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How and when to use dried blood spots in psychoneuroendocrinological research

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ABSTRACT

Objective: The term "dried blood spot" (DBS) refers to a sampling technique in which capillary whole blood is spotted on filter paper. Given the possibility to determine a wide range of hormones and related biomarkers in DBS, the method should be of interest to researchers in psychoneuroendocrinology. So far, however, the how and when of using DBS in this context have not been outlined.

Methods: A review of the literature was conducted in order to describe the materials and procedures necessary to determine relevant biological markers from DBS (how to use DBS). In addition, a comparison of the DBS method with other sampling techniques was undertaken and examples of its previous use in psychoneuroendocrinology were provided (when to use DBS).

Results: Both dyadic and DBS self-sampling are feasible, and a number of protocols are available to determine endocrine and immune, genetic and epigenetic markers. Decisions to use DBS instead of venous blood or saliva sampling should mainly be guided by whether it is sensible and feasible to determine the parameter of interest in whole blood obtained from DBS. In addition, DBS are well suited for large study populations with specific vulnerabilities, and for remotely located studies with budgetary constraints.

Conclusion: Dried blood spots are a promising material as well as a simple sampling technique for psychoneuroendocrinological research. Future efforts should be directed at continuing to adapt existing serum and plasma analysis protocols for use with DBS, and at testing the feasibility of DBS self-sampling in field studies.

1. Introduction

How is it possible to determine whether a newborn infant is suffering from phenylketonuria by obtaining a single drop of blood from the infant's heel? The term "dried blood spot" (DBS) refers to a sampling technique in which capillary whole blood is spotted on filter paper. More than 100 years ago, the physician Bang developed DBS as a technique to determine glucose levels (Schmidt, 1986). Fifty years later, Guthrie and Susi (1963) began to use DBS for the above-outlined purpose: By providing evidence that phenylalanine assayed from DBS was highly sensitive in detecting phenylketonuria, they laid the groundwork for population-based newborn screening for markers of various diseases. Outside the field of newborn screening, biological anthropologists also began to use DBS. For instance, Worthman and Stallings (1994, 1997) developed assays to quantify various hormones from DBS in order to be able to expand their research to remote locations and populations. Today, DBS collection and analysis is used in a wide range of areas, including newborn screening for congenital diseases, infectious disease management, and therapeutic drug monitoring. Given the possibility to determine a wide range of hormones and related biomarkers in DBS, the method should be of great interest to the psychoneuroendocrinological scientific community. However, to date, there has been no review of the literature pointing out how and when DBS are best used in psychoneuroendocrinological studies.

The aim of this review was to support researchers in psychoneuroendocrinology in a) deciding whether they should use DBS or other tissues (e.g., saliva) to measure relevant biological parameters (i.e., when to use DBS), and b) in optimally implementing the methodology in their studies (i.e., how to use DBS). To this end, the present review will start out by describing the necessary materials for DBS sampling and the general sampling procedures, and will give an overview of parameters for which validated DBS assays exist. It will continue by comparing the DBS method with conventional methods for biological sampling (i.e., venous blood sampling, saliva sampling) and by providing examples of DBS use in psychoneuroendocrinological research. The findings will be summarized and integrated in the final part of the

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review.

2. How to use dried blood spots

2.1. Materials

In general, two main objects are necessary to conduct DBS sampling: a lancing device to puncture participants' skin, and filter paper to collect the blood drops. In terms of the lancing device, larger lancets with blades are preferable to lancets with needles, with the latter being commonly used by patients with diabetes. However, both blades and needles work if researchers or participants have been sufficiently instructed on how to perform DBS sampling. Of greater importance is that the lancing device is sterilized and single-use (e.g., Microtainer® contact-activated lancets; BD, Franklin Lakes, USA). In terms of the filter paper, Whatman® 903 protein saver cards (GE Healthcare, Cardiff, UK) and PerkinElmer 226 spot saver cards (PerkinElmer, Waltham, USA) are most commonly used, both of which are approved as class II medical devices by the US Food and Drug Administration (FDA). These papers are made of pure cellulose and are usually marked with four or five circles of 12 mm diameter, each allowing for approximately 50 µl of capillary whole blood to be spotted on for later punching and processing. Complementary materials include 70% isopropyl alcohol wipes, sterile gauze, and plasters for sample collection, and desiccant bags, impermeable plastic bags, and secondary containers for sample transportation and storage.

