The use of dried blood spots in antidoping: advantages and limitations

Abstract The adoption of dried blood spots (DBS) in antidoping has marked a transformative shift in the landscape of sample collection and analysis. This review explores the compelling advantages and inherent limitations associated with the use of DBS in antidoping efforts. One of the most notable advantages of DBS lies in its streamlined sample collection, transportation, and storage processes. The simplicity of DBS sample collection minimizes invasiveness, rendering it athlete-friendly and promoting more frequent testing. Furthermore, DBS samples are exceptionally stable, preserving the integrity of analytes within the dried matrix. This stability allows for prolonged sample storage without significant degradation, offering flexibility and cost-efficiency in sample management. In addition to these advantages, DBS simplifies the analytical process, facilitating large-scale screenings for prohibited substances. The method's practicality and efficiency make it a valuable tool for antidoping organizations seeking to conduct widespread analyses. Moreover, the information obtained from DBS can complement the interpretation of urine samples, enhancing result management. In cases of suspicious or non-conclusive findings, DBS data can serve as valuable supplementary evidence, aiding antidoping authorities in making informed decisions. However, DBS is not without its challenges. The hematocrit effect, a critical consideration, can lead to variations in analyte quantification due to fluctuations in individual hematocrit levels. This poses a significant hurdle in maintaining consistent sensitivity and accuracy. The limited volume of blood collected in DBS samples can also restrict sensitivity, particularly when detecting substances present in minute concentrations. Furthermore, the current regulatory framework confines the use of DBS to the detection of substances without predefined thresholds or minimum reporting levels, limiting its applicability to specific doping agents. It is important to recognize that the implementation of DBS in antidoping is still in its nascent stage. While initial experiences with DBS have shown promise, the true impact of this innovative approach on antidoping efforts will become increasingly apparent over the next two to three years. Importantly, DBS is not intended to replace conventional urine or blood collection methods but rather to complement them. This integration promises to enhance the comprehensiveness of antidoping practices and improve the fairness and integrity of sports antidoping measures.

Keywords **Dried blood spots (DBS), microsampling, capillary blood, direct detection.**

Résumé **L'utilisation des « dried blood spots » dans l'antidopage : avantages et limitations**

L'adoption des gouttelettes de sang séché (« dried blood spots », DBS) dans la lutte antidopage a marqué un changement fondamental dans la collecte et l'analyse des échantillons. Cet article explore les avantages considérables et les limitations inhérentes à l'utilisation des DBS dans la lutte antidopage. L'un des avantages les plus notables des DBS réside dans leur facilité de collecte, de transport et de stockage des échantillons. La simplicité de la collecte des échantillons de DBS réduit l'invasivité, en faisant une option respectueuse des athlètes et en favorisant des tests plus fréquents. De plus, les échantillons de DBS sont exceptionnellement stables, préservant l'intégrité des composants dans la matrice sèche. Cette stabilité permet de stocker les échantillons sur une période prolongée sans dégradation significative, offrant ainsi de la flexibilité et une gestion économique des échantillons. Outre ces avantages, les DBS simplifient le processus analytique, facilitant le dépistage à grande échelle de substances interdites. La praticité et l'efficacité de cette méthode en font un outil précieux pour les organisations antidopage cherchant à mener des analyses à grande échelle. De plus, les informations obtenues à partir des DBS peuvent compléter l'interprétation des échantillons d'urine, améliorant ainsi la gestion des résultats. En cas de résultats suspects ou non concluants, les données des DBS peuvent servir de précieuses preuves supplémentaires, aidant les autorités antidopage à prendre des décisions éclairées. Cependant, les DBS ne sont pas exempts de défis. L'effet hématocrite, en raison des fluctuations des taux d'hématocrite individuels, peut ainsi entraîner des variations cruciales dans l'analyse quantitative des substances détectées. Cela représente un obstacle important à la préservation de la sensibilité et de la précision. De plus, le faible volume de sang collecté dans les échantillons de DBS peut limiter la sensibilité, en particulier lors de la détection de substances présentes à de faibles concentrations. En outre, le cadre réglementaire actuel restreint l'utilisation des DBS à la détection de substances sans seuils prédéfinis ni niveaux minimaux de notification, limitant son applicabilité à des agents dopants spécifiques. Il est important de reconnaître que la mise en œuvre des DBS dans la lutte antidopage en est encore à ses débuts. Bien que les premières expériences avec les DBS soient très prometteuses, l'impact réel de cette approche novatrice sur les efforts antidopage deviendra de plus en plus évident au cours des deux à trois années à venir. Il est essentiel de souligner que les DBS ne visent pas à remplacer les méthodes conventionnelles de collecte d'urine ou de sang, mais plutôt à les compléter. Cette intégration promet de renforcer la globalité des pratiques antidopage et d'améliorer l'équité et l'intégrité des mesures antidopage dans le sport.

