DEVELOPMENT OF DRIED BLOOD SPOT REFERENCE MATERIAL

Reiner JL[1]*, Long SE[1], Bryan CE[2], Schantz MM[1]

[1]Analytical Chemistry Division, National Institute of Standards and Technology, 100 Bureau Dr., Gaithersburg, MD, USA [2]Analytical Chemistry Division, National Institute of Standards and Technology, 331 Fort Johnson Rd., Charleston, SC, USA

Introduction

The practice of collecting human blood on filter paper and using it for population screening dates back to the early 1960s when Guthrie and Susi[1] developed an assay which measured phenylalanine in dried blood spots (DBS). Though this innovated approach was developed for determination of metabolic diseases, some studies now report the use of DBS for the quantification of pharmaceuticals, organic contaminants, and elements[2-7]. With improvements in analytical instrument sensitivity, there is expected to be a surge in the use of DBS as a resource for chemical measurements. In anticipation of the surge, we aim to develop a reference material that will help to determine the recoverability and stability of different analytes from blood spots.

Materials and methods

Organic compounds

NIST Standard Reference Material (SRM) 1958 Organic Contaminants in Fortified Human Serum was reconstituted in 10.7 mL (mass known) of water according to the Certificate of Analysis. After reconstitution, 50 μL (mass known) of SRM 1958 was applied to a blood spot space on either a Whatman 903 protein saver card or an Agilent DMS card. For the Whatman cards, a total of five spots were available for application of the serum spot; however, only four spots were used. The Agilent cards offered four spots for the application of the serum; however, only three spots were used. One spot on each card was intentionally left blank to determine potential background levels of organic compounds from the card. The DBS cards were allowed to dry at room temperature overnight. After drying, the cards were placed in individual bags with a silica gel desiccant package until chemical analysis.

Perfluorooctane sulfonate (PFOS) was chosen as a marker compound for perfluorinated alkyl acids (PFAAs), since it is the PFAA occurring at highest concentrations in SRM 1958. Measurements of PFOS and other detectable PFAAs were made in the blood spot material using methods previously developed for serum[8, 9]. Briefly, for PFAA determination, a known mass of punched serum (approximately 0.03 g) from the cards was extracted with acetonitrile. The extract was concentrated, and the concentrated extracts were injected onto a liquid chromatograph (Agilent 1100 HPLC, Palo Alto, CA) interfaced to a negative electrospray ionization tandem mass spectrometer (LC-MS/MS) (API 4000, Applied Biosystems-MDS Sciex, Foster City, CA). The calibrants, a subsample of reconsitituted SRM 1958 (as a control), and blank samples were analyzed alongside the blood spot materials.

Inorganic compounds

NIST SRM 955c Caprine Blood, Level 4 was applied to the blood spot spaces on both the Whatman 903 and the Agilent DMS cards. Similar to the organic compound method, at least one spot was intentionally left blank to determine the potential background levels on the cards. Mercury (Hg) was chosen for preliminary measurements of inorganic compounds. In the Level 4 caprine blood Hg is detectable and has been quantified with a reference value of 33.9 ± 2.1 μg/L (32.2 ± 2.0 μg/kg wet mass, corrected for the SRM's density at 22 ºC). Measurements of Hg were made directly using a direct mercury analyzer DMA 80 (Milestone Scientific, Shelton, CT). Briefly, a known mass punch of the blood applied to the cards was removed and analyzed with the DMA 80. Calibrants, blanks, and a subsample of SRM 955c (as a control) were analyzed alongside the blood spot materials.

Results and discussion

Organic compounds

Preliminary results show that PFOS is measureable from the extraction of the blood spots (Table 1). The two blood spotting cards yielded different concentrations of PFOS. The concentrations reported here are based on the amount measured from the dry punch removed from the card. The PFOS concentration measured in the Whatman card is similar to the reference concentration of PFOS reported on the Certificate of Analysis for SRM 1958 (16.5 ± 0.6 ng/g, reconstituted serum). In comparison, the concentration of PFOS reported for the Agilent DMS card is elevated compared to SRM 1958. The two blood spot card types are made up of different materials, possibly explaining the

difference in concentrations seen from the two card types. Other PFAAs were detectable in the blood spot (Table 1). Perfluorohexane sulfonate (PFHxS) was measureable in the blood spots punched from both cards, while perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) were measureable in extracted serum from the Agilent cards.

