Approaches to Microsample Collections

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Although the application of microsamples in metabolic phenotyping exists, it is still in its infancy, with whole blood being overwhelmingly the primary biofluid collected through the collection method of dried blood spots.

Keywords: microsampling ; sample miniaturisation ; metabolic phenotyping ; metabolomics

1. Traditional Dried Samples: Dry Blood Spots (DBSs)

Historically, DBSs have been the flagbearer for microsampling. The concept for the preservation of dried human biological samples as a spot is attributed to Ivar Christian Bang in 1913 [1][2]. Typically, these microsamples can be collected via a skin prick with a lancet or created by transferring from a phlebotomy tube with a micro-pipette [3][4]. Currently, such samples are collected on a specialised filter paper, which can contain anywhere from 15 to 50 µL of blood, and take approximately three to four hours to completely dry at room temperature [1]. This can make DBSs amenable to collections where cold-chain shipping protocols may not be feasible. However, it should be noted that below-zero temperatures (−20 °C, −80 °C) are still recommended for the long-term storage of DBSs [1][3][4].

DBS samples have been evaluated in mass spectrometry (MS)-based metabolic phenotyping and have been used in applications of cancer diagnostics [5], cancer treatments [6], air pollution [7], drug discovery [8], acidemia [9], and pyruvate kinase deficiency [10]. Despite this wide range of applications of DBS samples, there still remain concerns around their volumetric accuracy, particularly in metabolic phenotyping, where the standardisation of sample volume is required. For example, the uneven spread of collected blood across the spot can introduce metabolite variation due to differences in viscosity/haematocrit (hct %; the fraction of blood made up of red blood cells). This has been observed in DBS studies using liquid scintillation analysis, where sub-punches from an individual sample were taken and the number of metabolites measured varied vastly from sample to sample [11]. DBS collections are remarkably susceptible to variation based on an individual's haematocrit. Varying haematocrit levels are of particular concern due to its propensity to undergo rapid changes in the body. This is a widely known phenomenon in DBS microsampling and is commonly referred to as the *'haematocrit effect'* [12], where external factors such as dehydration, polycythaemia, anaemia, overhydration, kidney failure, or chronic inflammatory conditions can cause plasma volume perturbations. Additionally, pregnancy may also cause slightly decreased hct due to an increase in blood volume [3][13]. For reference, the normal range of hct is 36–48% for women and 42–52% for men [14]. Put simply, increases in hct beyond the normal range affect blood viscosity and therefore reduce the spread of the blood spotted on the carrier material, whereas decreases in hct (i.e., reduced viscosity) can create greater spread [15]. As such, the sample's spotted area has a linear, inverse relationship with hct [12][13].

In MS-based studies, normalisation techniques have been employed in order to address the *'haematocrit effect'* [16]. However, it remains one of the most prevalent challenges faced for translating the wide use of microsamples in metabolic phenotyping workflows. To date, studies have successfully performed DBS haematocrit normalisation through potassium content [17], using near-infrared (NIR) spectroscopy [18], haemoglobin measurement using non-contact diffuse reflectance spectroscopy [19], and using wax barriers on DBSs [20]. Despite the development of these normalisation techniques for DBSs, they are yet to be widely adopted in metabolic phenotyping workflows, with many literature examples not implementing a normalisation step in their protocol descriptions [6][7][9][10][18][21][22][23]. Only two studies have openly reported hct normalisation of their DBS samples as part of their metabolic phenotyping workflow. The first was by Koulman et al., who analysed infant heel-prick DBS samples, utilising a volumetric and hct-independent Liquid chromatography–mass spectrometry (LC–MS) method [24]. This was performed by relatively expressing the extracted lipid intensity of a given DBS sub-punch to its summed intensity. Interestingly, this method revealed that lipid profiles in DBSs showed comparable or better precision to plasma and whole blood samples, which the authors propose could be attributed to the halting of the oxidative process in dried samples compared to traditional venous whole blood and plasma samples [24]. Another study successfully employed an automated haematology analyser for the analysis of hct in serum to

normalise steroid concentrations obtained from traditional DBSs [25]. This correction, by Salamin et al., used the following equation [25]:

$$Corrected\ concentration = (DBS\ concentration)/(1 - hct) \quad (1)$$

Another biological factor that affects DBS sample quality includes the nature of the analyte(s) of interest, because blood cells can cause variations in the amount of analyte that is extractable from the surface of the DBS card itself [26]. The reason for this is that analyte partitioning can occur between plasma and blood cells, which significantly influences the concentrations of analytes in plasma or whole blood samples taken from a DBS, although this is most commonly seen in monoclonal antibodies for pharmacokinetic studies [27][28]. Additionally, prominent sources of variation in DBS homogeneity have been attributed to the paper substrate used [12]; inconsistencies in the storage, packaging, and transport of samples [5][6]; and contact of DBSs with other surfaces [29].

