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REVIEW

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Dried blood spot analysis in antidoping: Technical challenges, analytical advances, and future perspectives

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Abstract

Dried blood spot (DBS) analysis is a sample collection strategy that requires minimal volume of blood while providing practical advantages such as a simple collection procedure, enhanced sample stability, and efficient transport and storage. Due to these benefits, DBS has been applied in newborn screening, therapeutic drug monitoring, and pharmacokinetic studies for clinical and life sciences. Recently, the potential of DBS has gained attention for antidoping; therefore, the World Anti-Doping Agency (WADA) has advanced the introduction and application of DBS-based analytical approaches as a complementary strategy to conventional urine and venous blood samples. Consequently, various studies have been conducted for the development of high-sensitivity analytical methods for prohibited substances, evaluation of DBS sampling devices, and the advancement of sample preparation focused on overcoming matrix-related challenges. The development of DBS analysis and its clinical applications are overviewed, followed by a discussion of the rationale for its adoption in antidoping analysis and WADA's strategy. The challenges in DBS sampling and instrumental analysis were also investigated comprehensively, and recent advances in addressing limitations were also investigated. The analysis of steroid esters and other methods for substances in WADA technical documents was mainly focused on, and emerging approaches for erythropoietin and its markers in blood doping. Perspectives for the DBS analysis in antidoping operation in the large sports events were discussed.

INTRODUCTION

Dried blood spot (DBS) is a novel sampling strategy for practical and clinical purposes and originated from population-scale newborn screening, where a few drops of capillary blood enabled reliable detection of inborn errors of metabolism at an unprecedented scale.[1] This legacy is relevant to antidoping research because it demonstrates that collection, drying, storage, and analytical workflows are standardized. DBS could support robust information under practical conditions, often outside controlled clinical settings.

Over the last two decades, DBS has been expanded beyond screening programs into quantitative bioanalysis, therapeutic drug monitoring, infectious disease diagnostics, and multi-omics research.[2–6] Methodologically, DBS is now connected to liquid chromatography–mass spectrometry (LC–MS) and high-resolution MS (HRMS) platforms because these techniques can compensate for limited sample volume and enable multiresidue detection with high selectivity.[2,3] At the same time, many researchers showed that DBS was not simply "small-volume whole blood": hematocrit (Hct)-driven spot spreading, punch-position effects, variable deposited volume, and heterogeneous drying could introduce pre-analytical variability that has to be actively controlled or overcome.[4] These considerations have been addressed in wide healthcare contexts, including viral hepatitis and HIV

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testing, where DBS logistics such as remote collection and ambient shipment can be transformative when stability and chain-of-custody expectations could be clarified.[5] Likewise, in protein quantification and proteomics, DBS has been positioned as an enabling tool for longitudinal or decentralized sampling, with clear recognition that extraction efficiency, adsorption to cards, and matrix effects require fit-for-purpose validation.[6]

In parallel, antidoping science has entered an era in which analytical scope, sensitivity, and interpretive rigor continue to increase, driven by both evolving prohibited lists and increasingly sophisticated doping practices.[7] Recent perspectives highlight how next-generation antidoping can benefit from microscale separation, advanced MS workflows, and integrated data interpretation approaches, including metabolomics and machine learning.[8,9] Within this landscape, DBS provides a practical bridge between conventional venous blood and urine testing by increasing access to blood-based markers while reducing logistical burdens and enabling more frequent or strategically timed collections that are difficult to implement with phlebotomy.[10–12] This positioning has been emphasized in antidoping-oriented DBS reviews and commentaries, which frame DBS not as a replacement for urine or venous blood, but as a complementary matrix that expands what is feasible operationally.[13]

From collecting DBS samples from a finger or upper arm, a lancet or TASSO® device is used as illustrated in Figure 1, which reduces athlete burden. From an operational standpoint, DBS is attractive because capillary collection can be performed rapidly with minimal equipment, reducing barriers associated with venipuncture (staffing, cold chain, and sharps handling).[14] Emerging collection devices have further expanded this concept by enabling more standardized volumetric deposition or alternative anatomical collection sites (e.g., upper arm), aiming to reduce variability while maintaining the practical advantages of microsampling.[15] In the antidoping setting, such device innovation is directly linked to the need for laboratory and results-management confidence that DBS findings can be supported with the same level of defensibility expected for established matrices. Achieving this confidence requires both analytical performance and explicit operational rules for sample adequacy, documentation, storage, reanalysis, and confirmation testing.[16]

DBS could provide additional detection opportunities for substances such as anabolic agents, steroid esters, peptide hormones, and emerging biologics, and more direct physiological properties than urine.[10–12] Studies of

