DOI: 10.4149/BLL_2019_035

Bratisl Med J 2019; 120 (3)

223 – 234

LABORATORY MEDICINE

The dried blood spot sampling method in the laboratory medicine

Andrlova L, Kandar R

University of Pardubice, Faculty of Chemical Technology, Department of Biological and Biochemical Sciences, Pardubice, Czech Republic. **roman.kandar@upce.cz**

ABSTRACT

The minimization is currently expanding in all fields of human life. The smaller size of analytical instruments and their higher sensitivity enables an analysis of small amounts of a biological material with a very low consumption of chemicals, what is economically and also environmentally beneficial. A patient friendly and minimal invasive sample collection is therefore more than required. The dried blood spot (DBS) sampling standardly used in the newborn screening (NS) may be an option. The sample collection is simple, non-invasive, does not require trained medical personnel assistance and also storing and transportation of these samples is much easier in comparison with the whole blood samples. The DBS sampling method is used in the therapeutic drug monitoring or in the diagnosis of infectious diseases and its usage is still spreading. In some cases, it is still only a research object, but it shows a big potential for a future use. It could completely replace the whole venous blood collection in some cases, for example in the metabolic screening of diabetic patients or monitoring of treatment response, and it can overall simplify the sample collection and the transportation process *(Tab. 1, Ref. 152)*. Text in PDF *www.elis.sk.*

KEY WORDS: dried blood spot, DBS, dried samples, sample collection, laboratory medicine.

Introduction

The dried blood spot (DBS), a new sampling technique, was first introduced by Ivar Christian Bang in 1913 for the determination of blood sugar levels in rabbits (1). Subsequently, several scientists used this type of sample collection in their studies, but it became popular only in 1963, thanks to Robert Guthrie, who used it for sample collection in the neonatal screening of phenylketonuria. The capillary blood from a newborn's heel was saturated onto cellulose filter paper to detect levels of phenylalanine that is typically elevated in the disease. A disc was punched out from DBS sample and transferred to an agar plate containing *Bacillus subtilis* and a competitive inhibitor (beta-2-thienylalanine). The phenylalanine from the sample competed with the inhibitor, and the disease was detected by a zone of growth around the disc (2).

Currently, DBS is a standard sampling method in newborn screening of different diseases all over the world (the number and type of screened diseases vary from country to country). It allows us to analyze not only biochemical markers, but also nucleic acids. The whole process of blood collection, sample handling and transport conditions, to ensure the maximal quality, is published in a standard manual (3).

University of Pardubice, Faculty of Chemical Technology, Department of Biological and Biochemical Sciences, Pardubice, Czech Republic

Address for correspondence: R. Kandar, University of Pardubice, Faculty of Chemical Technology, Department of Biological and Biochemical Sciences, Studentska 573, CZ-532 10 Pardubice, Czech Republic.
Phone: +420.40.466037714

Due to a lot of advantages, in comparison with plasma or whole blood samples, DBS has become a very interesting sampling method in other analytical fields as well, like therapeutic drug monitoring (TDM), diagnostics of infectious diseases, metabolomics and so on. In contrast to newborn screening, the methodology of sample collection, handling and processing in these cases has not been unified yet. If the analytes of interest are defined with long-term stability, also the cards from newborn screening, that have been stored for years, could be used for retrospective studies (4, 5).

Advantages and disadvantages

In a comparison with classic liquid samples, DBS sampling has several advantages. One of the most important is a less invasive sample collection that is acceptable mainly in case of newborns, small children, and older people or needle-phobic patients. The small incision of a finger pad by a special lancelet is nearly painless and without special practicing can be performed by patients themselves or by other adults. In the case of newborns, even parents can prick the heel, thus, the need for qualified medical personnel is minimized. DBS cards can be stored very easily. After drying and eventual overlaying with a protective layer, the cards can be put on top of each other, thereby requiring less space. With the drying process, the most analytes become more stable and the need for low temperatures during storage then disappears. So, the transportation of those samples is much easier. It can be said, with exaggeration, that DBS samples can be collected by patients themselves and sent to a laboratory by post without the need of freezing. However, particular attention must be taken, because the temperature and humidity in mailboxes can rise according to the

weather conditions, which could harm the samples. In one of the studies van Amsterdam and Waldrop pointed out that depending on local conditions, distance and weight, more or less 200 euros per shipment could be saved by shipping DBS samples instead of plasma. It is also mentioned that approximately 30 % of all plasma samples are shipped in an incorrect way (incorrect packaging, documentation and so on) (6). On account of drying, viruses, such as HIV (human immunodeficiency virus) or HBV (hepatitis B virus), are not able to survive due to the disruption of the virus envelope, thus the risk of the transfer of blood borne diseases is minimized. Where animal experiments are necessary, DBS sample collection refines and reduces them. So, instead of taking several milliliters of blood, only few drops are necessary. For example, the total blood volume of a laboratory mouse is only 1.5 ml on average, so DBS sampling is suitable if more than one sample is needed. The low volume of biological material requires more sensitive analytical techniques, but, on the other hand, the amount of chemicals used in the processing of such samples is significantly lower, which is environmentally beneficial. Due to a very low sensitivity or problematic stability, the DBS sampling method is not 100 % appropriate for all analytes and the determination of some of them can be challenging. Most of all, the influence of other blood components must be considered (mainly different haematocrit levels among individuals – see Effect of matrix). A DBS sample is the mixture of plasma and blood elements, thus the concentration values cannot correspond to routinely used plasma reference levels and must be set, when establishing a method.

DBS sample collection

The skin must be disinfected with alcohol before being punctured and then allowed to air-dry. The blood can be taken from the finger, heel or ear lobe, by pricking with an automatic lancet. The first drop should be wiped away. The other blood drop can then be applied onto a sampling paper/card within the marked area that should be touched neither before, nor after blood application (especially while the sample is still wet). The indicated area should be homogeneously filled with blood (preferably with only one drop if it is possible) and show the same red color from both sides of filter paper. A DBS sample prepared like this should be left to air-dry in the horizontal position at ambient temperature for a minimum of 2–3 hours. Contact with other surfaces, direct sunlight exposure or the presence of any other heat sources, should be avoided (4, 7–9).

DBS sample can also be prepared by pipetting whole-venous blood from the tube with an anticoagulant in the middle of the indicated area. The blood is dosed in a particular amount with a calibrated pipette and disposable plastic tip. Thus, the error, due to the variable volume of blood on the filter paper, is minimized. The DBS sample must be prepared as soon as possible after anticoagulated whole-venous blood collection (not more than 24 hours after venepuncture). Drying conditions are the same as in the previous case. With this approach, the benefit of a less invasive sample collection is cancelled, but, on the other hand, there are benefits if venepuncture is required for some other examination. For example, collection of samples for research can be done in this way in a medical laboratory, with only the written consent of the patient, and without any additional punctures (4, 7).

Type of a filter paper

The DBS samples can be collected on the different types of filter papers that are available on the market. They are all made from 100 % pure cotton linters, but can vary in pore size or thickness and influence the absorptivity of blood (7). It was investigated if the type of filter paper can influence the results of DBS analysis. Some studies indicate that differences can be observed, mainly at extremely high or low analyte concentrations or extreme haematocrit levels (10, 11). However, according to Koster et al, all types of filter papers tested reported the same haematocrit level and the same pattern of recovery dependence on concentration. Mei et al propose that the results of analysis among different DBS cards are comparable (12). In the newborn screening programs, the untreated filter papers are mostly used and they are controlled also among different lots of the same manufacturer to ensure that the same conditions are preserved (8, 13). The untreated papers can be modified when soaked with various chemical solutions and allowed to air-dry in order to increase stability of analytes or in any other way to positively affect the character of DBS sample. The chemically-treated papers can also be found in the market. They were initially designed for nucleic acid analysis and they should cause cell lysis, protein denaturation or pathogen inactivation (13). The use of alternative filter paper for DBS sample collection, for example chromatographic (14), or even non-cellulose-based materials, like glass (15), are also mentioned in certain studies, but their usage is not significant.

DBS storage and stability

The quality of DBS samples can be negatively affected by moisture and humidity, which can cause bacterial growth, a degradation of molecules of interest or a variation in extraction recovery. Complete drying is therefore necessary before storing. The DBS samples should be protected even during storage either by adding desiccant to gas permeable zip-closure packaging or by overlaying them with paper. With the aim to increase stability, samples with less stable analytes should be stored at lower temperatures (4 °C, –20 °C or –80 °C), but if the molecules in the DBS samples are stable, room temperature is also appropriate (7-9).