The circulating blood metabolome is a tightly controlled homeostatic system, where preanalytical variation (paper substrate, storage, packaging, transport) can unavoidably lead to inaccurate and possibly misleading results [30]. This is a current limitation in pre-analytical workflows for DBS microsamples, particularly when considering the inherently heterogenous nature of biological samples, where physiological conditions (i.e., hct) already contribute dynamic changes [31]. As such, efforts seeking to enhance accuracy, sensitivity, and specificity during the analytical phase are in vain if the technical aspects that underpin microsample workflows are not reproducible. Thus, a lack of reproducibility and accuracy is detrimental to achieving outcomes of Predictive, preventive, personalised, and participatory medicine (P4 medicine) using DBS samples in metabolic phenotyping pipelines [32].

2. Improving Microsample Collection: Are Advanced Devices the Future for Metabolic Phenotyping?

Advancements in microsampling devices have allowed for improvements in blood collections by removing some of the inconsistencies experienced in DBS collections whilst maintaining the convenience of microsampling (**Table 1**). These devices can be classified into three broad classes: advanced dried samples; passive separation devices; and whole biofluid collectors, which will be discussed in the context of metabolic phenotyping below.

Table 1. Microsampling devices.

Sampling Technology	Device (Manufacturer)	Reps (Model); Volume	Biofluids	Description	Refs.
Traditional dried samples	Whatman (Cytiva; Marlborough, MA, USA)	5 (903 Protein Saver Card); 75–80 µL	Urine, Whole blood (WB)	Traditional paper-based carrier for collection of dried samples.	[33] [34]
	PerkinElmer (PerkinElmer; Waltham, MA, USA)	5 (226 Spot Saver Card); Up to 70 µL	WB	Traditional paper-based carrier for collection of dried samples.	[35]
	Ahlstrom-Munksjö (Ahlstrom-Munksjö; Helsinki, Finland)	5 (BioSample Card); Up to 70 µL	WB	Traditional paper-based carrier for collection of dried samples.	[36]

Sampling Technology	Device (Manufacturer)	Reps (Model); Volume	Biofluids	Description	Refs.
Advanced dried samples	TASSO (HemoLink; Seattle, WA, USA)	4 (M20); 20 µL	WB	M20 device collects four dried whole blood samples. The sample pod can be removed from the button and sent to the lab.	[25] [37] [38]
	Capitainer (Capitainer AB; Solna, Sweden)	2 (qDBS); 10 µL 2 (B Vanadate); 10 µL	WB	The qDBS (quantitative dried blood spot) collects two fixed-volume DBSs. A drop of blood is applied to the two microchannels within the device. After filling, the film at the capillary outlet dissolves, emptying the blood on the pre-punched Ahlstrom filter paper discs via capillary action. Successful sampling is indicated by the exit area of the device turning dark. Discs are then dried at room temperature for a minimum of 2 h. Then, the DBSs can be removed with tweezers for analysis. B Vanadate operates under the same mechanism with an additional phospholipase D inhibitor, sodium metavanadate ($NaVO_3$), in the DBS disc, to allow for accurate phosphatidylethanol testing.	[39] [40]
	Volumetric Absorptive Microsampling (VAMS) Mitra (Neoteryx; Torrance, CA, USA)	2 (Cartridge); 10, 20, or 30 µL 4 (Clamshell) 96 (Autorack)	Urine, WB (incl. serum and plasma)	A porous, absorbent white tip attached to a plastic handler. Tip consists of hydrophilic polymer and permits haematocrit-independent collection of desired fluid by wicking up an accurate volume via capillary action. Different sample volumes can be collected depending on tip size. Sample preparation can be performed manually by detaching the tip to transfer for extraction.	[41] [42] [43]
	hemaPEN (Trajan; Melbourne, VIC, Australia)	4; 2.74 µL	WB	Haematocrit-independent sampling system for volumetric collection of capillary blood on pre-punched filter paper discs. Contains four Ethylenediaminetetraacetic (EDTA)-coated capillaries, which fill by touching surface of blood drop of minimally 20 µL. Subsequently, clicking device into plastic base and inverting it allows capillaries to transfer blood onto four integrated pre-punched filter paper discs. Device is available with both Whatman and Perkin Elmer discs. TGA approved.	[44]
	HemaXis (DBS System SA; Gland, Switzerland)	4 (DB10); 10 µL	WB	DB10 utilises a conventional Whatman or Perkin Elmer card combined with 4 volumetric capillary channels. Following fingerstick lancing, a blood drop is applied to the inlet of the capillary, collecting 10 µL of blood per channel. When filling of a channel is completed, blood can be observed at the outlet of the channel. This indicates sufficient application. The device is then manually closed to allow contact between the capillary outlet and the card, taking approximately 5 s for transfer of blood.	[45]
	HemaSpot (Spot On Sciences; San Francisco, CA, USA)	Multiple (HD); 160 µL 8 (HF); 9.2 µL	WB	The HD is a large DBS that allows for multiple technical replicates upon performing a sub-punch; HF fan-shaped device has eight identical blades in protective plastic cassette. Two–three drops of blood must be applied to the centre of the device to ensure equal distribution of blood (taking approximately one minute). Each blade holds 1/8th of the total sample volume (~9.2 µL). The device can be closed after filling and is then left to dry, facilitated by the integrated desiccant, before it is ready for extraction.	[46] [47]

