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Clinical Chemistry and Dried Blood Spots: Increasing Laboratory Utilization by Improved Understanding of Quantitative Challenges

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Introduction

To best understand the use of dried blood spots (DBS) in clinical chemistry, we must first differentiate laboratory screening from diagnostics. Newborn screening (NBS) utilizes DBS to detect and quantify biomarkers indicative of more than 50 congenital diseases primarily of metabolic origin [1]. Many different methods are used, including modern analytical technologies such as tandem mass spectrometry (MS/MS) [2, 3] and molecular analyses [4, 5]. Each method is designed specifically to analyze DBS specimens with laboratory protocols and systems that begin with a paper hole puncher rather than a pipette. Unfortunately, NBS is often differentiated from clinical chemistry because it is “screening” tool rather than a “diagnostic” application. This is a common misconception because no method is diagnostic, but rather leads to a physician diagnosis that is based, in part, on laboratory results. Like any clinical test, NBS results take into consideration additional data such as age of newborn, birth weight, gestational age, and nutritional status [6–8]. Additionally, NBS is regulated by a comprehensive quality assurance/quality control (QA/QC) network that is shared worldwide [9]. There are a small number of traditional clinical laboratories that use DBS in a diagnostic setting, primarily in specialized metabolic applications, further supporting that DBS utilization is not restricted to “screening” laboratories, nor does it involve relaxed laboratory standards. The question is: why are DBS not used to a greater extent in traditional clinical laboratories? It is likely due to the analytical challenges and common misperceptions of the DBS. Specifically, the challenges

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of DBS implementation such as cross-validation of a DBS assay from a liquid sample analysis, logistical systems limitations for handling DBS versus liquid samples, and clinical laboratory bias towards single metabolite analysis versus metabolic profiles.

Advantages of DBS

DBS are whole blood specimens obtained directly from the patient being tested. For newborns DBS are collected as a heel stick and in adults, a finger prick. Approximately 200–300 μL of blood (4–6 drops) is needed for DBS analysis as compared to 5–10 mL of blood by venipuncture for most clinical assays. A reduction of blood volume required for collection and analysis is the primary advantage of DBS in clinical chemistry, especially for newborns and premature infants. Although there are new methods of sample collection that utilize small volumes of blood or plasma, a DBS card has the added advantage in that it can easily be sampled multiple times for a variety of assays; which is more challenging than in any small-volume liquid specimen collection.

One major advantage of DBS versus liquid specimens is shipping and storage requirements [10]. DBS can be shipped in a standard business envelope following proper labeling requirements, while blood or plasma requires refrigeration or packaging with dry ice which can be expensive and often requires overnight shipping. In terms of storage, enzymes are inactivated in the dry state, thus enhancing their stability on the DBS matrix and many proteins and metabolites have been shown to be stable in DBS [11]. Furthermore, the storage space requirements are dramatically reduced, refrigeration is not necessarily required, and the potential for infection is less than with liquid specimens. We return to the obvious question, why don’t clinical laboratories embrace the DBS specimen? The answer, in part, is the perception that sampling issues (volume obtained) of DBS specimens are problematic [12–14].

DBS Challenges and Solutions

Most sampling of DBS is done by obtaining a punch (a portion of an entire blood spot) from the filter paper specimen collection card and the blood volume in this punch can be estimated by the diameter of the punch. Three factors influence the estimated volume from a specific diameter punch, filter paper absorptivity, blood volume applied to the paper, and hematocrit influences, and must be taken into account for any successful DBS analysis. First is the absorptivity of the filter paper, which is controlled by standardization of all paper used in NBS [15]. That same standard can be applied to clinical laboratory tests. Second, the volume of blood applied to the paper can impact the saturation of the paper. While the exact volume of the blood drop applied to filter paper may vary, by printing a target on the filter paper (dashed circles) and filling these circles completely can ensure that 50–75 μL whole blood is present in each target area. Double-spotting (layering) can also be a problem but is usually obvious on inspection. Third, the hematocrit influences the surface area that blood will spread for any given volume applied. Hematocrit variations only affect blood volume significantly at very high or low values. When blood is collected properly and these concerns are addressed, then a DBS specimen is accurate to within 15%. However,

quantification and perceived inaccuracies are still partly to blame for the lack of adoption of DBS in clinical chemistry outside of NBS.

Solutions to DBS implementation in clinical chemistry begin by understanding the analytical approaches used in NBS [16].