Mots-clés **Gouttelette de sang séché (DBS), microéchantillonnage, sang capillaire, détection directe.**

From finger to filter: unraveling the magic of dried blood spots

Dried blood spots (DBS) have become a pivotal form of biosampling over the years, offering a less invasive and more practical method than traditional venous blood draws. The concept of DBS dates back several decades, originally finding applications in neonatal screening. This innovative technique has since evolved, finding utility in various fields, including clinical diagnostics, toxicological analyses, and epidemiological research.

The process of collecting DBS involves a small, controlled finger prick (heel in the context of neonatal screening) to obtain a few drops of capillary blood (*figure 1*). Rather than the conventional liquid form, the collected blood is either applied directly or transferred via pipetting onto specialized filter paper in small, defined spots. Subsequently, these spots are left to air-dry, preserving the blood components in a stable state. The resulting dried blood spots are compact, lightweight, and easily handled, offering a simple solution for both sample storage and transportation.

This method's simplicity and versatility have made it increasingly popular, with recent applications extending to antidoping efforts in sports. In this review, we will explore the advantages and limitations of using DBS in antidoping analyses. We will delve into the technical aspects of DBS collection, storage, and analysis, highlighting the potential benefits for both antidoping authorities and athletes. Additionally, we will discuss the challenges and considerations associated with DBS in the context of antidoping, ensuring a comprehensive understanding of this innovative approach to sample collection and analysis.

Implementing dried blood spots in antidoping: current approaches

In 2021, the World Anti-Doping Agency (WADA) created guidelines for using DBS in antidoping tests. These guidelines cover everything from collecting and transporting samples to the actual testing and storage of the samples. These rules were updated in 2023, giving official recognition to any suspicious findings in dried blood, creating a clear legal framework for dealing with such results in antidoping efforts. Here is an overview of these guidelines for each particular aspect.

Sample collection and transport

Due to the absence of venipuncture for the collection of DBS, the samples can be collected by a regular doping control officer (DCO) without the need for a specialized blood control officer (BCO). Alternatively, the collection of DBS may be performed by the athletes themselves under close supervision. While the volume of capillary blood deposited onto filter is approximately 10-50 µL, the WADA guidelines require a minimum total of approximately 40 µL of capillary blood in the "A" spot(s) and with a minimum total of approximately 20 µL of capillary blood in the "B" spot(s) to satisfy relevant analytical requirements. Nevertheless, it is recommended to collect at least 60 µL and 40 µL, respectively [1]. To facilitate rapid drying of the spots once they are sealed and to shield the sample from potential early degradation or contamination, it is essential for the DBS sample container to include a desiccant. The samples shall be collected using disposable lancets in conjunction with cellulose cards. When collecting the sample from the fingertip, it is required to discard the initial drop after puncturing to prevent any potential dilution from interstitial fluids. Although numerous chemically treated filter cards can be found on the market, in the context of antidoping, it is essential that the absorbent sample support is made of untreated cellulose paper or alternative absorbent material (*e.g.* synthetic polymer). Following DBS collection, the sample is sealed in a tamper evident kit and DBS samples can either be transported at ambient temperature using regular mail or courier services or shipped refrigerated if other blood samples were also collected.