Sampling Technology	Device (Manufacturer)	Reps (Model); Volume	Biofluids	Description	Refs.
Passive separation devices	Capitainer (Capitainer AB; Solna, Sweden)	1 (Dried plasma spot (DPS) *); 11.6 µL	WB (separated into plasma)	The autonomous microfluidic DPS device collects plasma samples in under 6 min. It consists of a filtration membrane, a capillary metering channel, absorbent paper, and a drainage valve to remove excess plasma. A blood drop is applied to the filtration membrane, and the filtered plasma fills the capillary metering channel before it is absorbed by the DPS paper. Excess plasma is removed by the drainage valve.	[48]
	Book-Type Dried Plasma Spot Cards (Q2 Solutions; Morrisville, NC, USA)	4 (DPS *); variable (8–14 mm spots)	WB (separated into plasma)	Device consists of two layers that filter erythrocytes from WB to produce plasma. Blood is applied to the card in the closed-book configuration. After approximately 3 min, the sample book can be opened to collect the DPSs by removing the paper substrate. Volume of the DPS obtained depends on applied WB volume (approximately 0.303 µL plasma per 1 µL of WB).	[49]
	HemaXis (DBS System SA; Gland, Switzerland)	1 (DX *); 2µL	WB (separated into plasma)	The DX operates in the same way; however, it creates DPSs under the process of sedimentation once the device is closed. This is achieved through capillary pressure in a microfluidic system created with two polydimethylsiloxane sub-units.	[50]
	Telimmune Plasma Separation Cards (Novilytic; West Lafayette, IN, USA)	1 (Uno); 2.5 µL (plasma) 2 (Duo); 3.8 µL (plasma)	WB (separated into plasma)	Telimmune (formerly Noviplex) Uno and Duo collect dried plasma through membrane filtration. Blood is applied to the top layer of the device. Respectively, 25 µL of blood and 60 µL of blood are required for application on the devices; an indicator control spot on the top layer changes colour once this amount is reached. A separation membrane beneath the top layer retains erythrocytes through size filtration. This allows plasma to flow through. The process takes approximately 3 min, after which the top layer is peeled and discarded before drying the DPS for an additional 15 min. The DPS can then be removed using tweezers.	[51]
	HemaSpot (Spot On Sciences; San Francisco, CA, USA)	Multiple (SE); ~4 µL	WB (separated into serum)	SE device has a spiral-shaped design. It separates large whole blood components such as RBCs, platelets, and leukocytes from serum. Three–four blood drops applied to the centre allow the spiral-shaped membrane to separate different blood components through lateral flow. The serum components flow freely over the membrane, whereas the larger components are retained near the centre of the spiral. After sample is dried for two minutes, the cartridge is closed and is ready for analysis. Sub-punches can be made in different parts of spiral.	[52]