For example, enzymes can be degraded very quickly, especially with higher temperatures (16, 17). However, lysosomal enzymes show a very good stability even at ambient temperature, so the DBS technique can be easily used for the diagnosis of lysosomal storage diseases (18, 19). The stability of nucleic acids in DBS varies depending on the type of nucleic acid. Studies for HIV detection show that DNA is more stable in DBS samples than RNA. Whereas DNA is stable at least one year even, when stored at 37 °C, in most cases RNA is unstable under these conditions. Otherwise, RNA shows a good stability at –70 °C (6–9 months, depending on viral load) (20, 21). C-reactive protein (CRP) lev-

els decrease quickly in DBS, when stored at ambient temperature, since its stability under these conditions is only 3 days. However, storage at –20 °C prolongs stability for at least one year (22, 23). Immunoglobulins (IgG, IgM, IgA) for hypogammaglobulinemia detection are stable in DBS for 14 days at ambient temperature, but they degrade after 3 days, when stored at 37 °C (24).

The knowledge of analyte stability is crucial, when using the DBS method. Stable molecules are well preserved by drying, but the degradation or modification of less stable molecules presents a problem. Then, it must be considered very carefully if DBS is a suitable method for sample collection (7, 25–27). The stability can be positively affected by adding other substances to the filter paper before sample collection. For example, glutathione is subjected to non-enzymatic autoxidation and enzymatic conversion that can be stopped by pre-treating the filter paper with a reducing agent (for instance dithiothreitol) (28). Similarly, peroxidation of polyunsaturated fatty acids can be limited by adding butylated hydroxytoluene (BHT) (29).

Effect of the matrix

The most frequently reported component of blood, affecting the DBS samples, is haematocrit (HCT) (30–37). Its value indicates the percentage of erythrocytes in total blood volume and differs not only among individuals, but also during different disease states of one organism. The typical HCT level for men is 46 ± 6 % and for women is 41 ± 5 %, but it can vary from 28–67 % in some certain populations (neonates, 2–12 year old children) (7, 32, 38, 39). Moreover, capillary blood tends to have a higher HCT level than venous blood (40). HCT is closely related to blood properties, and that is why it can affect its behaviour, when spotting onto filter paper and can cause significant assay bias, mainly at very low or at very high concentrations. The blood becomes more viscous with high HCT levels, which means that the absorptivity and spreading of blood on filter paper is very poor in comparison with low HCT levels and the volume of blood in a punched disc may vary (7, 9, 25, 37, 39). The recommended, but not frequently used, manner of how to overcome this problem is an involvement of different HCT levels in method validation. The interval, in which the results are still valid, should be defined. Then, it should be decided if the HCT of the DBS samples is within this range or not. The measurement of the HCT level is not problematic, when using venous anticoagulated blood for preparation of DBS samples. However, this practice excludes the possibility of blood sampling by patients themselves at home, which is reported as one of the main advantages of using the DBS sampling method (32, 37). An alternative approach must be used, when samples are prepared from capillary blood or are already dried. The measurement of some other parameter that correlates with the HCT level (for example, potassium) can be performed (30, 41). Moreover, with the aim to minimize the HCT effect, the calibrators should be prepared from the blood with HCT levels corresponding to the expected median of the screened population. Another recommendation of how to deal with haematocrit problem is to analyse the whole DBS sample after pipetting the particular volume of blood on filter paper. However, again, this approach is not approved for the patient self-sampling procedure (37).

Some studies suggest that the punch location is also an important factor affecting the assay bias (33, 35). Holub et al inferred that the location of disc punch influences the levels of some analytes in conjunction with HCT levels. For example, in case the level of HCT is low in the blood used for a DBS sample preparation, higher concentrations of amino acids can be found on the edge of a blood spot. In the center of the blood spot, their concentration is lower. It is due to the chromatographic effect causing different distribution of the analyte in the DBS sample (35).

If the extraction procedure is not optimal, particularity of the DBS sampling method can result in other problems and it is a problem with recovery at different concentration levels. Because of the presence of –OH groups on the surface of cellulose filter paper, some analytes can soak into the paper in higher concentrations and stay on the surface in lower concentrations. This could lead to lower extraction recovery in higher concentrations, and conversely, higher extraction recovery in lower concentrations. The recovery of the method can be improved by impregnating the paper with some agents that decrease the hydrogen-bond activity (7, 42).

Use of the internal standard

An internal standard (IS) is required for monitoring the behaviour of analytes in a sample and for compensation of losses during sample handling or due to the matrix effect. Ideally, it should be a stable isotope-labelled substance, but a more cost-effective solution is to use any substance with the structure and properties most similar to the tested analyte, that is not naturally presented in the sample. The proper addition of an IS into the DBS sample is challenging. Even if the most commonly used technique – addition of an IS into an extraction solvent – has reproducible results and is very easy to perform, it actually does not fully reflect the behaviour of the analyte. Firstly, it cannot reflect the changes of the analyte during storage, and, secondly, it does not indicate the losses during the extraction procedure. From an analytical point of view, an IS should be added to the sample as soon as possible; that means before spotting blood onto filter paper. Ideally, it should perfectly interact with blood and paper matrix, without influencing the original sample (for example with chromatographic effects). Therefore, some other approaches of IS additions have been investigated (43–46).

According to van Baar et al, slightly better results, in a comparison with the widely used technique of adding an IS into an extraction solution, are achieved by adding an IS directly to a liquid blood sample. The complete incorporation of the IS into the blood matrix is supposed to occur this way. In practice, this approach is difficult to perform, because of increased complexity of all procedures and untrained personnel (44, 45). Liu et al proposed the use of small glass tubes covered with the IS on the inner wall, for blood collection and DBS preparation, which could simplify the process of sample-handling (47).

The results, comparable with those, when the IS is a part of an extraction solvent, are obtained by an application of the IS via

Tab. 1. Examples of the usage of DBS samples (except of newborn screening).

	Analytes	Sample type		Storing		Stability	Extraction	Derivatization	Separation and detection	Source
				Time	Temperature					
Elements and ions	As, Ca, Hg, Pb, Se, Tl	animal	N.A.	N.A.	N.A.	2 y, -70 °C	HNO_3	-	ICP-MS	(113)
	Na, Mg, K, Ca, Fe, Co, Cu, Zn, As, Se, Cd, Pb	human	finger prick	3 m	-20 °C	N.A.	Triton X-100 and HNO_3 mixture	-	ICP-MS	(114)
	As, Pb, Hg, Cd	human	finger prick	prior to shipment	-20 °C	N.A.	Triton X-100 and acetic acid mixture	-	ICP-MS	(115)
	Hg	animal	venous anticoagulated/ heparinized capillary tube blood	prior to analysis	RT	1 y at RT/3 m at 30 °C	-	-	AAS	(116)
	Pb, Hg	human	newborns heel prick	N.A.	N.A.	8.5 m at RT	2% double-distilled HCl solution containing 0.05% 2-mercaptoethanol, 0.001% L-cysteine and 10 p.p.b. Ir and Rh	-	ICP-MS	(117)
	K^+	human	venous anticoagulated blood	2 d	RT	2 d RT/20 h 60 °C	2.5 mM KCl in ultrapure water	-	ISE	(41)
	K^+	human	N.A.	N.A.	N.A.	3.5 h RT/200 d 4 °C	EtOH	-	LC-MS/MS	(30)
	ClO_4^-	human	specially processed venous anticoagulated blood	N.A.	-20 °C	N.A.	aqueous IS solution	-	IC-MS/MS	(118)
Amino acids	Cit, Arg, Orn	human	N.A.	N.A.	N.A.	N.A.	IS solution in 30% citrate phosphate buffer 0.1 M in MetOH	-/ BuOH:acetylchloride (10:1)	LC-MS/MS	(119)
	23 amino acids	human	N.A.	N.A.	N.A.	N.A.	IS solution in MetOH	acetylchloride:BuOH (10:90,v/v)	ESI-MS	(65)
	Phe, Tyr	human	N.A.	N.A.	RT	N.A.	5% $HClO_4$ in water	-	HPLC-PDA	(120)
	Phe, Tyr	human	N.A.	N.A.	N.A.	N.A.	70% EtOH	AQC	HPLC-FL	(121)
	Phe, Tyr	human	N.A.	N.A.	N.A.	N.A.	MetOH	butanolic HCl	HPLC-MS/MS	(121)
	Phe, Tyr, Trp	human	finger prick	N.A.	N.A.	N.A.	MetOH	acetylchloride in BuOH	LC-MS/MS	(122)
	cystathionine, tHcys, Met	human	N.A.	prior to analysis	RT	minimal 2w at 25 °C	MetOH:formic acid:DTT	-	LC-MS/MS	(123)
	Val, Leu, Ile, Met, Phe, Tyr	human	newborns heel prick	-/prior to analysis	-/4 °C	N.A.	HCl/HPO_3/$HClO_4$/ $C_2HCl_3O_2$/ 5-sulfosalicylic acid/ MetOH/EtOH/acetonitrile	OPA	HPLC-FL	(124)
Fatty acids	8 fatty acids	human	N.A.	-/prior to analysis	-/4 °C	N.A.	-	MetOH/HCl	GC-FID	(66)
	17 fatty acids	human	N.A.	15 d	4 °C	N.A.	saline and isopropanol:chloroform (11:7)	MetOH/acetylchloride (20:1)	GC-FID	(125)
	7 fatty acids	human	finger prick	N.A.	-25 °C	N.A.	-	HCl/MetOH	GLC-FID	(126)
	24 fatty acids	human	finger prick/venous anticoagulated blood	28 d	4 °C/-20 °C	1 m/-20 °C	-	HCl/MetOH	GC-FID	(127)
	γ-hydroxybutyric acid	human	finger prick/venous anticoagulated blood	N.A.	RT	N.A.	-	TFAA:heptafluoro-butanol (2:1)	GC-MS	(128)
	14 fatty acids	human	finger prick	prior to analysis	RT	N.A.	-	BF_3-MetOH	GC-FID	(129)
	4 fatty acids	human	newborns heel prick	17 d	N.A.	N.A.	HCl/acetonitrile	DMAE	ESI-MS/MS	(130)
	22 fatty acids	human	finger prick	1 d	-80 °C	N.A.	-	BF3-MetOH	GC-FID	(131)
proteins	CRP	human	finger prick/venous blood with no additives	3 w then another 7/14/28/ 42 d	-20 °C then 22 °C/37 °C/ -20 °C	up to 1 w at 22 °C	assay buffer (0.01M PBS, 0.5M NaCl, 0,1% v/v Tween20, pH 7.2)	-	ELISA	(22)
	CRP	human	finger prick	N.A.	N.A.	N.A.	assay buffer (0.01M PBS, 0.5M NaCl, 0,1% v/v Tween20, pH 7.2)	-	ELISA	(132)
	thyroglobulin	human	finger prick	prior to analysis	-20 °C	N.A.	N.A.	-	ELISA	(133)
	hCG	human	venous anticoagulated blood	7/14/45 d	RT/8 °C/ -25 °C	minimal 45 d at ≤ 8 °C	PBS (pH 7.4)	-	LC-MS/MS	(134)
	hCG	human	N.A.	none	none	N.A.	PBS (pH 7.4)	-	nanoLC-MS	(135)
	97 proteins	human	venous anticoagulated blood	overnight	RT	majority 22 w at -20 °C or 37 °C	25mM NH_4HCO_3, 10% (W/v) sodium deoxycholate and 5mM tris(2-carboxyethyl)phospine mixture	-	UHPLC-MS/MS	(136)
	measles specific IgG	human	heel/finger prick	15-20 d, then up to 12 m	10-12 °C, then -20 °C	N.A.	PBS	-	ELISA	(137)
	IgG, IgM, IgA	human	finger prick	N.A.	N.A.	14 d/RT 14 d/2-8 °C 3-4 d/37 °C 10-14 d/ -25 °C	PBS, 0.05% Tween-20	-	ELISA	(24)