Analytical testing and storage

In the present context, as outlined in the technical document governing the analysis of DBS samples, the analytical testing procedures are specifically designed for detecting substances that do not have threshold values without minimum reporting levels (MRL). In simpler terms, DBS samples are presently suitable only for identifying substances where the mere

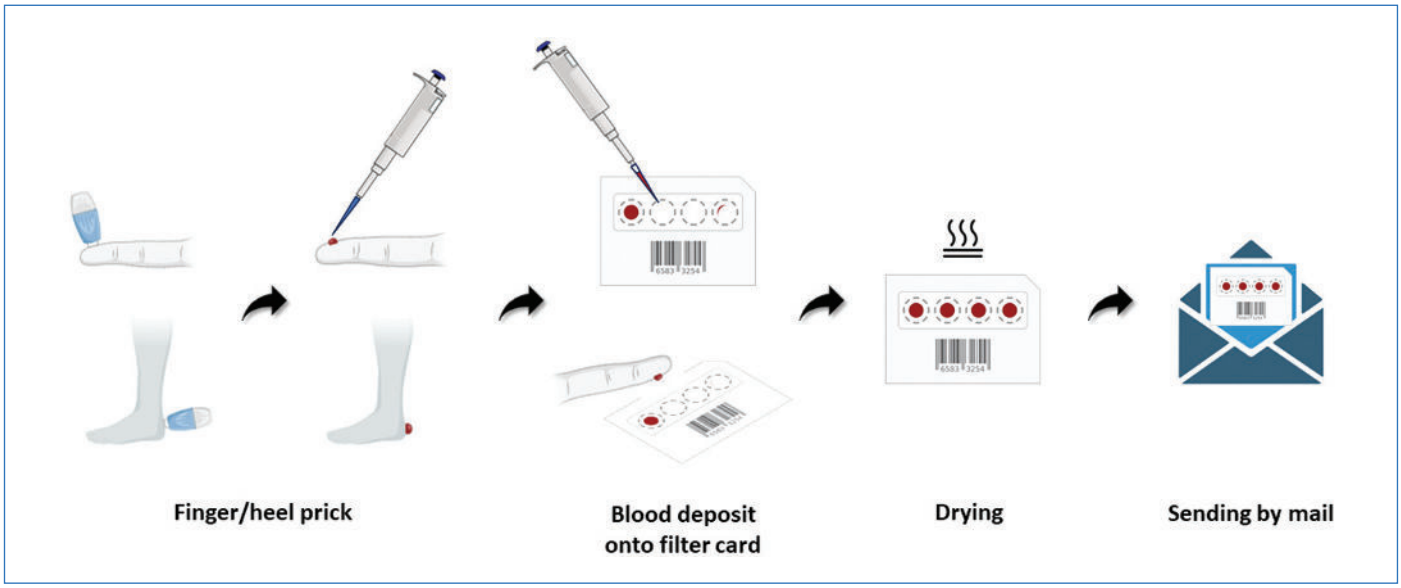

Figure 1 - Dried blood spot collection. First, a prick is performed with a specific lancet on the finger or heel (for neonates), the capillary blood is then transferred onto a filter card in pre-defined spots. After a proper air-drying process, the DBS card can be sent by regular mail. (created in BioRender.com)

presence is considered conclusive evidence of an antidoping rule violation. For instance, this includes a wide range of substances falling under the S1 category (anabolic steroids, excluding endogenous steroids), S2 (peptide hormones, growth factors, related compounds, and mimetics) and S4 (hormones and metabolic modulators) of the prohibited list.
Upon receiving DBS samples, the laboratory assesses for any potential irregularities, such as the absence of desiccant, attachment to the container, or inadequate drying. The DBS "A" sample should be refrigerated and protected from light until analysis while the "B" sample should be stored frozen (-20°C) after reception until analysis. Upon analysis, the samples are first brought to room temperature in an airtight and dry container (*e.g.* desiccator, plastic box containing desiccant) to avoid condensation. If spots are still present in the "A" sample, it should be placed back into refrigerated storage until both the initial testing procedure and, if applicable, the confirmation procedure are finished. Afterward, it should be stored in a frozen state at approximately -20°C.