Another, less recognized issue in the quantification of metabolites from DBS is the efficiency of analyte extraction into a liquid solvent that contains the reference standards [17]. With liquid analysis, these standards can be added to the specimen. Errors in the extraction process will affect both internal standards and metabolites. In stable-isotope dilution mass spectrometry (IDMS) methods, the standard is often an isotopologue and behaves like the metabolite measured. In DBS analysis, it is presumed no standard was added during the collection process but rather during the extraction or sample preparation. A reduction in the extraction of metabolites often occurs if DBS cannot be completely reconstituted in a solvent or liquid matrix. This can lead to errors if the extraction efficiency (recovery) is not taken into account or is not reproducible.

Newborn screening utilizes DBS exclusively while most clinical chemistry tests use plasma obtained from whole blood. To see a significant utilization of DBS in clinical chemistry, there would have to be a demonstrable need for its use based on its advantages as described here. Furthermore, DBS widespread adoption in the near future is challenging as the existing infrastructure in clinical laboratories, especially hospital-based are heavily dependent on clinical auto analyzers, which utilize colorimetric assays, as the main tools in the clinical laboratory which are not DBS compatible. These systems are highly automated and perform many different assays, and have dedicated staff, laboratory technicians trained in their use and significant capital invested in them. However, DBS can make inroads into clinical use in laboratories that use specialized analytical tools such as gas chromatography/mass spectrometry, liquid chromatography/mass spectrometry, and molecular analysis, rather than large auto analyzers. Many specialized laboratories have been embracing these technologies and will make it easier to introduce DBS analysis rather than whole blood or plasma. The use of mass spectrometry-based analyzers in particular enables detection of many biomarkers present in blood at very low levels [18–20]; laboratory personnel must be trained on DBS sampling and handling after a method has been properly validated.

Cross-validation of DBS with comparison to existing whole blood/plasma-based assays is key for its introduction into a clinical laboratory workflow. Analytically it is a challenge to compare whole blood versus plasma, thus most assay developments compare plasma to DBS. It is important to understand that metabolite levels in blood may be different than in plasma and must be considered.

In terms of solving the limitations of DBS such as hematocrit, a focus on more accurately measuring the blood volume of the sample obtained would be useful regardless of spot size or hematocrit. Delivering known volumes of blood to a DBS card using a calibrated pipette will help, provided there was no hemolysis, clotting, or disruption of the paper upon spotting. Addition of internal standards during the collection process would all but eliminate

extraction efficiency errors in filter paper specimen collection, if mixing could be assured and costs kept low.

Some metabolic analyses in liquid are problematic if these metabolites are unstable or converted to other metabolites prior to analysis. These issues can often be addressed when using DBS [21]. However, if an analysis in a liquid specimen, with high precision (low coefficients of variation), is not accurate because of sample degradation, why isn't the alternative solution (DBS) considered? Is accuracy in the measurement better than precision when it comes to disease diagnostics? Clinical chemists should weigh the tradeoff between accuracy and precision and develop new approaches to interpretation of data such as concentration ratios of metabolites [22], detection of multiple markers supporting a disease diagnosis [7, 23], and markers that may represent liquid compartments (i.e., extracellular fluid, intracellular fluid).

DBS Outlook in Clinical Chemistry

There are many novel solutions that will improve the use of DBS in both screening and clinical laboratories [24]. This improvement may be observed when the advantages of DBS far outweigh the perceived disadvantages [12]. Clearly this is the case with newborn screening and metabolic diagnostic laboratories. Perhaps areas of clinical chemistry such as disease and drug monitoring or clinical trials will be where the evidence is obtained for its expansion [25]. In both these areas the need for offsite, small volume collection with stable storage and biosafety may outweigh any analytical concerns.

References

1. Watson MS, Lloyd-Puryear MA, Mann MY, Rinaldo P, Howell RR. Newborn screening: Toward a uniform screening panel and system. Genetics in Medicine. 2006; 8(5S):12S–252S.
2. De Jesus VR, Chace DH, Lim TH, Mei JV, Hannon WH. Comparison of amino acids and acylcarnitines assay methods used in newborn screening assays by tandem mass spectrometry. Clinica chimica acta international journal of clinical chemistry. 2010; 411(9–10):684–689. [PubMed: 20122909]
3. Chace DH, Diperna JC, Mitchell BL, Sgroi B, Hofman LF, Naylor EW. Electrospray tandem mass spectrometry for analysis of acylcarnitines in dried postmortem blood specimens collected at autopsy from infants with unexplained cause of death. Clinical chemistry. 2001; 47(7):1166–1182. [PubMed: 11427446]
4. Wang LY, Chen NI, Chen PW, et al. Newborn screening for citrin deficiency and carnitine uptake defect using second-tier molecular tests. BMC medical genetics. 2013; 14:24. [PubMed: 23394329]
5. Puckett RL, Orsini JJ, Pastores GM, et al. Krabbe disease: clinical, biochemical and molecular information on six new patients and successful retrospective diagnosis using stored newborn screening cards. Molecular genetics and metabolism. 2012; 105(1):126–131. [PubMed: 22115770]
6. Chan CL, Mcfann K, Taylor L, Wright D, Zeitler PS, Barker JM. Congenital adrenal hyperplasia and the second newborn screen. The Journal of pediatrics. 2013; 163(1):109–113. e101. [PubMed: 23414665]
7. Sarafoglou K, Himes JH, Lacey JM, Netzel BC, Singh RJ, Matern D. Comparison of multiple steroid concentrations in serum and dried blood spots throughout the day of patients with congenital adrenal hyperplasia. Hormone research in paediatrics. 2011; 75(1):19–25. [PubMed: 20798478]
8. CLSI. Newborn Screening for Preterm, Low Birth Weight, and Sick Newborns: Approved Guideline. Wayne, PA: CLSI; 2009. I/LA31-A