	Analytes	Sample type		Storing		Stability	Extraction	Derivatization	Separation and detection	Source
				Time	Temperature					
nucleic acids	Rubella virus RNA	human	N.A.	5 m/30 m	-20 °C/ -70 °C	N.A.	Tri-reagent	-	RT-PCR/ nested RT-PCR/ sequencing	(138)
	Dengue virus RNA	human	non-heparinized blood	1 y	-70 °C	minimal 9 w at 20 - 25 °C	RNA-ase free water	-	RT-PCR/ nested PCR	(139)
	HIV nucleic acid	human	finger prick/venous anticoagulated blood	2-4 w	RT/-80 °C	2 w at RT	QIAamp viral kit	-	RT-PCR/ nested PCR	(140)
	Plasmodium DNA	human	venous anticoagulated blood	N.A.	N.A.	N.A.	Bio-Rad Instagene matrix	-	RT-PCR	(141)
	Epstein-Barr virus DNA	human	venous anticoagulated blood	34-64 d	RT	N.A.	Qiagen DNA Micro-kit	-	RT-PCR	(142)
	HCV RNA	human	venous anticoagulated blood	N.A.	2-8 °C	N.A.	Abbot diagnostics extraction buffer and elution mix	-	RT-PCR	(143)
	HCV RNA	human	N.A.	N.A.	N.A.	minimal 12 m at RT	NucliSENS lysis buffer	-	RT-PCR	(144)
	S. pneumoniae DNA	human	finger prick	prior to shipment	-80 °C	N.A.	TrisEDTA (pH 8.5)	-	RT-PCR	(145)
drugs and their metabolites	THC, THC-OH, THC-COOH	human	finger prick	N.A.	N.A.	3 m at RT	MetOH	-	HPLC-MS/MS	(83)
	PEth	human	venous anticoagulated blood	N.A.	N.A.	110 d at 22 °C	MetOH	-	LC-MS/MS	(146)
	salicylic acid	human	finger prick/venous anticoagulated blood	N.A.	N.A.	N.A.	-	-	DESI-MS	(59)
	tricyclic antidepressants (amitriptyline, nortriptyline, imipramine, clomipramine)	human	finger prick	N.A.	N.A.	1-3 m at RT 3 m at 4 °C	acetonitrile:MetOH (1:3)	-	LC-MS/MS	(147)
	antipsychotics (citalopram, mirtazapine, risperidone, 9-OH-risperidone)	human	finger prick/venous anticoagulated blood	N.A.	N.A.	1-3 m at 8 °C	IS solution in MetOH	-	HPLC-MS	(81)
	everolimus	human	finger prick	overnight	RT	3 d at 60 °C 1 m at RT	IS solution in MetOH	-	HPLC-MS/MS	(42)
	oseltamivir and oseltamivir-carboxylate	human	finger prick/venous anticoagulated blood	N.A.	N.A.	minimal 7 d at RT or 4 °C	5% MetOH in water	-	UHPLC-MS/MS	(148)
	rifapentine	human	venous anticoagulated blood	N.A.	-70 °C	11 w at RT	MetOH:50 mM ammonium formate buffer (90:10), containing ascorbic acid and IS	-	LC-MS/MS	(149)
enzymes	N-acetylgalactosamine-6-sulphatase activity	human	N.A.	N.A.	4 °C	minimal 30 d at 4 °C	distilled water and 10mM 4-methylumbelliferyl-β-galactoside-6-sulphate in 0.1M Na-acetate/0.1 M acetic acid buffer with 0.02% NaN_3 pH 4.3	-	spectral fluorimeter	(16)
	palmitoyl protein thioesterase 1	human	N.A.	N.A.	N.A.	2 w at RT 3 h at 50 °C	substrate buffer and phosphate-citrate buffer	-	spectral fluorimeter	(18)
	tripeptidyl peptidase 1 activity	human	N.A.	N.A.	N.A.	2 w at RT 3 h at 50 °C	substrate buffer and NaCl	-	spectral fluorimeter	(18)
	lysosomal enzymes activity	animal	venous anticoagulated blood	up to 1 m	4 °C	6 m at 4 °C	distilled water/buffer containing substrate	-	spectral fluorimeter	(19)
	glucose-6-phosphate degydrogenase activity	human	finger prick	34-72 h	RT	4 d at RT	distilled water or commercially available elution buffer containing substrate	-	spectral fluorimeter	(150)
	glucocerebrosidase, sphingomyelinase, alpha-galactosidase A, acid alpha-glucosidase and galactosylceremidase activity	human	venous anticoagulated blood	up to 1 w/ longer periods	4 °C/ ≤20 °C	N.A.	20mM sodium phosphate buffer (pH 7.1)	-	MS/MS	(151)
	lysosomal acid lipase activity	human	venous anticoagulated blood	up to 2 w	-20 °C	N.A.	water	-	spectral fluorimeter	(152)
	pyridox(am)ine 5´-phosphate oxidase activity	human	venous or capillary blood	up to 1 w/ prior to analysis	RT/-20 °C/ -80 °C	12 w at -20 °C or -80 °C	40mM Tris-phosphate buffer (pH 7.6)	-	LC-MS/MS	(153)