Possible devices compliant with WADA technical documents

Most studies conducted thus far in the realm of DBS collection have predominantly utilized standard cellulose DBS cards like the DMPK-C cards or Whatman 903™ protein saver cards. However, recent advancements have ushered in a new era of DBS collection devices, many of which are volumetric in nature. This transition offers distinct advantages, allowing for precise and consistent sample volumes to be collected, reducing variability in sample collection, and simplifying analytical workflows.
Several innovative DBS collection devices have emerged on the market, each with their unique advantages. The *HemaXis DB10* (DBS System SA, Gland, Switzerland) incorporates a microfluidic chip to deliver an accurate amount of blood (10 µL) from the fingertip to a standard filter card (*Whatman 903*). A single cartridge allows for the collection of four spots. In accordance with WADA regulations, this necessitates the use of two kits to guarantee an adequate volume for both the "A" and "B" spots. The precision in volume control eliminates the necessity for a sub-punch since the entire spot can be utilized for extraction. The *Mitra* clamshell or cartridge (Neoteryx, LLC, Torrance, CA, USA) is a volumetric absorptive microsampling device using the VAMS technology allowing accurate collection of 10, 20 or 30 µL of capillary blood at the fingertip. The *Mitra 96-Autorack* compatible with 96-well plates allows for manual or high-throughput processing of DBS samples. The *Capitainer B* (Capitainer, Solna, Sweden) is another dried blood spot microsampling card device. The microfluidic qDBS technology of the *Capitainer B* card provides an exact sample volume (2×10 µL) to a pre-cut DBS disc. Capitainer's paper based drying pouch allows the sample to dry during transportation. The pre-cut disc allows for direct processing of the sample without the need to punch out. Like *Hemaxis DB10*, multiple devices should be used to ensure sufficient volume and spots in accordance with WADA requirements. Recently, the *Capitainer B50* was marketed for the collection of 2×50 µL spots. The *HemaSpot HF* capillary blood collection (Spot on Sciences, Austin, TX, USA) has a fan-shape design that allows even distribution across each of the eight blades with an approximate volume of 9 µL each. Each pre-cut fan with tracking notch filter (TNF) paper can be directly used for individual sample extraction and analysis with no punching required. In an alternative approach to fingertip collection, the *Tasso-M20* device (Tasso Inc., Seattle, USA) collects capillary blood volumetrically from the upper arm. This particular device stands out as the most user-friendly option, demanding minimal user manipulation. In essence, the procedure involves initially positioning the device on the skin. Once the user presses the button to activate the lancet, a precisely controlled vacuum is generated. This controlled negative pressure delicately draws four separate capillary blood samples, each measuring 20 µL, into the connected sample pod. In contrast to other kits, the absorptive *VAMS* tip (Mitra) and the *Tasso-M20* support are synthetic polymers. In addition to these advancements, it is crucial to combine these DBS collection devices with tamper-evident kits to ensure the integrity and authenticity of collected samples, particularly in antidoping settings. For instance, Versapak (Erith, UK) has developed a temper evident kit for the collection of *Hemaxis DB10* samples consisting of two ("A" and "B") sealed cases. Innovero has developed the *SAFESystem DBS kit* compatible with *Tasso-M20* devices. Innovero's *SAFESystem DBS kit* separates the sample from the sample pod into secure A/B samples, ensuring efficiency and consistency.
Before the implementation of DBS collection in antidoping, a study conducted by Solheim *et al.* compared the perception of different DBS collection kits (*HemaSpot HF* and *Tasso-M20*) and found a notable preference by both DCOs and athletes for the *Tasso-M20* device, likely due to its self-sampling capabilities and ease of use, which make it particularly appealing for a wide range of users [2]. These advancements in DBS collection technology underscore the importance of continuous innovation in simplifying sample collection processes and improving the overall experience for both professionals and individuals.

Unveiling the strengths of DBS in antidoping

Dried blood spot testing has emerged as a transformative approach in the realm of antidoping, bringing with it a plethora of advantages that enhance the efficiency, accessibility, and comprehensiveness of testing procedures.

Sample collection

One of the primary advantages of DBS lies in its minimally invasive nature during sample collection. Traditional venous blood sampling can be uncomfortable for athletes and requires trained personnel for extraction. In contrast, DBS allows for the collection of capillary blood through a simple finger prick, reducing discomfort and making it more athlete-friendly. This simplicity in sample collection contributes to increased compliance and a more positive testing experience for athletes. In addition, the collection of DBS would reduce the sampling time at the end of a competition (*e.g.* football game) and would allow for a larger number of athletes to be tested.