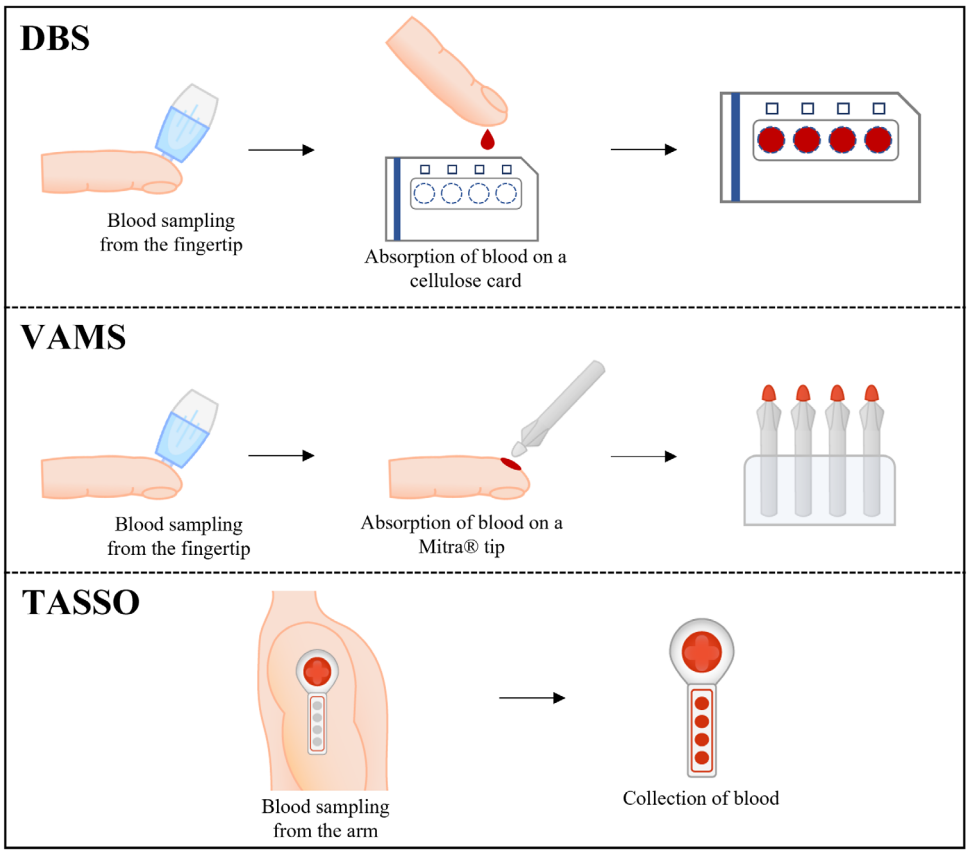

FIGURE 1 The schematic diagram of DBS collection methods. DBS, dried blood spot.

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comparison evaluated collection devices and DBS samples with urine for anabolic–androgenic steroid (AAS) and steroid esters detection, underestimating both the promise and the matrix-specific constraints, such as limited sample volume and the need for highly sensitive workflows.[17] Comprehensive reviews about AAS detection across biological matrices further support the role of blood-based samples as a complementary matrix to urine, which enables different detection windows, metabolite patterns, and analytical targets.[18] At the instrumental analysis, sensitivity can be significantly influenced by ionization efficiency and sample preparation, which has driven interest in approaches to chemical derivatization to enhance steroid sensitivity by LC–MS.[19] Because DBS typically provides only tens of microliters of whole blood per spot, stability and storage behavior of key analyte classes, including steroid hormones, become a central enabling factor rather than an ancillary consideration.[20] Similarly, work comparing quantitation of endogenous steroids across blood-related matrices reinforces that matrix definitions are analytically and physiologically consequential: whole blood, plasma/serum, and DBS differ in composition and behavior, which affects both method validation and downstream interpretation.[21]

An antidoping application depends on reliable identity, integrity, and the robustness of results. DBS has been studied for personal identification using associated samples, which has sparked interest in strengthening identity assurance through sampling scenarios.[22] In addition, the enlarged analytical scope of antidoping tests, providing backgrounds for multi-class, high-throughput, and scalable strategies, is regarded as important.[7] Communications from testing and results-management stakeholders noted a shift in testing patterns and an increasing emphasis on blood-based intelligence, aligning with the wide move toward matrices having more information.[23]

In order to harmonize, World Anti-Doping Agency (WADA) has announced DBS implementation through official technical documents (TDs). The TD-DBS has presented various standards for collection devices, sample suitability, storage conditions, analytical pathways, and the interfaces between laboratories and results management authorities. In TD2023DBS, a core expectation was established for DBS as a new matrix for routine testing.[24] Furthermore, TD2026DBS emphasized and defined a minimum requirement of device and sample spot, confirmation from independent aliquots, clarifying how limited spot resources interact with multiple analysis requests, and introducing minimum DBS testing menus including associated performance.[25]

Consequently, we summarized DBS technology in antidoping through an integrated point of view: (i) how DBS sample properties influence analytical variability and chain-of-custody, (ii) how modern analytical workflows enabled multi-class detection under the constraints of microsampling, (iii) what substance classes and analytical strategies have been demonstrated in DBS including steroids, steroid esters, small molecules, and peptides, and (iv) how WADA's TD-DBS requirements shape validation, confirmation, and routine applications. By aligning the methodological documents with the TD2023DBS and TD2026DBS expectations.[24,25]

CURRENT CHALLENGES AND METHODOLOGICAL ADVANCES IN DBS ANALYSIS

Although DBS sampling offers advantages for logistics and operations in antidoping activities, some technical challenges should be resolved for reliable analytical characteristics. Limitations of DBS are mainly associated with Hct variability, bias based on blood matrix, inaccurate sampling, material-derived interferences, or workflow constraints. Recent developments demonstrated that various issues could be remarkably mitigated through technological and procedural optimization.

Hct-dependent bias and volume variability

Hct variation became one of the most significant sources of bias in DBS analysis. Differences in red blood cell counts affect viscosity and spreading behavior on collecting material such as cellulose, therefore altering the distribution of the target analyte in a sample aliquot. Hct effect can lead to both bias in areas and recoveries, ultimately affecting accuracies of the results. Abu-Rabie et al. demonstrated that Hct-based bias in recovery could be reduced significantly by the application of an internal standard spray combined with whole-spot extraction and an automated elution procedure.[26] This strategy could compensate extraction related variability and represents a practical solution.