2.2. Procedures

Protocols for collecting DBS samples have been provided by the Clinical and Laboratory Standard Institute (CLSI, 2013) and by the World Health Organization (WHO, 2012). In addition, good blood-spotting practices have been formulated by the European Bioanalysis Forum (Timmerman et al., 2013, 2011), and several researchers have provided step-by-step instructions for DBS collection (e.g., Gruner et al., 2015; Ostler et al., 2014). A consolidation of these recommendations for researchers can be found in Table 1. In brief, participants' fingers are pricked with a lancing device and the first drop of blood is swiped off, while the following drops are applied to the filter paper to fill a prespecified number of circles. The paper is then dried for four hours and stored in small plastic bags with a desiccant until shipment to the laboratory.

In the case of self-sampling, participants can be provided with instructions via a face-to-face demonstration, video tutorials posted on laboratory or study websites (see e.g., https://www.psychologie.uzh.ch/de/bereiche/hea/klipsypt/labor/Dried-Blood-Spots.html), or written instructions. Ideally, written instructions are accompanied by illustrations. Table 2 provides an example of written instructions for self-sampling and Fig. 1 contains the accompanying illustrations.

Samples are shipped to the laboratory in the same plastic bags with a desiccant and sealed within a secondary container (e.g., an envelope). Interim storage at room temperature or in 4 °C refrigerators is recommended for no longer than one week (see 2.4 Analysis for exceptions), and long-term storage is recommended at −20 °C or −80 °C (Gruner et al., 2015; Ostler et al., 2014).

2.3. Processing

Before processing, visual inspection of samples is recommended to check that blood is symmetrically spread around the center and on both sides of the filter paper, as otherwise smearing or blotting may have occurred. Next, 3-8-mm diameter discs are cut from DBS by means of a circular punch. This can be done manually (i.e., using a hole punch) or automatically (e.g., using a DBS® puncher; PerkinElmer, Waltham, USA). A typical 12-mm diameter DBS will contain approximately 50 µl of whole blood and yield up to seven punched discs if a 3.2-mm diameter punch is being used. The discs are then placed in an elution solvent for extraction before biochemical analysis.

Table 1
Instructions for dried blood spot collection; researcher version (dyadic sampling).

1	Let the participant warm his/her own hands by clenching or shaking them, rubbing them together, or soaking them in warm water, or gently massage the participant's hands for him/her.
2	Prior to the blood collection, avoid any contact with the target site of the filter paper and wear disposable rubber gloves throughout the procedure.
3	Clean the tip distal phalanx of the participant's middle finger of the non-dominant hand with 70% isopropyl alcohol. Let the fingertip dry for at least ten seconds to avoid any contamination with alcohol.
4	Tighten the center of the fingertip with your thumb and index finger and puncture it with the lancing device by pressing it firmly against the fingertip and pushing the button.
5	Discard the first blood drop with a gauze pad to remove the interstitial fluid from the sample.
6	Apply constant pressure to the finger and allow a second drop to pool on the participant's finger.
7	Position the filter paper below the finger and let the drop fall from its own weight into the first circle. Do not let the finger touch the paper as this may cause smearing; however, the paper may be carefully brought to the blood drop for collection.
8	Massage the finger in an anterograde direction if necessary. Let the next blood drop fall into the second circle. Each circle should be filled with a single drop of blood only and spots should not overlap between circles.
9	Continue and fill the circles as quickly as possible to avoid clotting. If blood flow stops, repeat skin puncture on the ring or index finger. If it is not possible to fill all circles completely, collect one or two large drops of blood rather than several small ones.
10	Place a plaster on each punctured fingertip.
11	Place the filter paper horizontally on a non-absorbent surface and let it air dry for four hours at room temperature, out of direct sunlight.
12	Place the dry filter paper in a sealable plastic bag together with a desiccant to avoid bacterial growth.

Table 2
Instructions for dried blood spot collection; participant version (self-sampling).