Sampling Technology	Device (Manufacturer)	Reps (Model); Volume	Biofluids	Description	Refs.
Whole biofluid collectors	MSW2 (Shimadzu; Kyoto, Japan)	1 (Wing); 23 µL (WB) 5.6 µL (plasma) 14 (Windmill)	WB (separated into plasma)	Blood is collected at the tip of the Wing after performing a fingerstick (or similar) with a lancing device. The device is filled once blood reaches the reservoir (23 µL). A specialised centrifuge rotor is required (the Windmill), which can hold 14 Wings for centrifugation. Typically, the sample is then spun down as 2000 × G for 10 min. Following centrifugation, two plasma zones are created and can be snapped off with hands (i.e., does not require a specialised cutter) into a microtube for extraction.	[53]
	Touch Activated Phlebotomy (TAP) (Yourbio Health; Medford, MA, USA)	1 (TAP); 100 µL 1 (TAP II *); 250 µL	WB	Collects blood under vacuum pressure through microneedles, painlessly. Device sticks onto the skin (upper arm). Once button pushed, a ring of 30 small microneedles projects out of the device to micropuncture the skin. The needles immediately withdraw, creating a vacuum to induce collection of capillary blood. Blood flows through microfluidic channels into storage space, prefilled with lithium heparin anticoagulant. Subsequently, indicator window turns red when the reservoir is full (up to three minutes). Entire device can be sent to lab for collection and analysis of blood from device.	[54] [55]
	TASSO (HemoLink; Seattle, WA, USA)	1 (TASSO+); 200–600 µL 1 (SST); 200–300 µL	WB (incl. serum and plasma)	Placed on upper arm. Collects WB by pressing the button, which results in a lancet puncturing the skin. Blood collected from capillaries under vacuum pressure into the sample pod (different formats). TASSO+ device collects whole blood for a variety of standard collection tubes (i.e., EDTA and lithium-heparin) and passive separation tubes (serum and plasma); SST device pod collects liquid WB, which can be spun into serum at lab.	[56] [57]

Note. Reps = number of technical replicates that a given advanced microsampling device is able to produce; ***** = a device in development/not currently commercially

Advanced dried sample devices collect samples as a fluid and produce a dried sample either with a polymer tip (volumetric absorptive microsampling, “VAMS”—Neoteryx; Torrance, CA, USA) or carriers akin to DBS cards, such as the hemaPEN (Trajan; Melbourne, VIC, Australia), HemaXis DB10 (DBS System SA; Gland, Switzerland), Capitainer qDBS (quantitative dried blood spot) and B-Vanadate (Capitainer AB; Solna, Sweden), TASSO-M20 (HemoLink; Seattle, WA, USA), and HemaSpot HD and HF (Spot on Sciences; San Francisco, CA, USA) [25][58][59][60][61][62][63][64][65][66][67]. These technologies improve upon standard DBS cards by providing accurate/volumetric aspiration and thus heamatocrit-independent sample collection over a wide range of microsample sizes (2.74–30 µL).

Passive separation devices allow for the in situ separation of whole blood into its sub-components. e.g., serum or plasma, which can then store the resultant product in liquid form, e.g., TASSO+ (HemoLink; Seattle, WA, USA); as dried serum, e.g., HemaSpot SE (Spot on Sciences; San Francisco, CA, USA), or as dried plasma, e.g., Tellimmune Plasma Separation Cards (Novilytic; West Lafayette, IN, USA). Passive separation devices represent an expanding area commercially, with many dried plasma spot (DPS) devices currently in development, including the DPS (Capitainer AB; Solna, Sweden), Book-Type DPS (Q2 Solutions; Morrisville, NC, USA), and the Hemaxis DX (DBS System SA; Gland, Switzerland) [48][49][50]. It is not known how these devices will perform in metabolic phenotyping. However, as they produce samples akin to those obtained from venous whole blood separations, which are commonly used in metabolic phenotyping, they warrant further investigation in the field.

Whole biofluid collectors are advanced devices with the ability to collect and produce samples as liquid samples without the need for cellulose material. These devices can collect sample volumes as small as 23 µL, e.g., the MSW2 (Shimadzu; Kyoto, Japan), and extend to 100 µL, e.g., the TAP (Yourbio Health; Medford, MA, USA), and up to 600 µL, e.g., the TASSO+ and TASSO-SST (HemoLink; Seattle, WA, USA) [37][68][69].

All three broad classes of advanced microsampling devices are well positioned for direct implementation in metabolic phenotyping workflows in clinical and epidemiology studies. For example, they are already commercially available and therefore have advanced manufacturing consistency; they have been designed to counter specific challenges in DBS and traditional microsampling workflows, including the *‘haematocrit effect’* [3][65], and have already achieved translation to non-

metabolic phenotyping analytical chemistry protocols [58][70]. Despite these benefits, the translation of microsampling devices to metabolic phenotyping research is yet to be widely adopted by the field, as extensive studies investigating comparability to venipucture and metabolite stability are lacking [71]. This is important as validated microsampling methods that leverage the advances in device design have the potential to enhance metabolic phenotyping studies in clinical and epidemiology settings, facilitating greater sampling frequency and sample size, and thereby providing valuable gains in statistical power [71][72].