For chemical compensation approaches, nondestructive Hct estimation using an image analysis has also been proposed. Barroso et al. reported a grayscale scanning method capable of estimating Hct with acceptable precision, providing the identification and exclusion of samples with extreme Hct values prior to quantitation.[27] Those approaches were particularly relevant in antidoping purposes where inter-individual hematological variation will be common.

The development of certified reference material also contributed to harmonization efforts. Woo et al. reported the importance of whole-spot sampling to minimize heterogeneity among the spots and bias in sampling.[28] Their findings highlighted discrepancies between punching and whole-spot analysis, underscoring the importance of a standardized volumetric sampling strategy in practical situations.

Matrix effects in analysis and automation

Whole blood introduces significant matrix complexity compared to plasma or urine. Ion suppression or

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enhancement affects during liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis and has been a main concern for mass spectrometric approaches in antidoping. Studies comparing coated/uncoated cellulose cards have demonstrated observable differences in recoveries and matrix effects, which means substrate selection could impact significant quantitation performance.[29] Contaminants from collection devices represented an additional but often insufficiently considered challenge. Feng et al. reported false-positive screening results caused by benzene derivatives originating from printed ink on collection devices.[30]

Manual extraction of DBS could introduce variability and limit throughput, which could be a major issue in large-scale testing events. Recent advances in automated extraction systems have significantly enhanced reproducibility and efficiency in antidoping laboratories. Based on the hardware setup illustrated in Figure 2, Luginbühl et al. demonstrated a fully automated LC–MS/MS workflow for tramadol using robotic sample preparation, achieving high accuracy in approximately 4 min per single sample.[31] Similarly, Tretzel et al. reported the implementation of an online solid phase extraction approach, including LC–MS for the detection of nicotine and its metabolites, with fewer manual steps and improved consistency.[32]

Automation was also applied to large molecules such as antibodies. Knoop et al. demonstrated successful automated extraction of antibodies from DBS samples with reproducible results over extended storage periods.[17]

RECENT ADVANCES AND EXPANDING ANALYTICAL SCOPE OF DBS IN ANTIDOPING

This section describes an overview of recent technological progress in DBS analysis, focusing on the development of sample preparation and instrumental procedures and the expanding scope of target analytes for antidoping. Summary of multiplex DBS techniques introduces the methods for multiple prohibited substance classes listed in WADA TDs, supporting the transition of DBS from feasibility studies. In addition, developments of extending beyond the current coverage of TDs, including applications related to erythropoietin (EPO) and blood doping, or protein-based analytes such as biomarkers combining immunoassays, were introduced.

Advances in steroid ester analysis

Steroid esters have been complex and challenging substances in antidoping analysis. Esterified forms of testosterone, nandrolone, and boldenone are typically administered intramuscularly or transdermally, then circulate in the blood. However, rapid hydrolysis, extensive tissue distribution, and relatively short detection windows cause difficulties for identification from urine samples. While these challenges were already proposed in blood testing, the limited volume and whole-blood matrix inherent to DBS further increase analytical complexity, requiring highly sensitive and selective instrumental strategies.

Early investigations established the viability of monitoring steroid esters in DBSs using LC–MS/MS, demonstrating that various esters could be identified with acceptable sensitivity despite ion suppression effects caused by the matrix.[33] In parallel, pharmacokinetic or pharmacodynamic studies of DBS sampling were validated for depot steroid preparations, confirming that quantitative time-course profiling is also available.[34] Furthermore, earlier work demonstrated that administration of testosterone could be monitored in DBS samples through assessment of glucuronidation patterns,[35] illustrating that DBS supports both parent compound detection and metabolite-based interpretation.

Methodological expansion subsequently included adaptation of gas chromatography–tandem mass

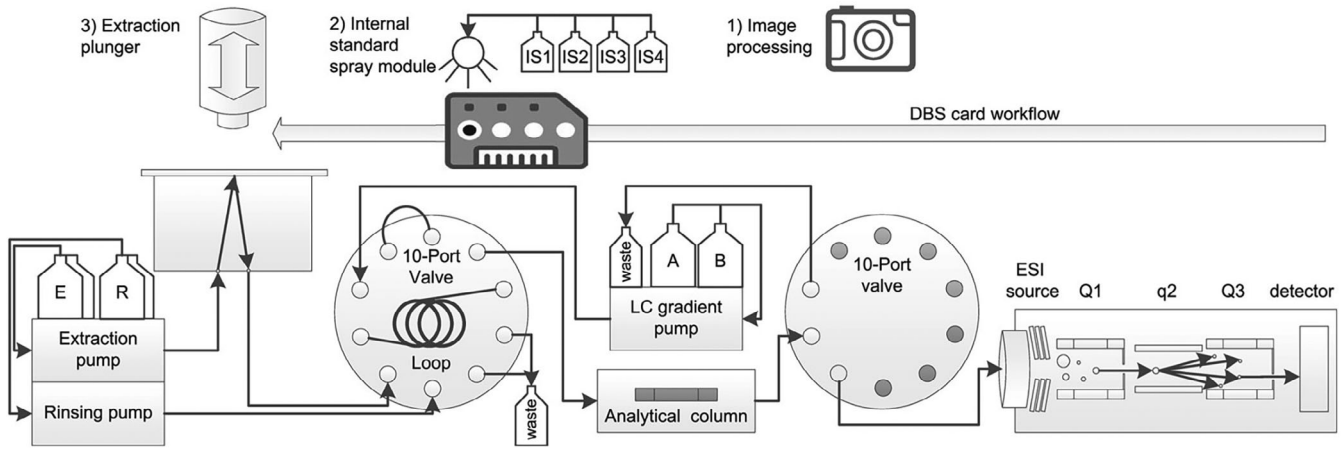

FIGURE 2 The hardware scheme of online-connected, fully automated DBS extraction to an LC–MS/MS system (reprinted from Reference 31, Copyright 2020, with permission from the Wiley-VCH). DBS, dried blood spot; LC–MS/MS, liquid chromatography–tandem mass spectrometry.