1	Warm your hands by clenching them, shaking them, rubbing them together, soaking them in warm water, or by massaging them.
2	Before taking blood, do not touch the circles on the filter paper.
3	Clean the tip of the middle finger of your non-writing hand with the alcohol wipe. For most people, this will be the left hand. Let the fingertip dry for at least ten seconds before you continue.
4	Put your non-writing arm on an even surface. Use your other hand to prick the center of the fingertip with the needle by pressing it firmly against the fingertip and pushing the button.
5	Wipe off the first blood drop with the gauze.
6	Milk the finger with your other hand from palm to tip. Keep the pressure until a large drop appears on your finger.
7	Put the filter paper right below the finger and let the drop fall from its own weight into the first circle. Do not let the finger touch the paper. However, you may carefully bring the paper to the blood drop if necessary.
8	Milk the finger from palm to tip until another blood drop appears. Let this drop fall into the second circle. Each circle should be filled with one drop of blood only. Make sure that spots do not overlap between circles.
9	Continue and fill the circles as quickly as possible. If blood stops flowing, prick another fingertip. Use your ring or index finger and a new needle for this. If you cannot fill all circles, it is better to have one or two large circles rather than several small ones.
10	Put a plaster on each used fingertip.
11	Put the filter paper on a plastic, glass or metallic surface and let it air dry for four hours at room temperature. Keep it out of direct sunlight.
12	Put the dry filter paper in the plastic bag together with the desiccant bags.

2.4. Analysis

Similar to venous blood or saliva sampling, two general approaches can be taken to analyze endocrine and immune parameters from DBS: immunoassays or chromatography combined with mass spectrometry. Several DBS immunoassays for measuring endocrine and immune markers in adults have been developed and validated over the past