References

1. Carpentieri, D.; Colvard, A.; Petersen, J.; Marsh, W.; David-Dirgo, V.; Huentelman, M.; Pirrotte, P.; Sivakumaran, T. Mind the Quality Gap When Banking on Dry Blood Spots. Biopreservation Biobanking 2021, 19, 136–142.
2. Grüner, N.; Stambouli, O.; Ross, R.S. Dried Blood Spots-Preparing and Processing for Use in Immunoassays and in Molecular Techniques. J. Vis. Exp. 2015, 97, e52619.
3. Delahaye, L.; Veenhof, H.; Koch, B.; Alffenaar, J.; Linden, R.; Stove, C. Alternative Sampling Devices to Collect Dried Blood Microsamples: State-of-the-Art. Ther. Drug Monit. 2021, 43, 310–321.
4. Lehmann, S.; Delaby, C.; Vialaret, J.; Ducos, J.; Hirtz, C. Current And future use of “dried blood spot” analyses in clinical chemistry. Clin. Chem. Lab. Med. CCLM 2013, 51, 1897–1909.
5. Cao, Y.; Wang, Q.; Gao, P.; Dong, J.; Zhu, Z.; Fang, Y.; Fang, Z.; Sun, X.; Sun, T. A dried blood spot mass spectrometry metabolomic approach for rapid breast cancer detection. Onco Targets Ther. 2016, 9, 1389–1398.
6. Hasanah, Y.I.F.; Harahap, Y.; Purwanto, D.J. Phenotyping Study of Cyclophosphamide 4-Hydroxylation in Malay Cancer Patients. Drug Des. Dev. Ther. 2021, 15, 305–313.
7. Loo, R.L.; Lu, Q.; Carter, E.M.; Liu, S.; Clark, S.; Wang, Y.; Baumgartner, J.; Tang, H.; Chan, Q. A feasibility study of metabolic phenotyping of dried blood spot specimens in rural Chinese women exposed to household air pollution. J. Expo. Sci. Environ. Epidemiol. 2020, 31, 328–344.
8. Lang, W.; Qi, J.; Caldwell, G.W. Drug, Lipid, and Acylcarnitine Profiling Using Dried Blood Spot (DBS) Technology in Drug Discovery. In Optimization in Drug Discovery; Humana Press: Totowa, NJ, USA, 2013; pp. 461–475.
9. Haijes, H.A.; Jans, J.; Van Der Ham, M.; Van Hasselt, P.M.; Verhoeven-Duif, N.M. Understanding acute metabolic decompensation in propionic and methylmalonic acidemias: A deep metabolic phenotyping approach. Orphanet J. Rare Dis. 2020, 15, 68.
10. Van Dooijeweert, B.; Broeks, M.H.; Verhoeven-Duif, N.M.; Van Beers, E.J.; Nieuwenhuis, E.E.S.; Van Solinge, W.W.; Bartels, M.; Jans, J.J.M.; van Wijk, R. Untargeted metabolic profiling in dried blood spots identifies disease fingerprint for pyruvate kinase deficiency. Haematologica 2020, 106, 2720.
11. O’Mara, M.; Hudson-Curtis, B.; Olson, K.; Yueh, Y.; Dunn, J.; Spooner, N. The effect of hematocrit and punch location on assay bias during quantitative bioanalysis of dried blood spot samples. Bioanalysis 2011, 3, 2335–2347.
12. Watson, P.; Maughan, R.J. Artifacts in Plasma Volume Changes due to Hematology Analyzer-Derived Hematocrit. Med. Sci. Sports Exerc. 2014, 46, 52–59.
13. Kundrapu, S.; Noguez, J. Chapter Six-Laboratory Assessment of Anemia. In Advances in Clinical Chemistry; Makowski, G.S., Ed.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 197–225.
14. Fischbach, F.T.; Fischbach, M.A. Fischbach’s Manual of Laboratory and Diagnostic Tests, 10th ed.; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2018.
15. Hematocrit. Nursing Critical Care. 2020, 15, 38.
16. Cvetko, A.; Tijardović, M.; Bilandžija-Kuš, I.; Gornik, O. Comparison of self-sampling blood collection for N-glycosylation analysis. BMC Res. Notes 2022, 15, 61.
17. Petrick, L.; Edmands, W.; Schiffman, C.; Grigoryan, H.; Perttula, K.; Yano, Y.; Dudoit, S.; Whitehead, T.; Metayer, C.; Rappaport, S. An untargeted metabolomics method for archived newborn dried blood spots in epidemiologic studies. Metabolomics 2017, 13, 27.
18. Palmer, E.A.; Cooper, H.J.; Dunn, W.B. Investigation of the 12-Month Stability of Dried Blood and Urine Spots Applying Untargeted UHPLC–MS Metabolomic Assays. Anal. Chem. 2019, 91, 14306–14313.
19. Yu, M.; Dolios, G.; Yong-Gonzalez, V.; Björkqvist, O.; Colicino, E.; Halfvarson, J.; Petrick, L. Untargeted metabolomics profiling and hemoglobin normalization for archived newborn dried blood spots from a refrigerated biorepository. J. Pharm. Biomed. Anal. 2020, 191, 113574.