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spectrometry (GC–MS/MS) platforms. GC–MS/MS methods combined with microsampling enabled reliable quantification of anabolic steroids in dried blood and were successfully applied to testosterone gel administration studies.[36] This demonstrated that DBS analysis can be integrated into established chromatographic infrastructures within antidoping laboratories, provided that extraction and derivatization steps are carefully optimized.

Recent advances have focused on improving sensitivity and separation efficiency. Derivatization strategies have demonstrated enhancing ionization efficiency for poorly ionizing steroid esters. Among them, charged-tag derivatization using Girard's reagent P significantly enhanced signal intensity and enabled detection of various types of steroid esters from DBS samples by coupling with HRMS, as described in Figure 3.[37] Complementary approaches implementing LC–MS^3 configurations provided additional structural information, reducing interference from isobaric or ester analogues with similar structures and improving analytical robustness.[38] These developments demonstrated the transition from simple targeted detection toward reliable information about structures.

The development of automation in DBS sample preparation has further improved reproducibility and throughput. Online extraction systems integrated with LC–MS/MS have been introduced for steroid ester detection, which minimizes human errors and offers high throughput.[39] Following studies demonstrated simultaneous detection of testosterone, nandrolone, boldenone, methyltestosterone, and testosterone propionate esters within a single LC–MS run.[40,41] Such integration of various ester targets within a single method suggested increasing analytical advances and operational suitability.

DBS has also proven valuable for interpretative applications as well as the presence of target substances. Combined analysis of urine and DBS showed differences between oral and transdermal administration routes of a steroid, highlighting the complementary information based on blood-based matrices for interpretation.[42] Studies for sample stability have ensured the detection ability of testosterone esters in DBS with intramuscular injection, supporting appropriate storage and transport conditions for routine antidoping operations.[43] These data could provide an essential dataset for defining pre-analytical handling requirements to collect practical samples.

Comparison studies among WADA laboratories have supported reliability in DBS methodology. Comparing serum and DBS analyses of testosterone esters demonstrated promising reproducibility and analytical consensus across multiple laboratories.[44] Investigations of alternative analytical workflows have also characterized key factors that could affect robustness and harmonization.[45] Additionally, studies about longitudinal monitoring for comparison of whole blood and DBS matrices for androstenedione, testosterone, and insulin-like growth factor-1 (IGF-1) have supported the suitability of DBS for endocrine monitoring related to the athlete biological passport (ABP).[46]

In endogenous AAS profiling, technological progress was achieved by using DBS platforms. Ultra-high performance liquid chromatography (UHPLC)–MS/MS-based steroid profiling workflow demonstrated sufficient sensitivity to detect physiologically relevant changes leading to testosterone administration.[47] Combining volumetric absorptive microsampling (VAMS) devices and LC–MS/MS showed improved analytical capabilities, allowing quantification of endogenous AAS and phase II metabolites in a limited volume of blood.[48] These developments could reduce Hct-related issues and provide information for the harmonization of steroid profiling.

The ambiguity in interpretation by structure isomerism has induced an important analytical challenge for steroid ester analysis. Investigations focusing on ultraviolet spectral properties and computational modeling of *E/Z* steroid isomers in DBS have provided applicability for isomer differentiation.[49] These techniques might become more important for identifying designer or structurally modified designer steroids that present out of current screening criteria.

Development of analytical methods for substances listed in WADA technical documents

The analytical scope of DBS testing in antidoping has been expanded rapidly from early feasibility demonstrations toward validated, operationally realistic methods that address multiple substance classes referenced in WADA TDs. This expansion has been driven by (i) the need for minimally invasive blood collection compatible with frequent testing, (ii) improvements in LC–MS/MS and LC–HRMS sensitivity in complex whole-blood matrices, and (iii) the emergence of automation and high-throughput workflows that reduce pre-analytical and analytical variability. Early conceptual and technical work established DBS as a complementary matrix for both in-competition and out-of-competition strategies, emphasizing practicality while acknowledging whole-blood specific constraints such as Hct effects and limited sample volume.[50]

Evolution from targeted assays to broad initial testing procedures

A notable trend is the transition from single-analyte or single-class assays to multi-class initial testing procedures (ITPs). Fully automated DBS sample preparation has been shown to enable sensitive detection of lower molecular mass compounds and to support high-throughput processing, strengthening the feasibility for routine antidoping operations.[51] In parallel, comprehensive DBS ITPs have been designed and validated specifically for doping control, integrating automated workflows to reduce operator dependence and to standardize extraction and analysis.[52] These approaches are complemented by

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HRMS-based screening methods capable of retrospective interrogation of full-scan data, which is particularly relevant as prohibited substances and reporting requirements evolve.[53]