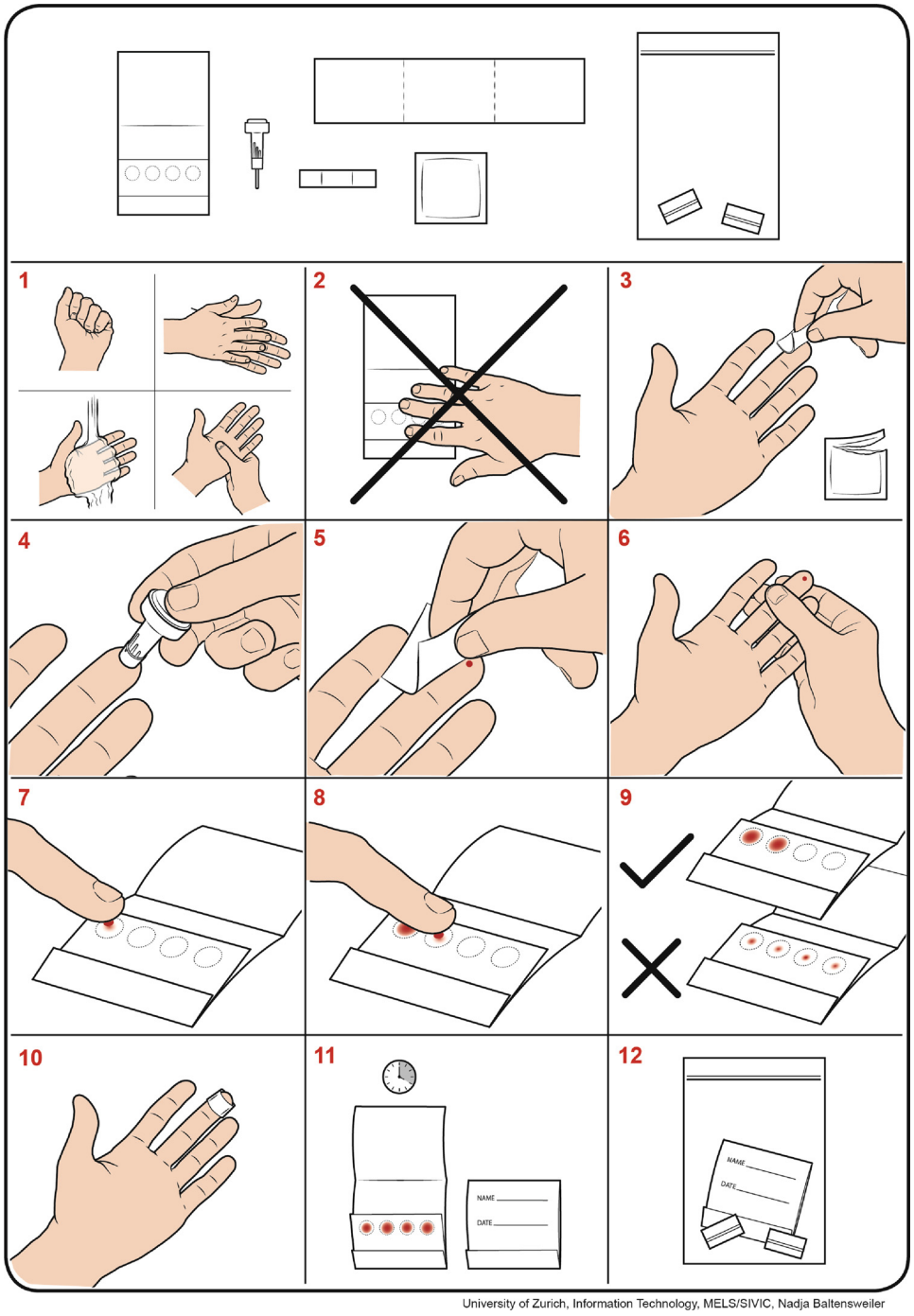

Fig. 1. Instructions for dried blood spot collection; accompanying illustrations.

decades (see also McDade, 2014). Protocols for endocrine markers, which are relevant to psychoneuroendocrinological research, are listed in Table 3. They use either radioimmunoassays, fluoroimmunometric assays, or enzyme assays, depending on the date of publication. There is a wide range of precision and reliability estimates; the best intra- and inter-assay variabilities are observed with anti-Müllerian hormone, leptin, and thyroxine (all coefficients < 10%), while insulin in particular appears to show high coefficients of variation (intra-assay: 14%, inter-assay: 25%). Stability seems to be highly dependent on the parameter under study: Storage at room temperature may occur for periods between three days and eight weeks, and storage at 4 °C may occur for between two and eight weeks. Correlation with serum or plasma values are high for almost all parameters (average r = 0.96). In terms of immune markers, highly sensitive protocols to analyze interleukin 6 (IL-6; Miller and McDade, 2012) and C-reactive protein (CRP; Brindle et al., 2010; Lacher et al., 2013; McDade et al., 2004) have been published. Notably, while in general, the precision, reliability and consistency of these protocols are satisfactory, they are lower for IL-6 than for CRP. In addition, a DBS protocol relying on a multiplex assay has recently been developed for use in adult populations (Skogstrand et al., 2008), although its validation is still pending.

Contrary to the abundance of DBS-adapted immunoassays, the development of DBS protocols for chromatography combined with mass spectrometry has been limited in psychoneuroendocrinological research. The use of these methods is likely to increase due to their high specificity and ever-increasing sensitivity. Antunes et al. (2016)

Table 3
Available immunoassay protocols for analyzing endocrine parameters from dried blood spots in adults.