Sample transport and storage

The ease of sample transportation and storage is another notable advantage of DBS. Liquid blood samples necessitate stringent temperature controls and timely analysis, posing logistical challenges. DBS samples, on the other hand, are dried and can be shipped at room temperature, eliminating the need for rigorous temperature control during transport. This feature reduces logistical complexities, making sample handling more convenient and cost-effective for antidoping organizations. Furthermore, in contrast to urine containers, DBS samples

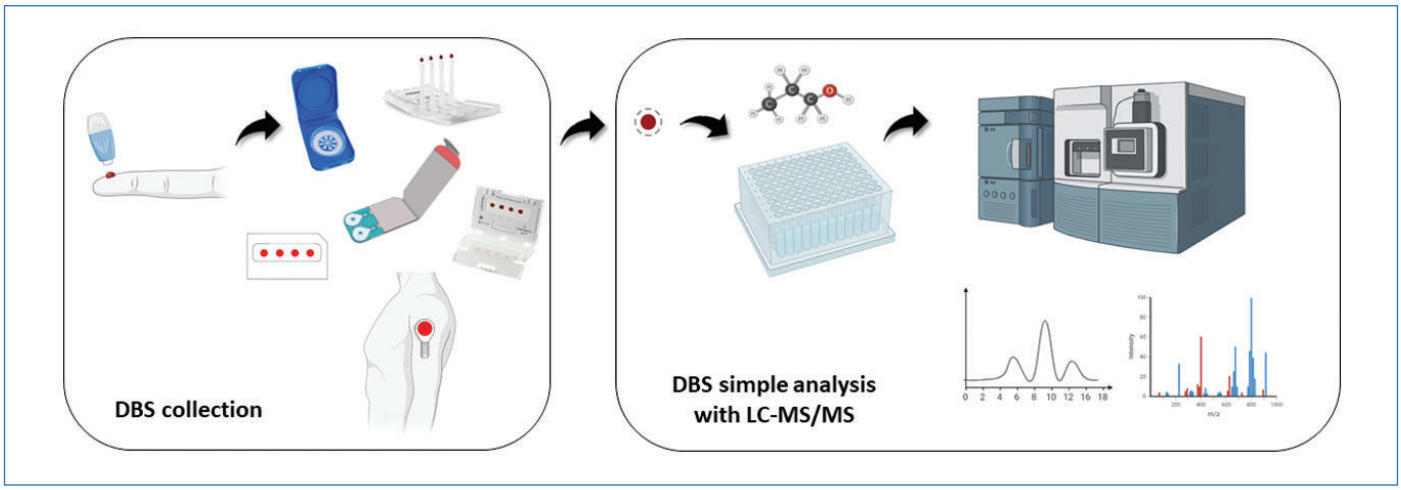

Figure 2 - From DBS collection (with the different devices presented above) to analysis using methanolic single-step extraction and injection with liquid-chromatography tandem mass spectrometry (created in BioRender.com).

occupy significantly less space due to their compact size. Consequently, a significantly greater number of DBS samples can be accommodated within the same storage area, thus expanding the available storage capacity considerably. This efficient use of space is advantageous for laboratories dealing with a high volume of samples, as it enables them to store and manage a larger quantity of specimens without requiring extensive storage facilities.

DBS samples are exceptionally stable, preserving the integrity of analytes within the dried matrix. This advantage stems from the inactivation of enzymatic activity due to the absence of water in the dried blood spots. This stability allows for prolonged sample storage without significant degradation, offering flexibility and cost-efficiency in sample management. The long-term stability of DBS samples ensures that they can be stored for extended periods without compromising the quality of the sample. This is particularly advantageous in situations where delayed analysis is required or when additional testing may be needed in the future. This feature proves especially advantageous when dealing with steroid esters that undergo swift hydrolysis due to the presence of esterase in the blood [3]. Similar considerations regarding the instability of hypoxia-inducible factor (HIF) activating agents in urine/blood highlight the use of DBS for the detection of such substances.

Sample analysis

Furthermore, DBS simplifies the analytical process, facilitating large-scale screenings for prohibited substances. The method's practicality and efficiency make it a valuable tool for antidoping organizations seeking to conduct widespread analyses. The incorporation of DBS analysis into the laboratory is facilitated by their minimal sample preparation demands (often a single-step extraction using organic solvent is sufficient) and their compatibility with mass spectrometry-based methods, offering a straightforward and effective approach for detecting prohibited substances (*figure 2*). This practicality offers the opportunity for full automation of sample extraction and analysis [4–6].