20. Baillargeon, K.R.; Brooks, J.C.; Miljanic, P.R.; Mace, C.R. Patterned Dried Blood Spot Cards for the Improved Sampling of Whole Blood. ACS Meas. Sci. Au 2022, 2, 31–38.
21. Kong, S.T.; Lin, H.-S.; Ching, J.; Ho, P.C. Evaluation of Dried Blood Spots as Sample Matrix for Gas Chromatography/Mass Spectrometry Based Metabolomic Profiling. Anal. Chem. 2011, 83, 4314–4318.
22. Michopoulos, F.; Theodoridis, G.; Smith, C.J.; Wilson, I.D. Metabolite profiles from dried blood spots for metabonomic studies using UPLC combined with orthogonal acceleration ToF-MS: Effects of different papers and sample storage stability. Bioanalysis 2011, 3, 2757–2767.
23. Ward, C.; Nallamshetty, S.; Watrous, J.D.; Acres, E.; Long, T.; Mathews, I.T.; Sharma, S.; Cheng, S.; Imam, F.; Jain, M. Nontargeted mass spectrometry of dried blood spots for interrogation of the human circulating metabolome. Biol. Mass Spectrom. 2021, 56, e4772.
24. Koulman, A.; Prentice, P.; Wong, M.C.; Matthews, L.; Bond, N.J.; Eiden, M.; Griffin, J.; Dunger, D. The development and validation of a fast and robust dried blood spot based lipid profiling method to study infant metabolism. Metabolomics 2020, 10, 1018–1025.
25. Salamin, O.; Nicoli, R.; Xu, C.; Boccard, J.; Rudaz, S.; Pitteloud, N.; Saugy, M.; Kuuranne, T. Steroid profiling by UHPLC–MS/MS in dried blood spots collected from healthy women with and without testosterone gel administration. J. Pharm. Biomed. Anal. 2021, 204, 114280.
26. Li, K.; Naviaux, J.C.; Monk, J.M.; Wang, L.; Naviaux, R.K. Improved Dried Blood Spot-Based Metabolomics: A Targeted, Broad-Spectrum, Single-Injection Method. Metabolites 2020, 10, 82.
27. Mingas, P.-D.; Zdovc, J.; Grabnar, I.; Vovk, T. The Evolving Role of Microsampling in Therapeutic Drug Monitoring of Monoclonal Antibodies in Inflammatory Diseases. Molecules 2021, 26, 1787.
28. Matsuda, K.C.; Hall, C.; Baker-Lee, C.; Soto, M.; Scott, G.; Prince, P.J.; Retter, M.W. Measurement of in vivo therapeutic mAb concentrations: Comparison of conventional serum/plasma collection and analysis to dried blood spot sampling. Bioanalysis 2013, 5, 1979–1990.
29. Evans, C.A.; Bowen, C.L.; Filali-Ansary, A. Dried blood spots: Challenges. Future Sci. Group (FSG) 2013, 1, 30–46.
30. Stevens, V.L.; Hoover, E.; Wang, Y.; Zanetti, K.A. Pre-Analytical Factors that Affect Metabolite Stability in Human Urine, Plasma, and Serum: A Review. Metabolites 2019, 9, 156.
31. Yin, P.; Lehmann, R.; Xu, G. Effects of pre-analytical processes on blood samples used in metabolomics studies. Anal. Bioanal. Chem. 2015, 407, 4879–4892.
32. Flores, M.; Glusman, G.; Brogaard, K.; Price, N.D.; Hood, L. P4 medicine: How systems medicine will transform the healthcare sector and society. Pers. Med. 2013, 10, 565–576.
33. Drolet, J.; Tolstikov, V.; Williams, B.A.; Greenwood, B.P.; Hill, C.; Vishnudas, V.K.; Sarangarajan, R.; Narain, N.R.; Kiebish, M.A. Integrated Metabolomics Assessment of Human Dried Blood Spots and Urine Strips. Metabolites 2017, 7, 35.
34. Cytiva. Whatman 903 Proteinsaver Card. 2022. Available online: https://www.cytivalifesciences.com/en/us/shop/whatman-laboratory-filtration/whatman-dx-components/blood-collection-cards-and-accessories/903-proteinsaver-card-p-01011 (accessed on 10 March 2022).
35. PerkinElmer Inc. PerkinElmer 226 Spot Saver RUO Card. 2022. Available online: https://www.perkinelmer.com/uk/product/pki-ruo-spot-saver-card-pack-of-100-gr2261005 (accessed on 10 March 2022).
36. Ahlstrom-Munksjö. Biosamples Collection Cards. 2022. Available online: https://www.ahlstrom-munksjo.com/products/medical-life-sciences-and-laboratory/specimen-collection-cards/Biosamples-collection-cards/ (accessed on 10 March 2022).
37. Roadcap, B.; Hussain, A.; Dreyer, D.; Carter, K.; Dube, N.; Xu, Y.; Anderson, M.; Berthier, E.; Vazvaei, F.; Bateman, K.; et al. Clinical application of volumetric absorptive microsampling to the gefapixant development program. Bioanalysis 2020, 12, 893–904.
38. Tasso, Inc. Tasso-M20. 2022. Available online: https://www.tassoinc.com/tasso-m20 (accessed on 10 March 2022).
39. Capitainer. Capitainer®B Vanadate. 2022. Available online: https://capitainer.se/capitainer-b-vanadate/ (accessed on 10 March 2022).
40. Capitainer. Capitainer®qDBS. 2022. Available online: https://capitainer.se/capitainer-qdbs/ (accessed on 10 March 2022).
41. Neoteryx. The Mitra 96-Autorack. 2022. Available online: https://www.neoteryx.com/mitra-high-throughput-rack-systems?hsLang=en (accessed on 10 March 2022).