Technology development has also addressed the identification demands of antidoping screening: high-resolution Orbitrap-based configurations demonstrated early capability for sensitive detection and

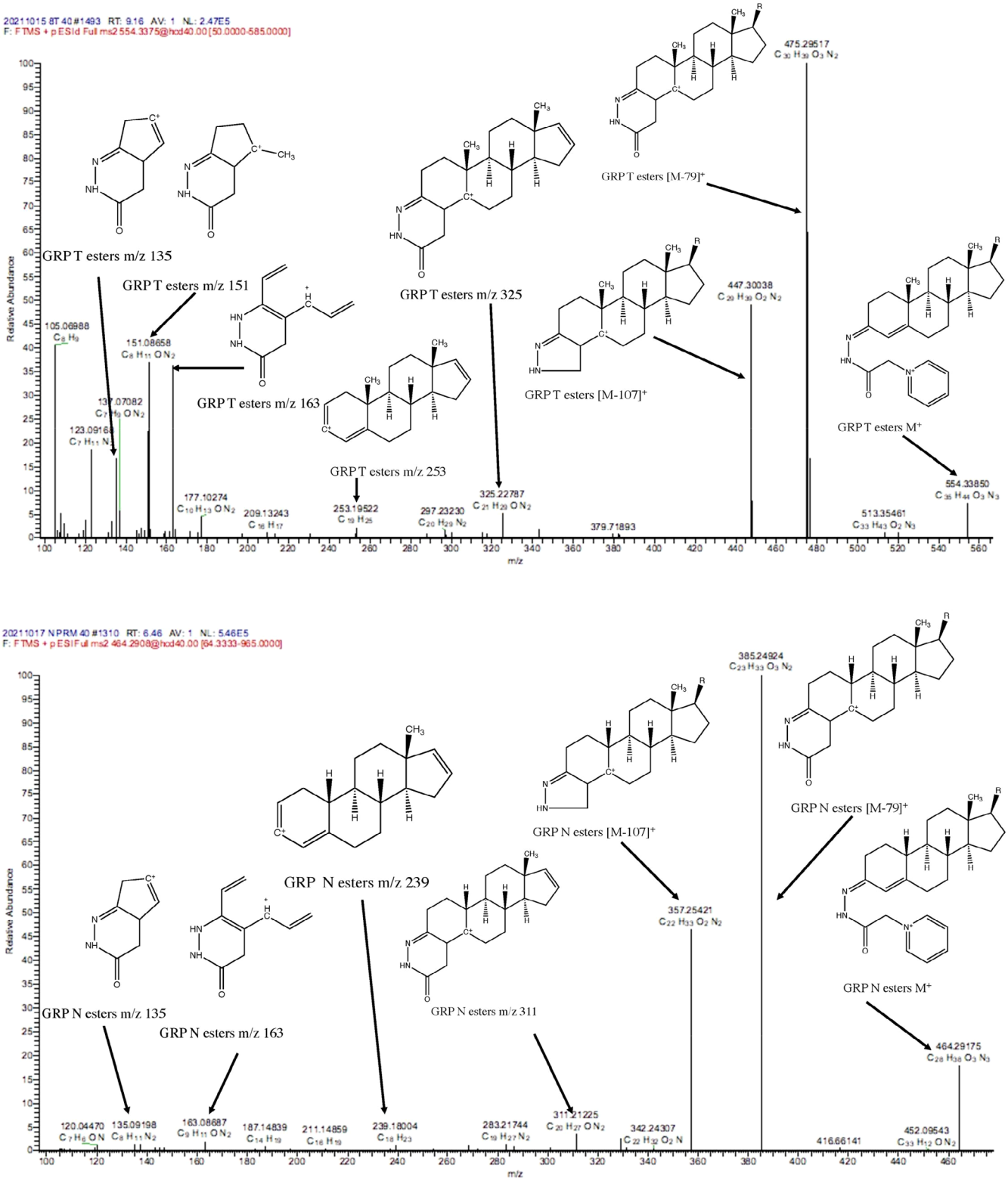

FIGURE 3 The fragment ions spectra of girard's reagent P-derivatized testosterone esters, nandrolone esters, and dehydroepiandrosterone acetate and the predicted structure of fragment ions (reprinted from Reference 37, Copyright 2022, with permission from the Elsevier.)

