuracy and precision of sample collection is dependent on the performance of VAMS tips.

As mentioned previously, the cost of a device and costs related to its use, for example, costs for the training to use the device and costs for sample storage, should also be taken into account in the decision process. However, the price of each of these devices may change, depending on their status of development, numbers produced, and the desire to purchase. Therefore, we are unable to make any comparative statements on this matter. Nonetheless, it would be interesting to evaluate the cost for each aspect related to the use of a certain device and compare this with the cost of a conventional (dried) blood sample, to evaluate the cost-effectiveness of alternative sampling methods. Furthermore, this has been performed for the use of DBS for TDM in pediatric renal transplant patients and pediatric hemato-oncology patients,⁹⁹ revealing that the use of DBS would lead to considerable cost savings in both patient populations.

CONCLUSION

New microsampling devices are continuously being developed. Whether or not these devices will effectively be used for home sampling, and more specifically for home sampling within the scope of TDM, is dependent on multiple factors, namely, ease of sampling, automatability of sample processing, cost, and technical performance.

Currently, for most devices, analytical performance has been evaluated. By contrast, data supporting their successful use by patients and clinical validation data regarding methods using these devices for capillary sampling are often limited or lacking. Further data regarding these topics should be generated to increase (confidence in) the use of these devices in daily practice. Moreover, patient education and patient motivation will be highly important to collect high-quality samples. This is a prerequisite, as the starting point to obtain reliable data is the availability of reliable samples.

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