42. Neoteryx. The Mitra Cartridge. 2022. Available online: https://www.neoteryx.com/mitra-cartridge-blood-sampling-device-dbs?hsLang=en (accessed on 10 March 2022).
43. Neoteryx. The Mitra Clamshell. 2022. Available online: https://www.neoteryx.com/mitra-clamshell-blood-collection-device?hsLang=en (accessed on 10 March 2022).
44. Medical TSa. Hemapen. 2022. Available online: https://www.trajanscimed.com/pages/hemapen (accessed on 10 March 2022).
45. HemaXis. HemaXis DB10 Whole Blood Collection Device. 2022. Available online: https://hemaxis.com/products/hemaxis-db10/ (accessed on 10 March 2022).
46. Spot On Sciences. HEMASPOT HD. Available online: https://www.spotonsciences.com/hemaspot-hd/ (accessed on 10 March 2022).
47. Spot On Sciences. HEMASPOT HF. Available online: https://www.spotonsciences.com/hemaspot-hf/ (accessed on 10 March 2022).
48. Hauser, J.; Lenk, G.; Ullah, S.; Beck, O.; Stemme, G.; Roxhed, N. An Autonomous Microfluidic Device for Generating Volume-Defined Dried Plasma Spots. Anal. Chem. 2019, 91, 7125–7130.
49. Ryona, I.; Henion, J. A Book-Type Dried Plasma Spot Card for Automated Flow-Through Elution Coupled with Online SPE-LC–MS/MS Bioanalysis of Opioids and Stimulants in blood. Anal. Chem. 2016, 88, 11229–11237.
50. Forchelet, D.; Béguin, S.; Sajic, T.; Bararpour, N.; Pataky, Z.; Frias, M.; Grabherr, S.; Augsburger, M.; Liu, Y.; Charnley, M.; et al. Separation of blood microsamples by exploiting sedimentation at the microscale. Sci. Rep. 2018, 8, 14101.
51. Telimmune. Telimmune Plasma Separation Cards (Formerly Noviplex). 2022. Available online: https://www.telimmune.com/plasma-separation-cards (accessed on 10 March 2022).
52. Spot On Sciences. HEMASPOT SE. 2022. Available online: https://www.spotonsciences.com/hemaspot-se/ (accessed on 10 March 2022).
53. Shimadzu. MSW² Type Udck. 2022. Available online: https://www.shimadzu.eu/msw2 (accessed on 10 March 2022).
54. YourBioHealth. Touch Activated Phlebotomy (TAP). 2022. Available online: https://company.yourbiohealth.com/products/tap/ (accessed on 10 March 2022).
55. YourBioHealth. Touch Activated Phlebotomy II (TAP II). 2022. Available online: https://company.yourbiohealth.com/products/tap-ii/ (accessed on 10 March 2022).
56. Tasso, Inc. Tasso+. 2022. Available online: https://www.tassoinc.com/tasso-plus (accessed on 10 March 2022).
57. Tasso, Inc. Tasso-SST. 2022. Available online: https://www.tassoinc.com/tasso-sst (accessed on 10 March 2022).
58. Deprez, S.; Paniagua-González, L.; Velghe, S.; Stove, C.P. Evaluation of the Performance and Hematocrit Independence of the HemaPEN as a Volumetric Dried Blood Spot Collection Device. Anal. Chem. 2019, 91, 14467–14475.
59. Kocher, S.; Tshiananga, J.K.T.; Koubek, R. Comparison of Lancing Devices for Self-Monitoring of Blood Glucose regarding Lancing Pain. J. Diabetes Sci. Technol. 2009, 3, 1136–1143.
60. Mandrioli, R.; Mercolini, L.; Protti, M. Blood and Plasma Volumetric Absorptive Microsampling (VAMS) Coupled to LC–MS/MS for the Forensic Assessment of Cocaine Consumption. Molecules 2020, 25, 1046.
61. Jacobs, C.M.; Wagmann, L.; Meyer, M.R. Development, validation, and application of a quantitative volumetric absorptive microsampling–based method in finger prick blood by means of LC-HRMS/MS applicable for adherence monitoring of antipsychotics. Anal. Bioanal. Chem. 2021, 413, 1729–1737.
62. Protti, M.; Mandrioli, R.; Mercolini, L. Tutorial: Volumetric absorptive microsampling (VAMS). Anal. Chim. Acta 2018, 1046, 32–47.
63. Gao, X.; Chen, C.; Geng, D.; Bateman, K.P.; Shi, S.; Woolf, E.J.; Xu, Y. Volumetric absorptive microsampling (VAMS®) in therapeutic protein quantification by LC–MS/MS: Investigation of anticoagulant impact on assay performance and recommendations for best practices in method development. J. Pharm. Biomed. Anal. 2021, 196, 113895.
64. Denniff, P.; Spooner, N. Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis. Anal. Chem. 2014, 86, 8489–8495.
65. Kok, M.G.; Fillet, M. Volumetric absorptive microsampling: Current advances and applications. J. Pharm. Biomed. Anal. 2018, 147, 288–296.
66. Nix, C.; Hemmati, M.; Cobraiville, G.; Servais, A.-C.; Fillet, M. Blood Microsampling to Monitor Metabolic Profiles During Physical Exercise. Front. Mol. Biosci. 2021, 8, 681400.