AAS – atomic absorption spectrometry; AQC – 6-aminoquinolyl-N-hydroxysccinimidyl carbamate; Arg – arginine; BuOH – butanol; Cit – citrulline; CRP – C-reactive protein; DESI – desorption electrospray ionization; DMAE – N,N-dimethylethanolamine; DNA – deoxyribonucleic acid; DTT – dithiothreitol; ELISA – enzyme-linked immunosorbent assay; ESI – electrospray ionization; EtOH – ethanol; FID – flame ionization detector; FL – fluorescence detector; GC – gas chromatography; GLC – gas–liquid chromatography; hCG – human chorionic gonadotropin; HCV – hepatitis C virus; HIV – human immunodeficiency virus; HPLC – high-performance liquid chromatography; ICP – inductively coupled plasma; Ig – immunoglobulin; Ile isoleucine; IS – internal standard; ISE – ion selective electrode; LC – liquid chromatography; Leu – leucine; m – month(s); Met – methionine; MetOH – methanol; MS – mass spectrometry; MS/MS – tandem mass spectrometry; N.A. – not available; OPA – o-phthaldialdehyde; Orn – ornithine; PBS – phosphate-buffered saline; PCR – polymerase chain reaction; PDA – photodiode array; PEth – phosphatidylethanol; Phe – phenylalanine; tHcys – total homocysteine; Trp – tryptophan; Tyr – tyrosine; RNA – ribonucleic acid; RT-PCR – reverse transcription polymerase chain reaction; TFAA – trifluoroacetic anhydride; THC – Δ9-tetrahydrocannabinol; UHPLC – ultra-high performance liquid chromatography; Val – valine; w – week(s); y – year(s)

spray in separated step either before or after blood spotting onto filter paper. This technique results in a more homogenous distribution, when compared to IS pipetting. Additionally, potential chromatographic effects are avoided. It is assumed that better results could be achieved by applying the IS onto filter paper before blood spotting, hence avoiding time differences emerging from variable time between sample collection and IS application (43–45).

Calibration standards and quality of the control samples

Just as in all other types of samples, analysis of DBS samples also requires calibration standards and the quality control (QC) samples most similar to the real ones. It means that fresh blood, with haematocrit level within the range of the tested population, should be used for DBS QC and calibration standard sample preparation. It is not difficult to find non-interfering blood for the analysis of new molecules (for example, new pharmaceutical drugs), but it could be a problem in the case of small biomarker molecules, which are presented endogenously and whose level can vary from human to human or according to biorhythms (7). A possible solution to this problem is the preparation of artificial "whole blood" by adding washed blood cells to an artificial plasma or serum (48, 49). Since the calibration and QC stock/working solutions are not solved in the blood, their volume in the artificial whole blood should be minimized as much as possible (maximum 5 % of the final volume) (7).

Extraction procedure

The extraction from solid to the liquid phase represents one of the most critical steps in DBS sample processing. The extraction of whole DBS, prepared from the exact amount of blood (50), or usage of these so-called perforated (51) or even pre-cut (52) DBS are mentioned in literature, but their usage is rare. Usually, the extraction procedure consists of punching out a disc, extraction and derivatization (if necessary), while the latter two mentioned can be performed in any order. The diameter of the disc punched out from DBS can range from 3 to 8 mm (9, 27, 53) and the number of discs used can vary according to the sensitivity of the analytical method. The extraction is performed by adding a specific volume of the extraction solvent that plays a crucial role in re-solubilizing the analytes to the disc. For better effectiveness, shaking, vortexing or ultrasound can be used. The choice of the extraction solvent depends on the physicochemical properties. It should be strong enough to interrupt the bindings between the molecule of interest and the paper matrix (7, 9, 27). Usually, organic solvents (methanol, hexane etc.) (54, 55), aqueous buffers (saline, phosphate buffers etc.) (56, 57) or mixtures of organic and aqueous solvents, and alternatively the commercial kits (58), are used for this purpose. To increase the extraction efficiency, pH altering substances, surfactants (Tween, Triton X) or chelating agents (EDTA) can be added (27). Manual extraction of DBS samples is a bit laborious and time-consuming and therefore an alternative approach would be necessary. Either automation or sample analysis that does not require sample preparation at all (or at least minimizes it) (43). Direct analysis without a previous extraction could be an option, for example: desorption electrospray ionization (DESI) (59–61), direct analysis in real time (DART) (15, 62), matrix-assisted laser desorption/ionization (MALDI) (63) etc. Unfortunately, direct analysis means the loss of the separation step, which may result in lower sensitivity and possible interferences, while current technological status does not allow automatic analysis of a huge number of samples (46, 53, 64). That is the reason why most of the studies prefer manual offline extraction. The extracts can be directly injected into an instrument (58) or derivatized considering the properties of the analyte and the chosen detector (7, 65). Alternatively, the derivatization reagent can be added directly to the DBS sample and then, just prepared derivatives are extracted (66). This procedure is worthy of discussion because it is not known what reactions could occur between the derivatization reagent and the paper matrix. Especially very aggressive derivatization reagents, for example acetyl chloride, could cause some unexpected damage of the paper material and negatively affect the sample processing and analysis.

DBS and liquid sample results comparison

The essential part of all research on the application of DBS samples should be a comparison of the levels of tested analyte(s) between routinely used types of sample (most often liquid) and DBS samples and a search for possible correlations in the results. The finding of strong correlations indicates that DBS samples can be used as an effective alternative to sample collection (7, 67–69).

Applications of the DBS samples

The DBS sample is standardly used for the early detection of different genetic and metabolic disorders in newborns, to avoid irreversible changes or at least significantly improve symptoms of the disease. The capillary blood from a newborn's heel is spotted onto a filter paper card, optimally 24–48 hours after birth, and analyzed by tandem mass spectrometry. The changes in the concentration of individual molecules might not have such an informative character as the monitoring of several biomarker molecules and their comparison in relation to each other. These metabolic patterns are evaluated in the context of different disease states (7, 9, 70–73).

Metabolomics is a relatively new discipline that reflects the actual state of the organism and that can show diverse variations in time. The qualification and quantification of metabolites in time has a great potential in the early detection of cancer, in the prediction of disease-development or in the monitoring of the treatment response. With huge number of samples that must be analyzed to find new biomarkers, DBS seems to be a good alternative to sample collection (65, 74, 75). As Kang et al and Zukunft et al demonstrated in their studies, DBS samples could fully replace plasma in metabolite profiling (76, 77). Chace et al proved that DBS samples can also be used in post-mortem metabolomics screening in cases of unexpected or unknown infant and child death (78).

Toxicology is a wide field, including preclinical drug discovery process (toxicokinetics), as well as latter phases (pharmacokinetics

and TDM). The aim is to determine the relation between a chemical substance entering the body with a specific rate and its fate in an organism (the way of metabolizing and excreting it). Very often animal experiments are needed, and DBS sampling meets the requirements of the 3R principle (replacement, reduction, and refinement). The number of samples per one animal is restricted physiologically and from an ethical point of view. DBS not only reduces the volume of blood needed and thus the number of animals needed, but it also reduces the cost of experiments for pharmaceutical companies (79). TDM is used in humans to optimize the drug dosage for individuals, so that the highest effectiveness and security is preserved. The self-sampling and almost painless sample collection is a big advantage of DBS, even if the samples can be possibly contaminated, if the person preparing them and dosing the drug is the same. The results obtained by the DBS method are comparable with plasma, so DBS sampling is an excellent alternative (80–82). DBS can be used also in forensic toxicology, for example in screening the abuse substances, like cannabinoids (83), cocaine (84), opiates (85) and so on (86). Also the screening of phosphatidylethanol, the direct ethanol metabolite, in DBS samples, could be a promising way of controlling the alcohol level of drivers. The same metabolite can be analyzed also in newborns, if prenatal alcohol exposure is suspected (86–88).

The simplicity and affordability of DBS samples is advantageous also in epidemiological studies. The disruption of the virus envelope during the DBS drying process ensures biosafety, whereas well preserved antibodies and nucleic acids enable the detection of various infectious diseases (89), for example: measles (90), rubella (91) or even Ebola (92). DBS samples could also help with the actual summary of epidemiological situations concerning asymptomatic or long-incubation period diseases (for example, AIDS or chronic hepatitis, via HIV or HCV monitoring) (93).

Some of the concrete examples of huge usage of DBS samples can be seen in the Table 1.

Similar types of samples

Recently, there is a great interest in a new form of simple, rapid and non-invasive sample collection. The unique character of DBS sample collection inspires the search for new types of samples in dried form.

Dried plasma spot (DPS) or dried serum spot (DSS) samples are prepared exactly like DBS samples, but from patients' plasma/serum pipetted onto filter paper, which requires the additional step of blood centrifugation and automatically means the loss of the non-invasive sample collection status. However, DPS/DSS samples, which are more homogenous in comparison with DBS, do not contain blood cells and thus do not show the haematocrit dependence. These samples are often used in different fields of clinical and pre-clinical analyses (94–99).

The collection of dried urine spot (DUS) samples is even less invasive and in the case of newborns, the filter paper can be simply inserted into the diaper. This type of sample collection is suitable for all analytes excreted in urine. It can be used for example in the diagnosis of different diseases or for drug screening (100–105).