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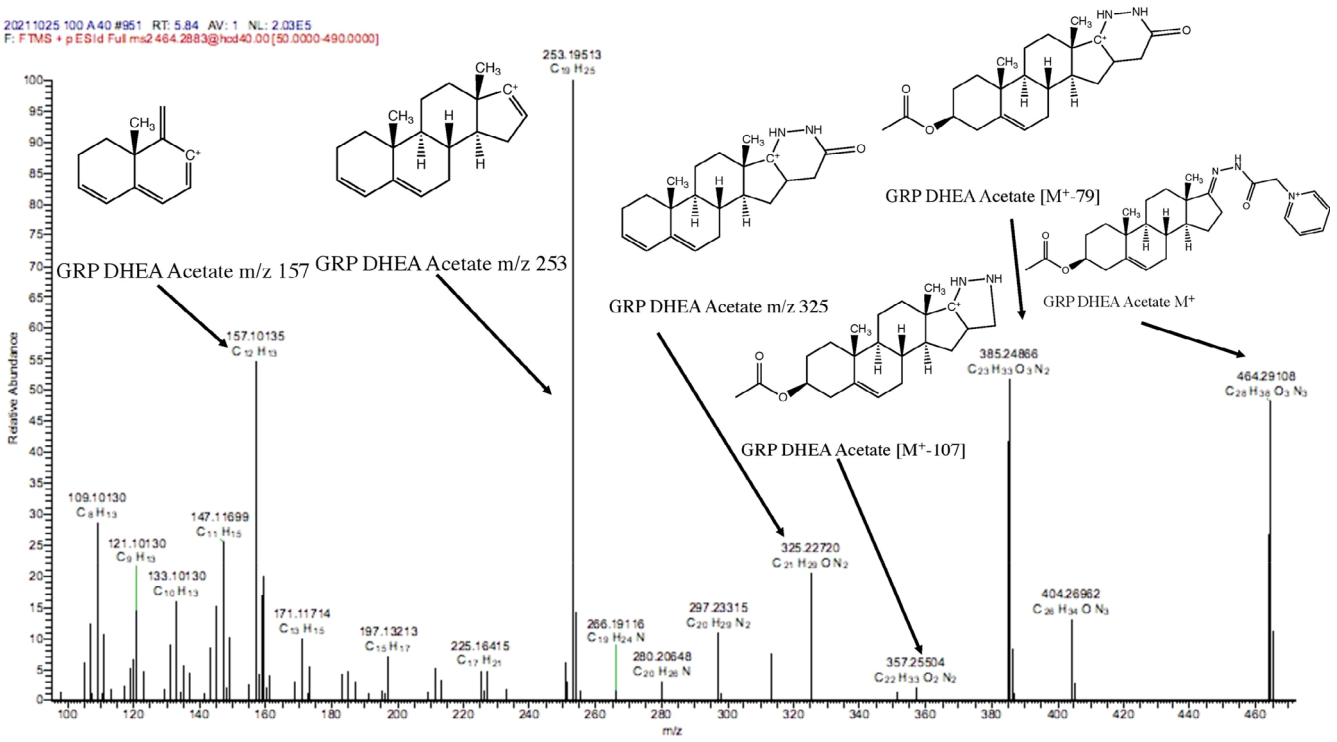

FIGURE 3 (Continued)

confirmatory-quality identification in DBS, supporting both targeted inclusion-list workflows and broader screening paradigms.[54] More recent studies further emphasize the value of comparing volumetric vs. non-volumetric spotting devices for large-panel screening in capillary blood, strengthening confidence that device selection can be aligned with analytical performance and operational constraints.[53]

Peptide hormones, protein biomarkers, and macromolecules

A major challenge for DBS in anti-doping is the extension from small molecules to peptides and proteins, where matrix complexity, adsorption, and stability can be limiting. Nonetheless, several studies reported meaningful progress. A multitarget screening method for small peptides in DBS indicates that optimized extraction and HRMS strategies can support broader peptide coverage within a single analytical framework.[55] This is complemented by DBS analysis workflows for growth hormone (GH) misuse detection, confirming that a single DBS can support the determination of recent GH abuse.[56]

Development of diverse analytical approaches focuses on targeting peptide therapeutics and analogues relevant to sports doping. Analysis of synacthen in DBS by LC–MS/MS showed a focused peptide assay with dried matrices.[57] Similarly, a mass spectrometric method for peginesatide in DBS demonstrated feasibility for erythropoiesis-related peptide targets, extending the applicability of DBS to EPO-relevant domains beyond small molecules.[58] Similarly, complementary LC–HRMS methods for sotatercept detection in DBS reported how targeted macromolecular approaches can be studied for doping control needs.[13]

Related biomarker-oriented work examined IGF-1 and GH axis markers using DBS. Quantification of insulin-like growth factor-1 in DBS provides the foundation for GH-related detection strategies, particularly when longitudinal interpretation may benefit from repeated microsampling.[59] Additional work on capillary dried blood for quantification of intact IGF-I by LC–HRMS further reinforces its feasibility for the detection of intact proteins in dried matrices.[60] Additional studies evaluating water-soluble DBS using insulin as a model protein provide broader insight into how DBS chemistry and substrate properties can be optimized to improve recovery and robustness for macromolecules.[61]

DBS applied to GH isoform testing using capillary DBSs. The dose of the substance administration and large-scale field collections are critical factors to define detection windows for the reliability of analyses under realistic scenarios.[62] For erythropoiesis-stimulating agents, a rapid screening method of continuous erythropoietin receptor activator in DBS concluded that even challenging biologic targets can be approached with DBS-compatible strategies when assay design is appropriate. Similarly, DBS-focused administration studies for peptide doping agents such as growth hormone-releasing

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peptide 2 (GHRP-2) showed that DBS can provide actionable detection windows, whereas also emphasizing differences between venous and capillary DBS and the importance of sampling-site considerations.[63]

β2-agonists and other small molecules relevant to WADA TDs

DBS has also been used to address substances where blood detection windows may be shorter than urine, but still operationally meaningful. For example, clenbuterol administration was shown to be detectable in DBS following a single dose, with stability and detection window assessments supporting practical utility.[53] Such work is particularly relevant for antidoping contexts where confirmatory identification and stability during shipment/storage are critical.

