Editorial

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The re-emergence of dried blood spot sampling – are we ready?

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"Everything old is new again" is an appropriate description for the recent re-emergence of dried blood spot (DBS)-based methods outside of the traditional realm of newborn screening. From its humble beginnings over a century ago [1], DBS sampling emerged 50 years later with the worldwide application of capillary sampling of babies for newborn bloodspot screening (NBS) [2, 3]. Stepping forward another 50 years, we now see an increase in translational research publications for dried matrices (primarily blood, but also urine, saliva and sweat) looking for their place as mainstream applications [4]. Whilst there are significant drivers for microsampling with DBS, we need to consider whether these emerging applications achieve appropriate quality goals and traceability to ensure they are fit for their clinical purpose [5, 6].

In this issue of *Clinical Chemistry and Laboratory Medicine* (CCLM), there are three timely articles highlighting the utility and continued challenges of DBS analysis [7–9]. The articles clearly demonstrate the emergence and growth of DBS applications from research to mainstream application and together they highlight specific considerations across the total testing process of the brain-to-brain loop [10]. The first article discusses at improving the pre-analytical process through the development of a phone-based application (app) for timely determination of DBS quality [8]. The other two articles discuss analytical considerations for mass spectrometry-based DBS methods and examine the post-analytical components by comparing plasma with DBS result interpretation [7, 9].

Pre-analytical errors associated with poor spot quality, which is a contributor to inaccurate results, can be high. The quality of the spot collection, even in controlled environments with experienced phlebotomists, can lead to a 4%–5% rejection rate [11]. This rejection rate can escalate to 20% for paediatric home-based sampling [12]. Even 4% is high compared to NBS, which has an achievable rejection rate guideline of less than 2% [13]. Hence, a lower error rate for these emerging applications is potentially achievable.

The report by Veenhof and colleagues describes a *Dried Blood Spot Photo App* that has been developed to determine if the quality of the DBS is acceptable or should be rejected (and therefore re-collected) prior to the sample being sent to the laboratory [8]. The app is reported to detect the size and overall appearance of the blood spot, but it does not detect haemolysis or humidity errors. The app was tested on the Apple IPhone 5s with an eight-megapixel camera in the laboratory with standard fluorescent lighting. Previously, spot quality was decided once the sample reached the laboratory. This retrospective quality check results in significant inconvenience for the patient and a delay in time for analysis. Although the current version of this app does not claim to detect all the possible pre-analytical errors associated with DBSs, it does provide an immediate indication as to whether a sufficient sample has been collected. In addition to the authors' proposed use of the app, it can potentially also be utilised for training and education to improve the analytical quality.

The continued emergence of improved sensitivity in mass spectrometry-based technologies has underpinned the recent rapid expansion in the availability of applications for microsampling [4]. Clinicians and consumers are driving the demand for this technology with the laboratory professional the guardian of performance quality. This is highlighted by both Polo and Veenhof in their respective decisions on acceptability of the methods for clinical use [7, 9]. Whilst the plasma sphingolipid method is described as the "gold standard", the DBS was described as "a useful tool" and considered acceptable for diagnosis. On the other hand, the transplant drug method was considered inadequate in the "strict clinical setting" for monitoring. Interestingly, whilst the authors apply the same decision and acceptance criteria for both plasma and DBSs, the method validation criteria presented are different between these two publications, the description of which ideally could be standardised [14] (Table 1).

Many consumers maintain an interest in DBS testing due to a desire to improve the long-term monitoring of particular health conditions. Whilst it is clear that the agreement between the plasma and DBS matrix is not ideal,

with the DBS described as "not quite as good as plasma in terms of discrimination" for the sphingolipid methods and "not good enough" for the transplant drugs, neither of the studies evaluated the intra-patient agreement longitudinally. This may be of value as there is evidence to suggest that the correlation between the DBS and plasma improves for repeat sampling within the one patient [15]. This is likely due, at least in part, to consistent haematocrit and extraction efficiency of the DBS sample. Hence, there is a potential need to reconsider the way in which we evaluate DBS methods moving forward.

Similar to the available pre-analytical DBS guideline, there is a need for DBS-specific method validation guidance [16]. For accuracy of quantitation, the method validation of DBS requires additional considerations over conventional liquid blood testing. This includes the influence of: (1) haematocrit and blood diffusion pattern; (2) punch location; (3) extraction efficiency; (4) traceability of the method; and (5) application of correction factors [17]. In addition, where the comparison is between spotted or liquid venous blood and a capillary DBS, the probable difference between capillary and venous levels of the target metabolite needs to be considered. The final part of the method validation that requires close consideration relates to the decision limits used and how they relate to the liquid matrix if applicable. Together with the development of a specific guideline, it will be the future availability of matrix-matched commercially available traceable reference materials for calibration of the assays and external quality assurance programs that will significantly enhance the harmonisation and standardisation of the DBS analysis [16, 18].

Table 1: Comparison of information provided for the discussed DBS mass spectrometry methods.

Method	Veenhof et al. [7]	Polo et al. [9]	Millington et al. [3]
Pre-analytical			
Subjects	Adults (app. n = 40)	Adults and paediatrics (app. n = 114)	Paediatrics (n = NS)
Specimen type	Venous whole blood and capillary finger prick DBS	Venous whole blood and capillary finger prick DBS	NBS cards
DBS collection	Finger prick/capillary	Finger prick/capillary OR 50 µL venous whole blood spotted on the filter paper	Heel prick/capillary
Storage	Whole blood <24 h, DBS <74 h ambient/DBS >29 h at −20 °C	Plasma at −80 °C, DBS at −20 °C	Ambient
Analytical			
Haematocrit correction	No	No	No
Punch size/location	8 mm/Central	3.2 mm/?	Whole spot/NS
Measurement	LC-MS/MS	LC-MS/MS	FAB[a]-MS/MS
Calibrator matrix	Dried spot	Dried spot	NS
Method validation			
Overall correlation	$R^2 = 0.93–0.97$	$R^2 = 0.59–0.98$	NS
Overall difference	Positive bias	Positive bias (except for $LysoGb_3$)	NS
FDA/EMA acceptance criteria	(67% ± 20%)	NA	NS
Limits of clinical relevance (85%–115%) criteria	>80%	NA	NS
QCs relative error (accuracy)	NA	75%–119%	NS
Median predicted percentage error criteria	<15%	NA	NA
Receiver-operating characteristic curve	NA	Plasma AUC[b] > DBS AUC	NA
Post-analytical			
Suggested conversion factor	NA	e.g. 2.7	NA
Primary purpose of the method	Diagnosis	Monitoring	Population screening
FFP[c] (Authors' discussion)	Rejected	Accepted	Accepted

For comparison, the original DBS mass spectrometry method for NBS of amino acids and acyl carnitines is provided. [a]FAB, fast atom bombardment; [b]AUC, area under the curve; [c]FFP, fitness for purpose; NA, not applicable. h, hours; QC, quality control; FDA, US Food and Drug Administration; EMA, European Medicines Agency; NS, not specified.

In summary, the three manuscripts in this addition of CCLM highlight the emergence of DBS as a standard matrix for testing. With this, the hybrid laboratory model, which facilitate consumer access, is likely to grow for mass spectrometry and other methods based on DBS micro-sampling [5, 19]. Part of the adjustment to the changing landscape will be a review of how we manage the quality of the total testing process (including method validation procedures) to ensure the results generated are fit for their clinical purpose.

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