Some drugs and metabolites can be present also in human saliva, therefore dried saliva spot (DSaS) samples were created (106–108).

Also, a research on human milk samples in dried form could be truly beneficial for mothers staying at home. Self-sampling, simple collection and ease of transportation are big pluses of this method. Dried milk spot (DMS) samples can be used also for TDM (109–111).

Another biological fluid, in which analytes can be determined, is sweat. Dried sweat spot (DSwS) samples can be used for example in the diagnosis of disturbances in lipid metabolism as they show a very good correlation with plasma samples in the analysis of fatty acids (112).

Conclusions

The advantages of the usage of DBS samples are noticeable. The minimization of the amount of biological material needed for analysis, the enhancement of biosafety and the simplification of the whole collection and transportation process are all the reasons, why DBS samples have spread so quickly (not only in newborn screening programs). The influence of HCT and the absence of the automation step for a huge number of samples can be the main pitfall causing the lack of common use in clinical practice and as a primary method for research. The analysis of some molecules can be challenging due to a very small sample volume and the need for more sensitive analytical methods. However, this problem appears to gradually fade with the development of new methods, like mass spectrometry. Some molecules are not even appropriate for DBS sample analysis due to their instability or the fast alteration of their structure. Thus, DBS samples would never be able to completely replace the whole blood or plasma samples, but in some specific cases, DBS can simplify all logistics around sample handling and storage, which is beneficial mainly for long-term studies. In animal experiments, it helps to follow the principle of the 3Rs (replacement, reduction and refinement) and, what is more, it is economical and environmentally-friendly (the low amount of samples means a low amount of chemicals needed for analysis).

References

1. Schmidt V. Ivar Christian Bang (1869–1918), founder of modern clinical microchemistry. Clin Chem 1986; 32 (1): 213–215.

2. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963; 32 (3): 338–343.

3. Hannon WH, De Jesus VR, Balance LO et al. Blood collection on filter paper for newborn screening programs; Approved standard – sixth edition. Wayne: Clinical and Laboratory Standards Institute, 2013: 1–35.

4. Gruner N, Stambouli O, Ross RS. Dried blood spots – preparing and processing for use in immunoassays and in molecular techniques. J Vis Exp 2015; 97: 1–9.

5. Strnadova KA, Holub M, Muhl A et al. Long-term stability of amino acids and acylcarnitines in dried blood spots. Clin Chem 2007; 53 (4), 717–722.

6. van Amsterdam P, Waldrop C. The application of dried blood spot sampling in global clinical trials. Bioanalysis 2010; 2 (11), 1783–1786.

7. Li W, Tse FLS. Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules. Biomed Chromatogr 2010; 24 (1), 49–65.

8. Mei JV, Alexander JR, Adam BW, Hannon WH. Use of filter paper for the collection and analysis of human whole blood specimens. J Nutr 2001; 131 (5), 1631–1636.

9. Deep A, Kumar P, Kumar A, Thakkar A. Dry blood spot technique: a review. Int J Pharm Sci Rev Res 2012; 15 (2), 90–94.

10. Koster RA, Botma R, Greijdanus B et al. The performance of five different dried blood spot cards for the analysis of six immunosupresants. Bioanalysis 2015; 7 (10): 1225–1235.

11. Rottinghaus EK, Beard RS, Bile E et al. Evaluation of dried blood spots collected on filter papers from three manufacturers stored at ambient temperature for application in HIV–1 drug resistance monitoring. PLoS One 2014; 9 (10): 1–5.

12. Mei JV, Zobel SD, Hall EM, De Jesus VR, Adam BW, Hannon WH. Performance properties of filter paper devices for whole blood collection. Bioanalysis 2010; 2 (8): 1397–1403.

13. Wagner M, Tonoli D, Varesio E, Hopfgartner G. The use of mass spectrometry to analyze dried blood spots. Mass Spectrom Rev 2016; 35 (3), 361–438.

14. Shen Z, Kang P, Rahavendran SV. Metabolite profiling of dasatinib dosed to Wistar Han rats using automated dried blood spot collection. J Pharm Biomed Anal 2012; 67–68, 92–97.

15. Crawford E, Gordon J, Wu JT, Musselman B, Liu R, Yu S. Direct analysis in real time coupled with dried spot sampling for bioanalysis in a drug-discovery setting. Bioanalysis 2011; 3 (11): 1217–1226.

16. Camelier MV, Burin MG, De Mari J, Vieira TA, Marasca G, Giugliani R. Practical and reliable enzyme test for the detection of mucopolysaccharidosis IVA (Morquio syndrome type A) in dried blood samples. Clin Chim Acta 2011; 412 (19–20): 1805–1808.

17. Tan MA, Dean CJ, Hopwood JJ, Meikle PJ. Diagnosis of metachromatic leukodystrophy by immune quantification of arylsulphatase a protein and activity in dried blood spots. Clin Chem 2008; 54 (11): 1925–1927.

18. Lukacs Z, Santavuori P, Keil A, Steinfeld R, Kohlschütter A. Rapid and simple assay for the determination of tripeptidyl peptidase and palmitoyl protein thioesterase activities in dried blood spots. Clin Chem 2003; 49 (3): 509–511.

19. Sewell AC, Haskins ME, Giger U. Dried blood spots for enzymatic diagnosis of lysosomal storage diseases in dogs and cats. Vet Clin Pathol 2012; 41 (4): 548–557.

20. Aitken SC, Wallis CL, Stevens W, de Wit TR, Schuurman R. Stability of HIV-1 nucleic acid in dried blood spot samples for HIV-1 drug resistance genotyping. PLoS ONE 2015; 10 (7): 1–10.

21. Leelawiwat W, Young NL, Chaowanachan T et al. Dried blood spots for the diagnosis and quantitaion of HIV–1: Stability studies and evaluation of sensitivity and specificity for the diagnosis of infant HIV-1 infection in Thailand. J Virol Methods 2009; 155 (2): 109–117.

22. Brindle E, Fujita M, Shofer J, O'Connor KA. Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research. J Immunol Methods 2010; 362 (1–2): 112–120.

23. McDade TW, Burhop J, Dohnal J. High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. Clin Chem 2004; 50 (3) 652–654.

24. Yel L, Rabbat CJ, Cunningham-Rundles C et al. A novel targeted screening tool for hypogammaglobulinemia: measurement of serum immunoglobulin (IgG, IgM, IgA) levels from dried blood spots (Ig-DBS Assay). J Clin Immunol 2015; 35 (6): 573–582.

25. Chace DH, De Jesus VR, Spitzer AR. Clinical chemistry and dried blood spots: increasing laboratory utilization by improved understanding of quantitative challenges. Bioanalysis 2014; 6 (21): 2791–2794.

26. 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.

27. 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 2013; 51 (10), 1897–1909.

28. Kandar R, Stramova X, Drabkova P, Brandtnerova M. Determination of total glutathione in dried blood spot samples using a high-performance liquid chromatography. J Chromatogr Sci 2015; 53 (6): 879–885.

29. Metherel AH, Hogg RC, Buzikievich LM, Stark KD. Butylated hydroxytoluene can protect polyunsaturated fatty acids in dried blood spots from degradation for up to 8 weeks at room temperature. Lipids Health Dis 2013; 12 (22): 1–9.

30. den Burger JC, Wilhelm AJ, Chahbouni AC, Vos RM, Sinjewel A, Swart EL. Haematocrit corrected analysis of creatinine in dried blood spots through potassium measurement. Anal Bioanal Chem 2015; 407 (2): 621–627.

31. Chao T, Trybala A, Starov VM, Das DB. Influence of haematocrit level on the kinetics of blood spreading on thin porous medium during dried blood spot sampling. Colloids Surf A Physicochem Eng Asp 2014; 451 (1): 38–47.

32. Denniff P, Spooner N. The effect of hematocrit on assay bias when using DBS samples for quantitative bioanalysis of drugs. Bioanalysis 2010; 2 (8): 1385–1395.

33. 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 samples. Bioanalysis 2011; 3 (20): 2335–2347.

34. Lawson AJ, Bernstone L, Hall SK. Newborn screening blood spot analysis in the UK: influence of spot size, punch location and hematocrit, J Med Screen 2016; 23 (1): 7–16.

35. Holub M, Tuschl K, Ratschmann R et al. Influence of hematocrit and localisation of punch in dried blood spots on levels of amino acids and acylcarnitines measured by tandem mass spectrometry. Clin Chim Acta 2006; 373 (1–2): 27–31.

36. Hall EM, Flores SR, De Jesus VR. Influence of hematocrit and total-spot volume on performance characteristics of dried blood spots for newborn screening. Int J Neonatal Screen 2015; 1 (2): 69–78.

37. De Kesel PM, Capiau S, Lambert WE, Stove CP. Current strategies for coping with the hematocrit problem in dried blood spot analysis. Bioanalysis 2014; 6 (14): 1871–1874.