The information obtained from DBS is not limited to the immediate testing scenario; it can also complement the interpretation of urine samples, enhancing result management. In cases of suspicious or inconclusive findings, DBS data can serve as valuable supplementary evidence, aiding antidoping authorities in making informed decisions. This is especially relevant for substances prohibited in-competition for which it is fundamental to determine the time of administration or exposure to the prohibited substance (*e.g.* cocaine, glucocorticosteroids, cannabinoids…). The estimated concentration of clenbuterol (a β-2-agonist with anabolic and lipolytic side effects) in DBS could also help to discern whether the doping incident was intentional or unintentional (ingestion of contaminated meat) [7].

Furthermore, DBS serve as valuable matrices for substances that incorporate into erythrocytes. An example of such a substance is meldonium, an anti-ischemic drug with cardioprotective properties, which has been prohibited in sports since 2016 under the category of a "hormone and metabolic modulator". Meldonium's significant incorporation into red blood cells (RBCs) and its subsequent gradual release from these cells lead to an unusual urinary excretion pattern characterized by an initial rapid phase followed by a slower phase, resulting in detection times spanning several weeks [8].

Navigating hurdles: exploring the limitations of DBS for antidoping

While DBS have emerged as a promising tool in antidoping analyses, it is crucial to acknowledge their inherent limitations. Understanding these constraints is vital for ensuring a nuanced and realistic assessment of the technology's applicability in the realm of antidoping efforts.

Hematocrit effect

One of the primary challenges associated with DBS is the hematocrit effect [9]. Hematocrit, the proportion of blood volume occupied by red blood cells, can vary among individuals. This variability introduces complexities in the quantification of substances present in the dried matrix. Fluctuations in hematocrit levels may impact the concentration of analytes, posing a challenge to consistent sensitivity and accuracy. In individuals with high hematocrit levels, DBS samples may yield higher analyte concentrations, while those with lower hematocrit levels may produce lower concentrations. This variability can complicate the interpretation of DBS results and necessitates careful consideration when analyzing samples. To overcome this effect, techniques for the nondestructive hematocrit determination in DBS have been developed [10–13].

Table - Summary of the advantages and limitations of the use of DBS in antidoping.

Dimension	Advantages	Limitations
Sample collection	Simple No need for phlebotomist Reduced sampling time Low invasiveness Large number of tested athletes Optional remote testing	Low number of available temper-evident kits Limited control on sample quality
Sample transport & storage	Low cost Logistic Reduced storage space High stability	
Sample analysis	Minimal sample preparation High complementarity with mass spectrometry Pharmacological information Possible automation of sample extraction and analysis Complementary information for result management Substances that incorporate into erythrocytes	Regulatory constraints Low sample volume Limited number of spots (replicates) Hematocrit effect Short detection windows Time-consuming manual sample preparation

Limited sample volume

The volume of blood collected in DBS samples is inherently limited. This constraint becomes particularly significant when detecting substances present in low concentrations in the bloodstream. The reduced sample volume may compromise the sensitivity of the analysis, potentially leading to the underdetection of certain doping agents. Moreover, the number of spots may also limit the coverage of analytes that can be screened. For example, achieving sensitive detection of recombinant human erythropoietin often necessitates the use of multiple spots or a larger spot with a greater volume [14]. This requirement poses certain challenges and limitations in this particular context.

Regulatory constraints

The current regulatory framework for DBS testing in antidoping is another limiting factor. Presently, DBS analysis is primarily applicable to substances without predefined thresholds or minimum reporting levels (MRLs) for blood. Therefore, they are currently only effective in detecting substances for which their mere presence is deemed sufficient evidence of an antidoping rule violation. This restriction narrows the scope of substances that can be effectively monitored using DBS, limiting its broader applicability.

Sensitivity challenges

Achieving high sensitivity in detecting prohibited substances is a paramount goal in antidoping analyses. While DBS offers practical advantages, such as ease of collection and transport, its sensitivity may be compromised compared to traditional sampling methods. The sensitivity is also impaired by the short detection windows of many prohibited substances in the blood. This compromise stems from the nature of the dried matrix and the need for highly sensitive detection methods. In addition to issues related to sensitivity, the potential for high throughput capacity may be restricted when manual sample preparation is the sole option for the laboratory.