Parameter	Type of assay	Precision and reliability	Stability	Consistency	Reference
Anti-Müllerian hormone	Enzyme immunoassay	Intra-assay variance: 5–7% Inter-assay variance: 4–7%	Storage at room temperature: 2 weeks Storage at 4 °C: 4 weeks	Correlation with serum: r = 0.97	McDade et al. (2012)
Cortisol	Enzyme immunoassay	Intra-assay variance: 4–7% Inter-assay variance: 6–13%	Not reported	Correlation with serum: r = 0.91	Konishi et al. (2012)
	Radioimmunoassay	Intra-assay variance: 6–12% Inter-assay variance: 6–13%	Storage at room temperature: 4 weeks Storage at 4 °C: 8 weeks	Correlation with plasma: r = 0.93	Worthman and Stallings (1997)
Dehydroepiandrosterone sulfate	Radioimmunoassay	Intra-assay variance: 7–9% Inter-assay variance: 12–14%	Storage at room temperature: 4 weeks Storage at 4 °C: 8 weeks	Correlation with plasma: r = 0.99	Worthman and Stallings (1997)
Estradiol	Enzyme immunoassay	Intra-assay variance: < 10% Inter-assay variance: 10–22%	Not reported	Correlation with plasma: r = 0.84	Edelman et al. (2007)
	Radioimmunoassay	Intra-assay variance: 2–16% Inter-assay variance: 8–10%	Not reported	Correlation with serum: r = 0.96	Shirtcliff et al. (2001)
	Radioimmunoassay	Intra-assay variance: 4–14% Inter-assay variance: 12–14%	Storage at room temperature: 3 weeks Storage at 4 °C: 8 weeks	Correlation with plasma: r = 0.98	Worthman and Stallings (1997)
Follicle-stimulating hormone	Fluoroimmunometric assay	Intra-assay variance: 6–16% Inter-assay variance: 6–16%	Not reported	Correlation with plasma: r = 0.95	Edelman et al. (2007)
	Fluoroimmunometric assay	Intra-assay variance: 5–10% Inter-assay variance: 6–9%	Storage at room temperature: 8 weeks Storage at 4 °C: 8 weeks	Correlation with plasma: r = 0.98	Worthman and Stallings (1997)
Insulin	Radioimmunoassay	Intra-assay variance: 14% Inter-assay variance: 25%	Not reported	Correlation with plasma: r = 0.96	Dowlati et al. (1998)
Leptin	Enzyme immunoassay	Intra-assay variance: 4–6% Inter-assay variance: 5–8%	Storage at room temperature: 3 days Storage at 4 °C: 14 days	Correlation with plasma: r = 0.98	Miller et al. (2006)
Luteinizing hormone	Fluoroimmunometric assay	Intra-assay variance: 7–18% Inter-assay variance: 7–18%	Not reported	Correlation with plasma: r = 0.96	Edelman et al. (2007)
	Fluoroimmunometric assay	Intra-assay variance: 4–11% Inter-assay variance: 7–12%	Storage at room temperature: 8 weeks Storage at 4 °C: 8 weeks	Correlation with plasma: r = 0.98	Worthman and Stallings (1997)
Progesterone	Fluoroimmunometric assay	Intra-assay variance: < 10% Inter-assay variance: 10–19%	Not reported	Correlation with plasma: r = 0.91	Edelman et al. (2007)
	Enzyme immunoassay	Intra-assay variance: 5–7% Inter-assay variance: 12–17%	Not reported	Correlation with serum: r = 0.99	Konishi et al. (2012)
	Radioimmunoassay	Intra-assay variance: 6–13% Inter-assay variance: 9–17%	Not reported	Correlation with serum: r = 0.98	Shirtcliff et al. (2001)
Prolactin	Fluoroimmunometric assay	Intra-assay variance: 3–11% Inter-assay variance: 6–8%	Storage at room temperature: 3 weeks Storage at 4 °C: 8 weeks	Correlation with plasma: r = 0.99	Worthman and Stallings (1997)
Testosterone	Radioimmunoassay	Intra-assay variance: 5–7% Inter-assay variance: 6–7%	Not reported	Correlation with serum: r = 0.86	Shirtcliff et al. (2002)
	Radioimmunoassay	Intra-assay variance: 7–8% Inter-assay variance: 12–14%	Storage at room temperature: 3 weeks Storage at 4 °C: 8 weeks	Correlation with plasma: r = 0.98	Worthman and Stallings (1997)
Thyroxine (fT4)	Radioimmunoassay	Intra-assay variance: 4–9% Inter-assay variance: 9%	Not reported	Correlation with serum: r = 0.97	Pacchiarotti et al. (1988)
	Enzyme immunoassay	Intra-assay variance: 6–9% Inter-assay variance: 6–7%	Storage at room temperature: 4 weeks	Correlation with serum: r = 0.98	Hata et al. (1985)
Triiodothyronine (fT3)	Radioimmunoassay	Intra-assay variance: 9–10% Inter-assay variance: 9–10%	Not reported	Correlation with serum: r = 0.96	Pacchiarotti et al. (1988)

recently summarized current recommendations on how to conduct validation studies when intending to use liquid chromatography combined with mass spectrometry (LC–MS/MS) to analyze DBS. However, a DBS protocol for use in adults has so far only been developed for cortisol, and while its precision and reliability appear satisfactory, a comparison with cortisol obtained from serum or plasma samples is still outstanding (Kim et al., 2015).