38. Walker HK, Hall WD, Hurst JW (Eds). Clinical Methods: The history, physical, and laboratory examinations. 3rd edition. Boston: Butterworths, 1990: 1–1087.

39. Arora R, Hudson W, Boguszewski P. Effect of hematocrit on analyte quantification using dried blood spot technology for pharmaceutical bioanalysis. Application Note. Agilent Technologies 2012; 1–13.

40. Kayiran SM, Ozbek N, Turan M, Gürakan B. Significant differences between capillary and venous complete blood counts in the neonatal period. Clin Lab Haem 2003; 25 (1): 9–16.

41. Capiau S, Stove VV, Lambert WE, Stove CP. Prediction of the hematocrit of dried blood spots via potassium measurement on a routine clinical chemistry analyzer. Anal Chem 2013; 85 (1): 404–410.

42. van der Heijden J, de Beer Y, Hoogtanders KJ et al. Therapeutic drug monitoring of everolimus using the dried blood spot method in combination with liquid chromatography–mass spectrometry. J Pharm Biomed Anal 2009; 50 (4): 664–670.

43. Zimmer D, Hassler S, Betschart B, Sack S, Fankhauser C, Loppacher M. Internal standard application to dried blood spots by spraying: investigation of the internal standard distribution. Bioanalysis 2013; 5 (6): 711–719.

44. van Baar BL, Verhaeghe T, Heudi O et al. IS addition in bioanalysis of DBS: results from EBF DBS-microsampling consortium. Bioanalysis 2013; 5 (17), 2137–2145.

45. Abu-Rabie P, Denniff P, Spooner N, Brynjolffssen J, Galluzzo P, Sanders G. Method of applying internal standard to dried matrix spot samples for use in quantitative bioanalysis. Anal Chem 2011; 83 (22), 8779–8786.

46. Abu-Rabie P. Direct analysis of DBS: emerging and desirable technologies. Bioanalysis 2011; 3 (15): 1675–1678.

47. Liu J, Cooks RG, Ouyang Z. Enabling quantitative analysis in ambient ionization mass spectrometry: internal standard coated capillary samplers. Anal Chem 2013; 85 (12), 5632−5636.

48. Higashi T, Nishio T, Uchida S, Shimada K, Fukushi M, Maeda M. Simultaneous determination of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone in dried blood spots from low birth weight infants using LC–MS/MS. J Pharm Biomed Anal 2008; 48 (1): 177–182.

49. Fallah E, Peighambardoust SH. Validation of the use of dried blood spot (DBS) method to assess vitamin A status. Health Promot Perspect 2012; 2 (2): 180–189.

50. Fan L, Lee JA. Managing the effect of hematocrit on DBS analysis in a regulated environment. Bioanalysis 2012; 4 (4): 345–347.

51. Li F, Zulkoski J, Fast D, Michael S. Perforated dried blood spots: a novel format for accurate microsampling. Bioanalysis 2011; 3 (20): 2321–2333.

52. Youhnovski N, Bergeron A, Furtado M, Garofolo F. Pre-cut dried blood spot (PCDBS): an alternative to dried blood spot (DBS) technique to overcome hematocrit impact. Rapid Commun Mass Spectrom 2011; 25 (19): 2951–2958.

53. Wong P, Pham R, Bruenner BA, James CA. Increasing efficiency for dried blood spot analysis: prospects for automation and simplified sample analysis. Bioanalysis 2010; 2 (11): 1787–1789.

54. Thomas A, Geyer H, Schänzer W et al. Sensitive determination of prohibited drugs in dried blood spots (DBS) for doping controls by means of a benchtop quadrupole/Orbitrap mass spectrometer. Anal Bioanal Chem 2012; 403 (5): 1279–1289.

55. Faller A, Richter B, Kluge M et al. LC-MS/MS analysis of phosphatidylethanol in dried blood spots versus conventional blood specimens. Anal Bioanal Chem 2011; 401 (4): 1163–1166.

56. Jacomelli G, Micheli V, Peruzzi L et al. Simple non-radiochemical HPLC-linked method for screening for purine metabolism disorders using dried blood spot. Clin Chim Acta 2002; 324 (1–2): 135–139.

57. Kapur S, Kapur S, Zava D. Cardiometabolic risk factors assessed by a finger stick dried blood spot method. J Diabetes Sci Technol 2008; 2 (2): 236–241.

58. Khan HA, Alhomida AS, Madani HA, Sobki SH. Carnitine and acylcarnitine profiles in dried blood spots of patients with acute myocardial infarction. Metabolomics 2013; 9 (4): 828–838.

59. Siebenhaar M, Kullmer K, Fernandes NM, Hullen V, Hopf C. Personalized monitoring of therapeutic salicylic acid in dried blood spots using a three-layer setup and desorption electrospray ionization mass spectrometry. Anal Bioanal Chem 2015; 407 (23): 7229–7238.

60. Wiseman JM, Evans CA, Bowen CL, Kennedy JH. Direct analysis of dried blood spots utilizing desorption electrospray ionization (DESI) mass spectrometry. Analyst 2010; 135 (4): 720–725.

61. Wiseman JM, Kennedy JH. Analysis of dried blood spots using DESI mass spectrometry. Methods Mol Biol 2014; 1198: 291–297.

62. Wang C, Zhu H, Cai Z, Song F, Liu Z, Liu S. Newborn screening of phenylketonuria using direct analysis in real time (DART) mass spectrometry. Anal Bioanal Chem 2013; 405 (10): 3159–3164.

63. Kobrynski LJ, Yazdanpanah GK, Koontz D, Lee FK, Vogt RF. MALDI-TOF-MS assay to detect the hemizygous 22q11.2 deletion in DNA from dried blood spots. Clin Chem 2016; 62 (1): 287–292.

64. Deglon J, Thomas A, Daali Y et al. Automated system for on-line desorption of dried blood spots applied to LC/MS/MS pharmacokinetic study of flurbiprofen and its metabolite. J Pharm Biomed Anal 2011; 54 (2): 359–367.

65. Wang Q, Sun T, Cao Y et al. A dried blood spot mass spectrometry metabolomic approach for rapid breast cancer detection. Onco Targets Ther 2016; 9: 1389–1398.

66. Marangoni F, Colombo C, Galli C. A method for the direct evaluation of the fatty acid status in a drop of blood from a fingertip in humans: applicability to nutritional and epidemiological studies. Anal Biochem 2004; 326 (2): 267–272.

67. Youhnovski N, Michon J, Latour S et al. Determination of naproxen using DBS: evaluation & pharmacokinetic comparison of human plasma versus human blood DBS. Bioanalysis 2010; 2 (8): 1501–1513.

68. David S, Sachithanandham J, Jerobin J, Parasuram S, Kannangai R. Comparison of HIV-1 RNA level estimated with plasma and DBS samples: a pilot study from India (South). Indian J Med Microbiol 2012; 30 (4): 403–406.

69. Taylor RR, Hoffman KL, Schniedewind B, Clavijo C, Galinkin JL, Christians U. Comparison of the quantification of acetaminophen in plasma, cerebrospinal fluid and dried blood spots using high-performance liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal 2013; 83: 1–9.

70. Morris M, Fischer K, Leydiker K, Elliott L, Newby J, Abdenur JE. Reduction in newborn screening metabolic false-positive results following a new collection protocol. Genet Med 2014; 16 (6): 477–483.

71. Carpenter KH, Wiley V. Application of tandem mass spectrometry to biochemical genetics and newborn screening. Clin Chim Acta 2002; 322 (1–2): 1–10.

72. Chace DH, Kalas TA, Naylor EW. Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clin Chem 2003; 49 (11): 1797–1817.

73. Rinaldo P, Cowan TM, Matern D. Acylcarnitine profile analysis. Genet Med 2008; 10 (2): 151–156.

74. Jayavelu ND, Bar NS. Metabolomic studies of human gastric cancer: Review. World J Gastroenterol 2014; 20 (25): 8092–8101.

75. Lima AR, Bastos ML, Carvalho M, de Pinho PG. Biomarker discovery in human prostate cancer: an update in metabolomics studies. Transl Oncol 2016; 9 (4): 357–370.

76. Kong ST, Lin HS, Ching J, Ho PC. Evaluation of dried blood spots as sample matrix for gas chromatography/mass spectrometry based metabolomic profiling. Anal Chem 2011; 83 (11): 4314–4318.

77. Zukunft S, Sorgenfrei M, Prehn C, Moller G, Adamski J. Targeted metabolomics of dried blood spot extracts. Chromatographia 2013; 76 (19): 1295–1305.

78. 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. Clin Chem 2001; 47 (7): 1166–1182.