Complementarity, not replacement

It's crucial to recognize that DBS is not intended to replace traditional urine or blood sampling methods. Instead, it serves as a complementary approach, offering unique advantages while coexisting with established practices. Urine sampling remains crucial for detecting long-term metabolites of certain substances, which can provide valuable evidence of doping practices even when the parent compound is no longer detectable in blood or urine. Therefore, a combination of both urine and blood-based testing methods is essential to ensure comprehensive and effective antidoping efforts.

Reflections on the future of antidoping with DBS

The use of DBS in antidoping is relatively new and holds considerable promise for the future of athlete monitoring. The prospects of utilizing DBS for quantitative analysis in antidoping are promising but come with the need to establish new minimum reporting levels (MRLs) specifically tailored for blood matrices. As DBS gains traction as a viable alternative for sample collection, the establishment of MRLs becomes imperative to ensure accurate and reliable quantitative results. This involves determining the minimum concentration of a substance in the blood that can be reported with confidence. Once these new benchmarks are established, DBS can play a pivotal role in enhancing the sensitivity and precision of quantitative analyses, providing a valuable tool for antidoping efforts. In line with this perspective, the use of DBS could be applied for indirect detection with the quantification of biomarkers indicative of doping. Some studies have already explored this option for the detection of blood doping, testosterone or growth hormone administration [15–18].

One perspective involves exploring dried plasma spots (DPS), which could offer improved accuracy and sensitivity for detecting prohibited substances without the issues related to hematocrit. Increasing the volume of blood collected in each DBS spot is another avenue to enhance sensitivity, particularly for substances administered in microdoses.

Furthermore, the integration of remote sampling methods using DBS would streamline the antidoping process, enabling for simplified logistics related to sample collection [19]. This approach could facilitate more frequent and surprise testing, bolstering the effectiveness of antidoping efforts.

Finally, more than 25000 venous whole blood samples are collected per year since 2016 for the analysis of hematological parameters included into the ABP. The collection and transportation of such samples impose a significant financial burden on antidoping organizations. Typically, these samples

undergo immediate analysis upon arrival, after which they are refrigerated for a brief period before disposal, often without fully exploiting their potential. Utilizing these blood samples to generate DBS offers an intriguing alternative. This approach eliminates the constraints associated with limited sample volume, enabling the creation of multiple DBS. Consequently, these DBS could be stored for an extended duration, offering improved stability and space-efficient storage solutions. Over time, DBS derived from whole blood samples could serve as valuable complementary evidence in antidoping efforts, providing additional insights into the potential use of prohibited substances by athletes. This innovative approach not only maximizes the utility of collected blood samples but also demonstrates the adaptability of DBS in optimizing resources and enhancing the capabilities of antidoping organizations. Alternatively, microsampling could also be used for the collection of capillary whole blood intended for the measurement of hematological parameters [20]. Devices like the "*Tasso + EDTA*" (Tasso) or the "*onflow*" (Loop Medical SA) allows for the near-painless collection of capillary whole blood in a volume (250-500 µL and 1.5 mL, respectively) that is sufficient for the automated full blood count.

DBS and the future landscape of antidoping

The utilization of dried blood spots (DBS) in antidoping represents a promising advancement in the fight against doping in sports. As evidenced by testing figures in 2021, DBS implementation has commenced gradually, with some adverse analytical findings (AAFs) underscoring its efficacy. However, the true potential of DBS in enhancing antidoping efforts is yet to be fully realized. The next 2-3 years are poised to be pivotal in highlighting the transformative impact of DBS in athlete monitoring. With ongoing research, method refinements, and the establishment of minimum reporting levels for blood, DBS has the potential to offer more frequent and athlete-friendly sampling strategies, ultimately strengthening the integrity and fairness of sports. It is important to emphasize that while DBS brings significant advantages, it should not be seen as a replacement for urine or blood sampling. Instead, its integration complements the existing methods, enhancing test targeting and results management. As antidoping authorities continue to explore and refine the application of DBS, the stage is set for this innovative approach to play a substantial role in safeguarding clean and fair competition in the world of sports.

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