Finally, DBS protocols suitable for adults have also been published for whole-genome (Poulsen et al., 2016) and –methylome analyses (Hollegaard et al., 2013). In addition, McDade et al. (2016) have developed and validated a DBS-based transcriptome analysis protocol. Finally, protocols to analyze telomere length have recently been adapted for DBS (Stout et al., 2017; Zanet et al., 2013). While preliminary evidence for the validity of these protocols has been provided by means of comparison with DNA extracted from venous whole blood or peripheral blood mononuclear cells (PBMCs), the sample sizes of these studies were small and more large-scale replication studies are thus warranted.

2.5. Excursion: blood volume, hematocrit, and chromatographic effects

One issue to be considered during assay development and validation are blood volume effects (see also Wagner et al., 2016). Generally, it has been observed that the higher the blood volume, the higher parameter estimates, which most likely is due to greater paper saturation. Another issue to consider is the hematocrit, which refers to the proportion of whole blood that is made up of erythrocytes. A higher hematocrit results in comparably less spreading of blood on the filter paper and thus in more concentrated DBS punches (i.e., they will contain larger volumes of blood), while the proportion of plasma is at the same time lower. This affects the measurable concentration of the parameter of interest. Finally, chromatographic effects refer to the heterogeneous spreading of blood on the filter paper. As a result, the proportion of plasma in a punched disc from the center of a DBS circle differs from a punched disc from the periphery, meaning that comparably higher parameter concentrations are observed in discs from the edges of DBS (this is sometimes referred to as the coffee stain or volcano effect).

A number of sampling materials have been developed over the last few years to avoid such effects in the first place. These can be used as an alternative to the conventional lancing devices and filter papers mentioned above. As most recently summarized by Enderle et al. (2016), three main strategies can be distinguished. The first strategy is to analyze whole DBS rather than punches taken from whole DBS. This requires volumetric sampling, that is, taking measured amounts of blood. The second strategy is to use special filter papers. This includes fan-shaped filter papers, which are composed of several blades and enforce an even distribution of blood on the paper. As a third strategy, capillary blood can be centrifuged for plasma to be spotted onto filter paper to create dried plasma spots (DPS). An alternative solution is provided by special filter papers, where the blood cells are filtered out through a porous membrane and the plasma diffuses on a pre-cut disc one layer below.

However, given that in most studies to date, conventional sampling as outlined above is performed, sample volume, hematocrit, and chromatographic effects are taken into account during the development and validation of assays for DBS analysis (Jager et al., 2014). The development and validation of such assays is a time-consuming and costly matter. It includes tests of whether different ranges of spot volumes, hematocrit levels, and spot homogeneity affect assay performance, with 15% of variance in parameter concentrations still being considered acceptable. This is usually done at two different levels of analyte concentration (low and high), and in triplicates. Luckily, all three effects appear to be negligible for most parameters that have been determined in DBS to date. In addition, spot-to-spot carry-over effects, extraction recovery, matrix effects, transport and storage stability, and dilution integrity need to be evaluated see (see Jager et al., 2014 for more specific recommendations on how to conduct such experiments). Importantly, all DBS protocols should ultimately be validated against concentrations obtained from serum or plasma samples, and researchers are well-advised to ask about assay validation procedures when employing the services of biochemical laboratories.