Analytical methods have also been developed for specific prohibited or monitored therapeutic agents, including trimetazidine. A DBS-based doping control method targeting trimetazidine demonstrates that robust quantification can be achieved even for compounds with challenging interpretative contexts, helping expand finding coverage beyond classical stimulant/opioid domains.[64]

In addition, paired DBS and urine sampling provides an important bridge between matrices, showing how DBS can be integrated with conventional urine testing to generate complementary evidence in practical doping-control settings and to refine interpretation alongside established urine-based decision frameworks. In the Danish fitness-center paired-sample study, DBS findings mirrored urine result patterns across multiple substance classes, including S1 anabolic agents (e.g., boldenone, nandrolone, trenbolone, stanozolol, and ostarine), clenbuterol, and S4 modulators such as anastrozole/letrozole and tamoxifen/clomifene.[65]

Stimulants and specified stimulants

Stimulants remain a key target class where blood-based information can complement urine findings, especially for in-competition-only substances. DBS feasibility for ephedrine has been demonstrated with attention to the relationship between DBS concentrations and conventional venous plasma measurements, supporting the interpretation of blood concentrations during competition.[31] Earlier comparative work examining urine analysis vs. DBS analysis for ephedrine also contributed to defining how DBS may support or refine detection strategies for threshold or short-acting stimulants.[66]

Beyond classical stimulant agents, DBS and other microsampling techniques have also been implemented for the analysis of emerging specified stimulants such as synthetic cathinones. LC–MS/MS methods coupled with volumetric absorptive VAMS enable the quantitative bioanalysis of cathinone analogues in dried matrices, facilitating the monitoring of such compounds in sports-related contexts.[67] In the broader context of continuously evolving drug markets, UHPLC–HRMS–Quadrupole time-of-flight workflows validated for large panels of new psychoactive substances in blood-based matrices provide a relevant methodological foundation since similar HRMS principles and validation approaches can be adapted for DBS-centric antidoping screening.[68]

Narcotics, opioids, and analgesics

Opioids and analgesics represent another class where DBS offers operational advantages for event testing while maintaining analytical reliability. Tramadol has been investigated extensively: field-relevant DBS detection in cycling competitions illustrates practical sample collection and analytical implementation under competition conditions.[69] Complementary high-throughput approaches have enabled automated analysis of tramadol and its active metabolite (*O*-desmethyltramadol) in DBS, supporting scalable workflows that can be deployed when testing volume increases.[31]

More broadly, a fully automated LC–MS/MS method with on-line DBS extraction was implemented for opioid analysis in complex matrices without conventional DBS punching.[70] For the analysis of oxycodone and its metabolites, dried micromatrices, such as DBS and VAMS, are compared with classic doping samples, such as plasma and urine. Considering the stability of samples and the sensitivity, dried micromatrices can also be used when designing fit-for-purpose anti-doping assays.[71] Dried micromatrices can also be employed for cannabinoid analysis. Comparing DBS and VAMS, VAMS was less affected by Hcts than DBS; complementary application of both microsampling approaches enables their extension to cannabinoid and broader narcotics analysis.[72] Together, DBS-based methods for narcotics analysis balance operational simplicity with rigorous quantification/identification requirements.

Interpretation, matrix behavior, and emerging directions

As analysis of DBS is widely adapted to several analytical fields, considerations in interpretation and matrix comparability are crucial. Comparative work of liquid vs. dried blood matrices in longitudinal monitoring emphasized that whole-blood drying and matrix complexity should be understood when applying plasma/serum practices to DBS.[46] The studies on analyte metabolism and distribution across matrices (urine, DBS, plasma) in controlled settings supported the idea that pharmacokinetics of substances can inform doping-control interpretation.[73]

To facilitate direct comparison across the 44 studies cited in this section, we summarized the targeted analytes, sampling method, instrumentation, and the reported analytical sensitivity in Table 1. Cellulose-based

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TABLE 1 The summary of analytical characteristics including instrumental methods, collecting devices, and limits of detection.