Reproducibility is important consideration for reference material. With a small sample size, as in this study (n=3), little can be inferred. However, these results show that the Whatman 903 card had better spot to spot reproducibility compared to the Agilent DMS card for this study. Future work will require spotting of multiple Whatman and Agilent cards, followed by extraction of PFAAs to assess the reproducibility of PFAA measurements.

Table 1. Concentration of PFAAs (ng/g dry mass) measured in blood spots from the Whatman 903 protein saver cards and Agilent DMS cards

	Whatman 903 protein saver					
	Spot 1	Spot 2	Spot 3	**Average**	**SD**	**% RSD**
PFOA	< RL	< RL	< RL			
PFNA	< RL	< RL	< RL			
PFHxS	2.25	2.20	2.08	2.18	0.08	4%
PFOS	15.1	14.1	14.0	14.4	0.6	4%
	Agilent DMS					
	Spot 1	Spot 2	Spot 3	**Average**	**SD**	**% RSD**
PFOA	< RL	8.62	6.13	7.37	1.76	24%
PFNA	3.25	3.27	< RL	3.26	0.02	1%
PFHxS	9.01	7.98	4.82	7.27	2.18	30%
PFOS	61.2	55.2	34.9	50.4	13.8	27%

Values shown as "< RL" are below the reporting limit

Blank spots were also measured for PFAA background concentrations on both cards (Table 2). The average concentrations of PFOS and PFHxS were well below the concentrations measured in the blood spots. PFOA was the major PFAA found in the blank spots with concentrations up to 6.60 ng/g dry mass of the spot.

Table 2. Range of background concentration of PFAAs (ng/g dry mass) measured from the Whatman 903 protein saver cards and Agilent DMS cards

	Whatman 903	**Agilent DBS**
PFOA	2.90 - 4.13	6.44 - 6.60
PFNA	0.876 - 1.25	1.95 - 1.99
PFHxS	0.330 - 0.470	0.732 - 0.750
PFOS	0.481 - 0.685	1.067 - 1.094

Inorganic compounds

Preliminary results show that Hg is a measurable element in dried blood spot material. The two blood spot cards yielded similar results (Table 3). The Whatman cards had average Hg concentration (33.5 μg/kg dry mass) that fell within the reference values for SRM 955c. The Agilent cards had mean Hg concentrations (34.4 μg/kg dry mass) that were slightly above the reference values fro SRM 955c. That being said, the Agilent cards did show lower relative standard deviation (as expressed by % RSD) compared to the Whatman cards. The performance and repeatability were considered acceptable for both dried blood spot card types. Additionally, both Whatman and Agilent cards had background Hg concentration in the blanks that were not detectable.

Table 3. Concentration of Hg (μg/kg dry mass) measured in blood spots from the Whatman 903 protein saver cards and Agilent DMS cards

	Whatman 903 protein saver					
	Card 1	Card 2	Card 3	Average	SD	% RSD
Hg	32.7 ± 0.4	33.5 ± 0.3	34.3 ± 0.4	33.5	0.8	2%
	Agilent DMS					
	Card 1	Card 2	Card 3	Average	SD	% RSD
Hg	34.2 ± 0.3	34.5 ± 0.3	34.5 ± 0.1	34.4	0.2	0.5%

Future work will include the measurement of additional organic and inorganic compounds. Three other marker compounds have been chosen to analyze in the blood spot material. 4,4'-DDE, the metabolite of 4,4'-DDT, was chosen as a marker compound for organochlorine pesticides. The polychlorinated biphenyl (PCB) congener 153, the most often found at relatively high concentrations of PCB congeners, was chosen as a marker compound for PCBs. Polybrominated diphenyl ether (PBDE) 47 will be used as a marker compound for PBDE exposure. Inorganic elements, such as arsenic, iodine, and lead will be analyzed in the blood spot material.

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