3. When to use dried blood spots

3.1. Advantages of dried blood spot sampling

The most frequently employed sampling methods in psychoneuroendocrinological research are venous blood sampling and saliva sampling. Dried blood spot sampling has several advantages over these methods. When compared to venous blood sampling, first, one of the most obvious differing features of DBS sampling is its low invasiveness. As a consequence, individuals may have less fear of pain and the perceived risks may be lower, which in turn may increase study participation rates. Second, no trained phlebotomists are required, enabling the possibility of self-sampling, thus reducing the costs substantially. Third, DBS sampling permits blood collection from a broader range of subjects who are not eligible for venous blood collection, such as children, elderly people, or patients suffering from vascular diseases. This enhances the representativeness of study samples. A fourth advantage of the DBS method is the low risk of infection, since after drying, several viruses (e.g., hepatitis C, HIV) are destroyed (e.g., Lehmann et al., 2013). This reduces the contamination risk for study personnel when handling samples. Fifth, the solid state of DBS also implies that no cold chain is necessary during transport via mail, which facilitates sample handling for staff and participants and is more economical. Sixth, and still related to this, most compounds are highly stable in DBS (however, please refer to Table 3 for more specific information on this issue). This enables long-term storage, which is ideal for longitudinal studies and allows for re-analyses of old samples when new methods or parameters emerge. Finally, due to the small sampling volumes, shipping is facilitated and only little storage space is warranted, which further reduces costs.

When compared to saliva sampling, one important advantage of DBS sampling is the fact that oral health does not play into sample quality. Thus, acute local infections or injuries at the time of sampling do not have to be considered, unless they are relevant to the research in question. This may result in fewer participant dropouts. A second advantage lies in the posting of samples via mail, which is more convenient with DBS, since the solid state lowers the risk of leaking and several filter papers may be shipped simultaneously within the same envelope. In larger studies, this may also reduce transport costs and costs for storage space, which are arguably higher with salivary tubes (e.g., Salivettes®; Sarstedt, Nümbrecht, Germany). A final advantage of DBS sampling is the fact that in psychoneuroendocrinological research, blood is still the preferred tissue for the determination of several biomarkers. This is particularly true of immune markers, for several of which highly sensitive salivary assays are not yet available. Moreover, with certain genetic and epigenetic markers, depending on the research question, there may be a preference for DNA to be extracted from whole blood, which precludes the use of saliva samples.

3.2. Disadvantages of dried blood spot sampling

When compared to venous blood sampling, the low sampling volumes obtained via DBS do not allow for a large number of parameters to be determined at the same time. The advent of multiplexing methods has somewhat alleviated this issue, and even small amounts of DNA can now be amplified; however, the maximum amount of whole blood available per participant is still only 200 to 250 µl, and researchers are thus advised to carefully check the minimum amounts of blood necessary to analyze all parameters of interest. Second, while a large number of assays to determine endocrine and immune markers are available for

plasma and serum samples, the number of assays that were adapted for whole blood as obtained from DBS is still limited, and validation in adult samples is sparse (see 2.4 Analysis). This means that researchers may have to rely on additional serum or plasma samples to determine all parameters of interest, especially if they are interested in comparing the obtained values with those of a reference population.

When compared to saliva sampling, the materials required for DBS are still more expensive (e.g., lancing devices, filter paper, and complementary materials together cost more than e.g. Salivettes®). Second, DBS sampling is invasive, if only minimally. Thus, potential dropouts due to oral health issues need to be weighed against potential dropouts due to fear of needles or blood. A third, related aspect is sampling feasibility, with the collection procedures to obtain DBS being arguably more complex when compared to saliva samples. It is therefore crucial to provide researchers and participants with clear instructions in order to avoid problems such as contamination, insufficient amounts of blood, smearing, or overlapping spots, which may otherwise compromise sample quantity and quality (see Tables 1 and 2 and Fig. 1 or https://www.psychologie.uzh.ch/de/bereiche/hea/klipsypt/labor.html for instructions).

3.3. Examples of previous use in psychoneuroendocrinological research

When reviewing the psychoneuroendocrinological literature, there are three aspects which appear to guide researchers' decision to choose DBS over venous or saliva sampling. First and foremost, the decision can simply be based on the *parameter* of interest. To illustrate this: In the Women 40+ Healthy Aging Study (Mernone et al., 2019), which comprised 130 healthy middle-aged and elderly women, we investigated how hypothalamic-pituitary-gonadal (HPG) axis functioningwas related to healthy aging. Since the HPG axis was to be studied as comprehensively as possible, it was necessary to consider epigenetic, central, and peripheral indicators of HPG axis integrity. For this purpose, DBS sampling turned out to be the method of choice, since it allowed us not only to extract DNA from whole blood to determine methylation levels of genes encoding estrogen receptors, but also to measure gonadal steroids, follicle-stimulating hormone, and luteinizing hormone from the same tissue, the latter two of which cannot be assayed from saliva. The fact that all women agreed to participate in DBS sampling (which in this case was dyadic) and that 100% of samples were of sufficiently high quantity and quality for subsequent analysis indicates that DBS is an interesting method when the chosen parameters render whole blood the tissue of choice, the sample volume (max. 200 µl in this case) is high enough to determine all parameters of interest, and when validated DBS assays exist.