67. Velghe, S.; Stove, C.P. Evaluation of the Capitainer-B Microfluidic Device as a New Hematocrit-Independent Alternative for Dried Blood Spot Collection. Anal. Chem. 2018, 90, 12893–12899.
68. Xing, J.; Loureiro, J.; Patel, M.T.; Mikhailov, D.; Gusev, A.I. Evaluation of a novel blood microsampling device for clinical trial sample collection and protein biomarker analysis. Bioanalysis 2020, 12, 919–935.
69. Hotta, K.; Ishida, T.; Noritake, K.-I.; Kita, K.; Mano, Y. Quantitative and qualitative application of a novel capillary microsampling device, Microsampling Wing™ (MSW), using antiepileptic drugs in rats. J. Pharm. Biomed. Anal. 2020, 194, 113788.
70. Protti, M.; Marasca, C.; Cirrincione, M.; Cavalli, A.; Mandrioli, R.; Mercolini, L. Assessment of capillary volumetric blood microsampling for the analysis of central nervous system drugs and metabolites. Analyst 2020, 145, 5744–5753.
71. Wilson, I. Global metabolic profiling (metabonomics/metabolomics) using dried blood spots: Advantages and pitfalls. Bioanalysis 2011, 3, 2255–2257.
72. Amsterdam, P.V.; Waldrop, C. The application of dried blood spot sampling in global clinical trials. Bioanalysis 2010, 2, 1783–1786.