9. De Jesus VR, Mei JV, Bell CJ, Hannon WH. Improving and assuring newborn screening laboratory quality worldwide: 30-year experience at the Centers for Disease Control and Prevention. Seminars in perinatology. 2010; 34(2):125–133. [PubMed: 20207262]
10. Mei, JV. Dried Blood Spot Sample Collection, Storage, and Transportation. In: Wenkui Li, MSL., editor. Dried Blood Spots: Applications and Techniques. Wiley; 2014. p. 21-31.
11. Adam BW, Hall EM, Sternberg M, et al. The stability of markers in dried-blood spots for recommended newborn screening disorders in the United States. Clinical biochemistry. 2011; 44(17–18):1445–1450. [PubMed: 21963384]
12. De Jesus VR, Chace DH. Commentary on the history and quantitative nature of filter paper used in blood collection devices. Bioanalysis. 2012; 4(6):645–647. author reply 649. [PubMed: 22452254]
13. Spooner N. A dried blood spot update: still an important bioanalytical technique? Bioanalysis. 2013; 5(8):879–883. [PubMed: 23590471]
14. 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(20):2335–2347. [PubMed: 22011181]
15. CLSI. NBS01-A6 Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard - Sixth Edition. Wayne, PA: CLSI; 2013.
16. La Marca G. Mass spectrometry in clinical chemistry: the case of newborn screening. Journal of pharmaceutical and biomedical analysis. 2014
17. Chace DH, Kalas TA, Naylor EW. Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clinical chemistry. 2003; 49(11):1797–1817. [PubMed: 14578311]
18. Mather J, Rainville PD, Spooner N, Evans CA, Smith NW, Plumb RS. Rapid analysis of dried blood spot samples with sub-2-microm LC-MS/MS. Bioanalysis. 2011; 3(4):411–420. [PubMed: 21338261]
19. Barfield M, Spooner N, Lad R, Parry S, Fowles S. Application of dried blood spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicokinetic studies. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences. 2008; 870(1):32–37. [PubMed: 18550454]
20. Otero-Santos SM, Delinsky AD, Valentin-Blasini L, Schiffer J, Blount BC. Analysis of perchlorate in dried blood spots using ion chromatography and tandem mass spectrometry. Analytical chemistry. 2009; 81(5):1931–1936. [PubMed: 19199567]
21. Blessborn DSK, Zeeberg D, Kaewkhao K, Sköld O, Ahnoff M. Heat stabilization of blood spot samples for determination of metabolically unstable drug compounds. Bioanalysis. 2013; 5(1):31–39. [PubMed: 23256470]
22. Chace DH, Sherwin JE, Hillman SL, Lorey F, Cunningham GC. Use of phenylalanine-to-tyrosine ratio determined by tandem mass spectrometry to improve newborn screening for phenylketonuria of early discharge specimens collected in the first 24 hours. Clinical chemistry. 1998; 44(12): 2405–2409. [PubMed: 9836704]
23. Van Hove JL, Zhang W, Kahler SG, et al. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: diagnosis by acylcarnitine analysis in blood. American journal of human genetics. 1993; 52(5):958–966. [PubMed: 8488845]
24. Spooner N. A glowing future for dried blood spot sampling. Bioanalysis. 2010; 2(8):1343–1344. [PubMed: 21083330]
25. Manicke NE, Abu-Rabie P, Spooner N, Ouyang Z, Cooks RG. Quantitative analysis of therapeutic drugs in dried blood spot samples by paper spray mass spectrometry: an avenue to therapeutic drug monitoring. Journal of the American Society for Mass Spectrometry. 2011; 22(9):1501–1507. [PubMed: 21953253]