A second factor contributing to the decision to use DBS sampling is the study *population*. An exemplary study in this regard is the National Social Life, Health, and Aging project, which among other measures aimed to assess CRP in a sample of nearly 2000 older individuals (Williams and McDade, 2009). Again, inflammatory parameters are preferably determined in blood, due to potential confounding with local infections in the oral cavity or gum bleeding when relying on saliva samples. In addition, DBS are particularly well suited for large populations and may also alleviate the issue of elderly people being prone to hematoma during venipuncture. According to the authors, 85% of their sample agreed to dyadic DBS sampling. Of the obtained samples, 99% were usable in that at least one punched disc (3.2 mm) was available, 89% were acceptable in that the sample yielded at least four punched discs, 56% were good in that at least ten discs were harvested, and 14% were excellent in allowing punching of at least 20 discs. These findings indicate that DBS sampling is a useful alternative to saliva sampling in large-scale studies in vulnerable populations, such as elderly people, children, or patients with vascular, viral, or oral diseases, provided that the proportion of participants with blood-injection-injury phobia is not expected to be significantly elevated (e.g., in patients with mental disorders).

Third, in addition to the study parameters and population, a decision may be influenced by the available *resources*. An example of this can be found in the Fertility Information Research Study, which aimed at assessing fertility by means of anti-Müllerian hormone in a geographically diverse population of 163 young female cancer survivors after undergoing gonadotoxic treatments (Roberts et al., 2016). The researchers were precluded from using saliva sampling since anti-Müllerian hormone cannot be determined in saliva. Moreover, they were faced with a large sample size, and their study population was to be recruited remotely and from numerous different sites. Dried blood spot sampling is particularly useful in such a setting, as no trained phlebotomists and no immediate access to facilities for sample storage and analysis are required. All women were therefore sent written instructions on how to collect DBS samples at home, which were later used to determine anti-Müllerian hormone. Of the initial sample, 75% ultimately provided written informed consent, and 91% of these provided DBS of adequate quantity and quality for endocrine analysis. Dried blood spot sampling is therefore a worthwhile method for any remotely located study relying on self-sampling (e.g., epidemiological, ambulatory assessment), or for studies planning a long-term storage of samples.

4. Conclusion

The aim of the present review was to provide an overview of the DBS method and its application in psychoneuroendocrinology. In sum, both dyadic and DBS self-sampling are feasible when adequate instructions are applied, and an ever-increasing number of protocols has been developed to determine endocrine and immune, and – more recently – genetic and epigenetic markers from DBS. The advantages of DBS over venous blood or saliva sampling mainly result from the tissue that is collected (whole blood), the low sampling volumes, and their solid state. These advantages need to be weighed against the challenge of adequate sample collection, especially when self-sampling is being performed. Decisions to use DBS instead of venous blood sampling or saliva sampling should mainly be guided by the parameter of interest, that is, whether it is sensible and feasible to determine it in whole blood as obtained from DBS. In addition, DBS are well suited for large study populations and study populations with specific vulnerabilities, such as individuals with certain vascular, viral, or oral diseases. Similarly, field studies and studies with budgetary constraints in terms of study personnel or laboratory facilities may benefit from using the DBS method. With respect to future research, efforts should be directed at adapting and validating existing serum and plasma analysis protocols for use with DBS, especially in terms of immune, genetic, and epigenetic markers. Moreover, feasibility testing of dyadic DBS sampling in experimental studies and self-sampling in field studies (e.g., ambulatory assessment studies) should be continued. Together, these efforts should enhance the utility and applicability of this promising method within psychoneuroendocrinological research.

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Declaration of Competing Interest

None.

Author contributions

UE conceived the study, and SF and RO conducted the literature review. SF drafted the article and RO and UE revised it critically for important intellectual content. All authors approved the final version for submission.

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