79. Stove CP, Ingels AS, De Kesel PM, Lambert WE. Dried blood spots in toxicology: From the cradle to the grave? Crit Rev Toxicol 2012; 42 (3): 230–243.

80. Rezonja Kukec R, Grabnar I, Mrhar A, Cebron Lipovec N, Cufer T, Vovk T. A simple dried blood spot method for clinical pharmacological analyses of etoposide in cancer patients using liquid chromatography and fluorescence detection. Clin Chim Acta 2016; 452: 99–105.

81. Weber J, Oberfeld S, Bonse A, Telger K, Lingg R, Hempel G. Validation of a dried blood spot method for therapeutic drug monitoring of citalopram, mirtazapine and risperidone and its active metabolite 9-hydroxyrisperidone using HPLC–MS. J Pharm Biomed Anal 2017; 140: 347–354.

82. Wilhelm AJ, den Burger JC, Swart EL. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. Clin Pharmacokinet 2014; 53 (11): 961–973.

83. Mercolini L, Mandrioli R, Sorella V et al. Dried blood spots: Liquid chromatography–mass spectrometry analysis of Δ9-tetrahydrocannabinol and its main metabolites. J Chromatogr A 2013; 1271 (1): 33–40.

84. Mercolini L, Mandrioli R, Gerra G, Raggi MA. Analysis of cocaine and two metabolites in dried blood spots by liquid chromatography with fluorescence detection: A novel test for cocaine and alcohol intake. J Chromatogr A 2010; 1217 (46): 7242–7248.

85. Antelo-Dominguez A, Cocho JA, Tabernero MJ, Bermejo AM, Bermejo-Barrera P, Moreda-Pineiro A. Simultaneous determination of cocaine and opiates in dried blood spots by electrospray ionization tandem mass spectrometry. Talanta 2013; 117: 235–241.

86. Sadones N, Capiau S, De Kesel PM, Lambert WE, Stove CP. Spot them in the spot: analysis of abused substances using dried blood spots. Bioanalysis 2014; 6 (17): 2211–2227.

87. Bakhireva LN, Shrestha S, Gutierrez HL, Berry M, Schmitt C, Sarangarm D. Stability of phosphatidylethanol in dry blood spot cards. Alcohol Alcohol 2016; 51 (3): 275–280.

88. Bakhireva LN, Leeman L, Savich RD et al. The validity of phosphatidylethanol in dried blood spots of newborns for the identification of prenatal alcohol exposure. Alcohol Clin Exp Res 2014; 38 (4): 1078–1085.

89. Parker SP, Cubitt WD. The use of the dried blood spot sample in epidemiological studies. J Clin Pathol 1999; 52: 633–639.

90. Uzicanin A, Lubega I, Nanuynja M et al. Dried blood spots on filter paper as an alternative specimen for measles diagnostics: detection of measles immunoglobulin M antibody by a commercial enzyme immunoassay. J Infect Dis 2011; 204 (1): 564–569.

91. Hardelid P, Williams D, Dezateux C et al. Agreement of rubella IgG antibody measured in serum and dried blood spots using two commercial enzyme-linked immunosorbent assays. J Med Virol 2008; 80 (2): 360–364.

92. Sarkar S, Singh MP, Ratho RK. Dried blood spot for Ebola testing in developing countries. Lancet Infect Dis 2015; 15 (9): 1005.

93. Toledo AC Jr, Januario JN, Rezende RM et al. Dried blood spots as a practical and inexpensive source for human immunodeficiency virus and hepatitis C virus surveillance. Mem Inst Oswaldo Cruz 2005; 100 (4): 365–370.

94. Rodriguez-Auad JP, Rojas-Montes O, Maldonado-Rodriguez A et al. Use of dried plasma spots for HIV-1 viral load determination and drug resistance genotyping in mexican patients. Biomed Res Int 2015; 2015: 1–9.

95. Kolocouri F, Dotsikas Y, Loukas YL. Dried plasma spots as an alternative sample collection technique for the quantitative LC-MS/MS determination of gabapentin. Anal Bioanal Chem 2010; 398 (3): 1339–1347.

96. Parker SL, Lipman J, Dimopoulos G, Roberts JA, Wallis SC. A validated method for the quantification of fosfomycin on dried plasma spots by HPLC-MS/MS: Application to a pilot pharmacokinetic study in humans. J Pharm Biomed Anal 2015; 115: 509–514.

97. Uchikata T, Matsubara A, Fukusaki E, Bamba T. High-throughput phospholipid profiling system based on supercritical fluid extraction–supercritical fluid chromatography/mass spectrometry for dried plasma spot analysis. J Chromatogr A 2012; 1250: 69–75.

98. Suzuki M, Nishiumi S, Kobayashi T et al. Use of on-line supercritical fluid extraction-supercritical fluid chromatography/tandem mass spectrometry to analyze disease biomarkers in dried serum spots compared with serum analysis using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2017; 31 (10): 886–894.

99. Li W, Doherty J, Favara S, Breen C, Flarakos J, Tse FL. Evaluation of plasma microsampling for dried plasma spots (DPS) inquantitative LC-MS/MS bioanalysis using ritonavir as a model compound. J Chromatogr B 2015; 991: 46–52.

100. Forman M, Valsamakis A, Arav-Boger A. Dried urine spots for detection and quantification of cytomegalovirus in newborns. Diagn Microbiol and Infect Dis 2012; 73 (4): 326–329.

101. Carreno Balcazar JS, Meesters RJ. Bioanalytical comparison between dried urine spots and liquid urine bioassays used for the quantitative analysis of urinary creatinine concentrations. Bioanalysis 2014; 6 (21): 2803–2814.

102. Blau N, Matasovic A, Lukasiewicz-Wedlechowicz A, Heizmann CW, Leumann E. Simultaneous determination of oxalate, glycolate, citrate, and sulfate from dried urine filter paper spots in a pediatric population. Clin Chem 1998; 44 (7): 1554–1556.

103. Resano M, Aramendia M, Rello L, Calvo ML, Beraild S, Pecheyrand C. Direct determination of Cu isotope ratios in dried urine spots by means of fs-LA-MC-ICPMS. Potential to diagnose Wilson's disease J Anal At Spectrom 2013, 28 (1): 98–106.

104. Michely JA, Meyer MR, Maurer HH. Dried urine spots – A novel sampling technique for comprehensive LC-MSn drug screening. Anal Chim Acta 2017; 982: 112–121.

105. Jacomelli G, Micheli V, Bernardini G, Millucci L, Santucci A. Quick diagnosis of alkaptonuria by homogentisic acid determination in urine paper spots. JIMD Rep 2017; 31: 51–56.

106. Abdel-Rehim A, Abdel-Rehim M. Dried saliva spot as a sampling technique for saliva samples. Biomed Chromatogr 2014; 28 (6): 875–877.

107. Numako M, Takayama T, Noge I et al. Dried saliva spot (DSS) as a convenient and reliable sampling for bioanalysis: an application for the diagnosis of diabetes mellitus. Anal Chem 2016; 88 (1): 635–639.

108. Zheng N, Zeng J, Ji QC et al. Bioanalysis of dried saliva spot (DSS) samples using detergent-assisted sample extraction with UHPLC-MS/MS detection. Anal Chim Acta 2016; 934: 170–179.

109. Jackson KH, Polreis J, Sanborn L, Chaima D, Harris WS. Analysis of breast milk fatty acid composition using dried milk samples. Int Breastfeed J 2016; 11: 1–7.

110. Rudolph MC, Young BE, Jackson KH, Krebs NF, Harris WS, MacLean PS. Human milk fatty acid composition: comparison of novel dried milk spot versus standard liquid extraction methods. J Mammary Gland Biol Neoplasia 2016; 21 (3–4): 131–138.

111. Olagunju A, Bolaji OO, Amara A et al. Development, validation and clinical application of a novel method for the quantification of efavirenz in dried breast milk spots using LC-MS/MS. J Antimicrob Chemother 2015; 70 (2): 555–561.

112. Kandar R, Drabkova P, Andrlova L, Kostelnik A, Cegan A. Determination of selected fatty acids in dried sweat spot using gas chromatography with flame ionization detection. J Sep Sci 2016; 39 (22): 4377–4383.

113. Lehner AF, Rumbeiha W, Shlosberg A et al. Diagnostic analysis of veterinary dried blood spots for toxic heavy metals exposure. J Anal Toxicol 2013; 37 (7): 406–422.

114. Pedersen L, Andersen-Ranberg K, Hollergaard M, Nybo M. Quantification of multiple elements in dried blood spot samples. Clin Biochem 2017; 50 (12): 703–709.

115. Funk WE, Pleil JD, Sauter DJ, McDade TW, Holl JL. Use of dried blood spots for estimating children's exposures to heavy metals in epidemiological research. J Environ Anal Toxicol 2015; S7: 1–9.

116. Perkins M, Basu N. Dried blood spots for estimating mercury exposure in birds. Environ Pollut 2018; 236: 236–246.

117. Chaudhuri SN, Butala SJ, Ball RW, Braniff CT. Pilot study for utilization of dried blood spots for screening of lead, mercury and cadmium in newborns. J Expo Sci Environ Epidemiol 2009; 19 (3): 298–316.

118. 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. Anal Chem 2009; 81 (5): 1931–1936.

119. Wuyts B, Stove V, Goossens L. Critical sample pretreatment in monitoring dried blood spot citrulline. Clin Chim Acta 2007; 386 (1–2): 105–109.

120. Haghighi F, Talebpour Z, Amini V, Ahmadzadehb A, Farhadpourc M. A fast high performance liquid chromatographic (HPLC) analysis of amino acid phenylketonuria disorder in dried blood spots and serum samples, employing C18 monolithic silica columns and photo diode array detection. Anal Methods 2014; 7: 7560–7567.

121. Gregory CO, Yu C, Singh RH. Blood phenylalanine monitoring for dietary compliance among patients with phenylketonuria: comparison of methods. Genet Med 2007; 9 (11): 761–765.

122. Bergwerff CE, Luman M, Blom HJ, Oosterlaan J. No tryptophan, tyrosine and phenylalanine abnormalities in children with attention-deficit/hyperactivity disorder. PLoS One 2016; 11 (3): 1–13.

123. Bartl J, Chrastina P, Krijt J, Hodik J, Peskova K, Kozich V. Simultaneous determination of cystathionine, total homocysteine, and methionine in dried blood spots by liquid chromatography/tandem mass spectrometry and its utility for the management of patients with homocystinuria. Clin Chim Acta 2014; 437: 211–217.

124. Kandar R, Zakova P, Jirosova J, Sladka M. Determination of branched chain amino acids, methionine, phenylalanine, tyrosine and alpha-keto acids in plasma and dried blood samples using HPLC with fluorescence detection. Clin Chem Lab Med 2009; 47 (5): 565–572.

125. Gupta R, Abraham RA, Dhatwalia S, Ramakrishnan L, Prabhakaran D, Reddy KS. Use of dried blood for measurement of trans fatty acids. Nutr J 2009; 8: 35.

126. Mashavave G, Kuona P, Tinago W, Stray-Pedersen B, Munjoma M, Musarurwa C. Dried blood spot omega-3 and omega-6 long chain polyunsaturated fatty acid levels in 7-9 year old Zimbabwean children: a cross sectional study. BMC Clin Pathol 2016; 16: 14.

127. Bell JG, Mackinlay EE, Dick JR, Younger I, Lands B, Gilhooly T. Using a fingertip whole blood sample for rapid fatty acid measurement: method validation and correlation with erythrocyte polar lipid compositions in UK subjects. Br J Nutr 2011; 106 (9): 1408–1415.

128. Ingels AS, De Paepe P, Anseeuw K et al. Dried blood spot punches for confirmation of suspected γ-hydroxybutyric acid intoxications: validation of an optimized GC-MS procedure. Bioanalysis 2011; 3 (20): 2271–2281.

129. Sarter B, Kelsey KS, Schwartz TA, Harris WS. Blood docosahexaenoic acid and eicosapentaenoic acid in vegans: Associations with age and gender and effects of an algal-derived omega-3 fatty acid supplement. Clin Nutr 2015; 34 (2): 212–218.

130. Johnson DW. A rapid screening procedure for the diagnosis of peroxisomal disorders: quantification of very long-chain fatty acids, as dimethylaminoethyl esters, in plasma and blood spots, by electrospray tandem mass spectrometry. J Inherit Metab Dis 2000; 23 (5): 475–486.

131. Bailey-Hall E, Nelson EB, Ryan AS. Validation of a rapid measure of blood PUFA levels in humans. Lipids 2008; 43 (2): 181–186.

132. Ironson G, Banerjee N, Fitch C, Krause N. Positive emotional well-being, health Behaviors, and inflammation measured by C-Reactive protein. Soc Sci Med 2018; 197: 235–243.

133. Stinca S, Andersson M, Weibel S et al. Dried blood spot thyroglobulin as a biomarker of iodine status in pregnant women. J Clin Endocrinol Metab 2017; 102 (1): 23–32.

134. Rosting C, Gjelstad A, Halvorsen TG. Water-soluble dried blood spot in protein analysis: a proof-of-concept study. Anal Chem 2015; 87 (15): 7918–7924.

135. Rosting C, Tran EV, Gjelstad A, Halvorsen TG. Determination of the low-abundant protein biomarker hCG from dried matrix spots using immunocapture and nano liquid chromatography mass spectrometry. J Chromatogr B 2018; 1077–1078: 44–51.

136. Chambers AG, Percy AJ, Yang J, Borchers CH. Multiple reaction monitoring enables precise quantification of 97 proteins in dried blood spots. Mol Cell Proteomics 2015; 14 (11): 3094–3104.

137. Colson KE, Potter A, Conde-Glez C et al. Use of a commercial ELISA for the detection of measles-specific immunoglobulin G (IgG) in

dried blood spots collected from children living in low-resource settings. J Med Virol 2015; 87 (9): 1491–1499.

138. Zheng Q, Abernathy ES, Sun H et al. Genotyping of rubella virus RNA in sera and dried blood spots collected during routine surveillance and in archival sera. J Virol Methods 2013; 187 (2): 284–287.

139. Prado I, Rosario D, Bernardo L et al. PCR detection of dengue virus using dried whole blood spotted on filter paper. J Virol Methods 2005; 125 (1): 75–81.

140. Parry CM, Parkin N, Diallo K et al. Field study of dried blood spot specimens for HIV-1 drug resistance genotyping. J Clin Microbiol 2014; 52 (8): 2868–2875.

141. Canier L, Khim N, Kim S et al. Malaria PCR detection in Cambodian low-transmission settings: dried blood spots versus venous blood samples. Am J Trop Med Hyg 2015; 92 (3): 573–577.

142. Petrara MR, Penazzato M, Massavon W et al. Epstein–Barr virus load in children infected with human immunodeficiency virus type 1 in Uganda. J Infect Dis 2014; 210 (3): 392–399.

143. Mahajan S, Choudhary MC, Kumar G, Gupta E. Evaluation of dried blood spot as an alternative sample collection method for hepatitis C virus RNA quantitation and genotyping using a commercial system. Virus Dis 2018: 1–6.

144. Bennett S, Gunson RN, McAllister GE et al. Detection of hepatitis C virus RNA in dried blood spots. J Clin Virol 2012; 54 (2): 106–109.

145. Iroh Tam PY, Hernandez-Alvarado N, Schleiss MR et al. Molecular detection of Streptococcus pneumoniae on dried blood spots from febrile Nigerian children compared to culture. PLoS One 2016; 11 (3): 1–12.

146. Beck O, Moden NK, Seferaj S, Lenk G, Helander A. Study of measurement of the alcohol biomarker phosphatidylethanol (PEth) in dried blood spot (DBS) samples and application of a volumetric DBS device. Clin Chim Acta 2018; 479: 38–42.

147. Berm EJJ, Paardekooper J, Brummel-Mulder E, Hak E, Wilffert B, Maring JG. A simple dried blood spot method for therapeutic drug monitoring of the tricyclic antidepressants amitriptyline, nortriptyline, imipramine, clomipramine, and their active metabolites using LC-MS/MS. Talanta 2015; 134: 165–172.

148. Hooff GP, Meesters RJ, van Kampen JJ et al. Dried blood spot UHPLC-MS/MS analysis of oseltamivir and oseltamivircarboxylate–a validated assay for the clinic. Anal Bioanal Chem 2011; 400 (10): 3473–3479.

149. Parsons TL, Marzinke MA, Hoang T et al. Quantification of rifapentine, a potent antituberculosis drug, from dried blood spot samples using liquid chromatographic-tandem mass spectrometric analysis. Antimicrob Agents Chemother 2014; 58 (11): 6747–6757.

150. De Niz M, Eziefula AC, Othieno L et al. Tools for mass screening of G6PD deficiency: validation of the WST8/1-methoxy-PMS enzymatic assay in Uganda. Malar J 2013; 12: 210.

151. Alcalay RN, Wolf P, Levy OA et al. Alpha galactosidase A activity in Parkinson's disease. Neurobiol Dis 2018; 112: 85–90.

152. Angelico F, Corradini SG, Pastori D et al. Severe reduction of blood lysosomal acid lipase activity in cryptogenic cirrhosis: A nationwide multicentre cohort study. Atherosclerosis 2017; 262: 179–184.

Received August 20, 2018.
Accepted January 9, 2019.