Prohibited list group	Substances	Methods	Sample type	LOD (ng/mL)	References
S0: Non-approved substances	AdipoR-agonists	LC–HRMS	DBS	5–10	74
	SIRT1 activating compounds	LC–HRMS, LC–MS/MS	DBS	10–50	73
S1: Anabolic agents	AAS	GC–MS	VAMS	0.1–0.78	36
	AAS	LC–MS/MS	VAMS	0.02–20	48
	AAS	LC–MS/MS	DBS	—	34
	AAS	LC–MS/MS	DBS	0.02–50 (LLOQ)	47
	AAS	LC–HRMS	DBS	—	49
	AAS	LC–HRMS	DBS, TASSO®	0.03–2.25	65
	AAS	LC–HRMS	DBS	0.05–0.5	54
	Clenbuterol	LC–MS/MS	DBS	0.03	75
	Methyltestosterone	LC–MS/MS	DBS	—	42
	Stanozolol, DHCMT	LC–MS/MS	DBS	0.02–0.8	50
	Steroid esters (testosterone, nandrolone, boldenone)	LC–HRMS	DBS	0.2–0.5	39
	Steroid esters (testosterone, nandrolone, boldenone)	LC–MS/MS	DBS	0.05–1.0	45
	Steroid esters (testosterone, nandrolone, androstanolone, boldenone)	LC–HRMS	DBS	0.03–125	37
	Steroid esters (testosterone, nandrolone, boldenone)	LC–MS/MS	DBS, TASSO®	0.05–0.25	38
	Steroid esters (testosterone, nandrolone, boldenone)	LC–MS/MS	DBS, TASSO®	<0.1–0.9	41
	Steroid esters (testosterone, nandrolone)	LC–MS/MS	DBS	0.1–0.5	33
	Testosterone	LC–HRMS	DBS	0.05–0.2	43
	Testosterone	LC–MS/MS	DBS	0.1	40
	Testosterone	LC–MS/MS	DBS	0.06–0.08	46
S2: Peptide hormones, growth factors, related substances, and mimetics	GHRP	LC–MS/MS	DBS	0.05	63
	hGH	Immunoassays	DBS	24–228	56
	Ibutamoren	LC–HRMS	DBS, TASSO®	0.25–0.5	65
	IGF-1	LC–MS/MS	DBS	25–47	46
	IGF-1	LC–MS/MS	DBS	50 (LLOQ)	59
	IGF-1	LC–HRMS	VAMS	50 (LLOQ)	60
	Peginesatide	LC–MS/MS	DBS	10	58
	Recombinant and pituitary GH	Immunoassays	TASSO®	0.041	62
	SERA	ELISA	DBS, VAMS	0.01462	76
	Small peptides	LC–MS/MS	DBS	0.5–20	51
	Small peptides	LC–HRMS	DBS, TASSO®	1–10	55
	Tetracosactide	LC–HRMS	DBS	0.05	64
S3: β2-agonists	β2-agonists	LC–HRMS	DBS	0.5	54
	Terbutaline	LC–HRMS	DBS, TASSO®	0.75–0.93	47
S4: Hormone and metabolic modulators	Aromatase inhibitor	LC–HRMS	DBS	0.05–0.125	54
	Hormones	LC–HRMS	DBS, TASSO®	0.2–1.9	65
	Insulin	LC–MS/MS	DBS	30	61
	Trimetazidine	LC–HRMS	DBS	0.05	64
S5: Diuretics and masking agents	Diuretics	LC–HRMS	DBS, TASSO®	0.2	65
	Hydrochlorothiazide	LC–HRMS	DBS	0.25	54
S6: Stimulants	Cathinone analogues	LC–MS/MS	VAMS	10	67

(Continues)

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TABLE 1 (Continued)

Prohibited list group	Substances	Methods	Sample type	LOD (ng/mL)	References
	Cocaine and metabolites	LC–MS/MS	VAMS	0.3–0.8	77
	Ephedrine	LC–HRMS	DBS, TASSO®	1	78
	Ephedrine and methylephedrine	LC–MS/MS	DBS	5	79
	Pseudoephedrine	LC–MS/MS	DBS	0.8	50
	Pseudoephedrine	LC–MS/MS	DBS	0.5	68
	Stimulants	LC–HRMS	DBS	0.05–0.5	54
S7: Narcotics	Opioids	LC–HRMS	DBS	1.3–6.3	68
	Opioids	LC–MS/MS	DBS, VAMS	0.1–1 (LLOQ)	70
	Oxycodone and metabolites	LC–MS/MS	DBS	0.15	71
	Tramadol	LC–MS/MS	DBS	2	31
	Tramadol	LC–MS/MS	DBS	5	69
S8: Cannabinoids	Synthetic cannabinoids	LC–MS/MS	DBS, VAMS	0.03–0.1	72
	THC	LC–HRMS	DBS	0.25	54
S9: Glucocorticoids	Corticosteroid	LC–HRMS	DBS	0.125–0.25	54
P1. Beta blockers	Beta blockers	LC–HRMS	DBS	0.05–0.125	54
	Propranolol	LC–MS/MS	DBS	0.5	80

Note: Each target or group was separated according to its corresponding references. Anabolic androgenic steroids (AASs) include androsterone, epitestosterone, etiocholanolone, testosterone, 5α-androstanediol, and 5β-androstanediol.

DBS cards, including DMPK-C cards and Whatman 903 protein saver cards, were used as sample-collecting devices, and volumetric microsampling devices such as TASSO® and VAMS, which are less susceptible to the Hct effect, were also applied. No significant differences in sensitivity were observed across sample types (38, 41, 63). According to WADA TD2026DBS, steroid esters are assigned a low Minimum Required Performance Levels of 1 ng/mL, and correspondingly, methods targeting this class tend to achieve low LODs. In contrast, small peptides such as GH-releasing peptides are subject to a higher MRPL of 20 ng/mL, which is reflected in the generally higher LODs reported for S2 group analytes. Although GC, immunoassays, and ELISA have also been employed, LC–MS-based methods were adopted for the DBS analysis. This overview enables rapid assessment of the analytical methods and performances of a substance class.

Emerging DBS applications beyond WADA TDs for novel antidoping analyses

DBS applications are increasingly extending beyond the small-molecule LC–MS/MS workflows, showing that DBS can serve as a practical platform for biologics, biomarker-driven research, and emerging gene-doping surveillance. A clear example is the adaptation of EPO receptor agonist (ERA) testing to low-volume DBS, where electrophoresis-based workflows met serum/plasma minimum performance expectations using a single DBS spot with conserved analytical stability under practical storage conditions.[81,82] Subsequent field and administration-oriented studies further strengthened the case by showing analysis of endogenous EPO using different microsampling formats, including comparisons with matched urine samples and evaluation of confounding biological patterns that can complicate interpretation.[83] Furthermore, controlled micro-dosing of a recombinant EPO and testosterone illustrated how DBS can be integrated with established urine-based approaches and confirmatory tools such as IRMS, emphasizing that sampling volume and format can influence practical sensitivity.[84]

Genetic characterization of the EPO c.577del variant addressed a potential ambiguity in electrophoretic EPO testing caused by non-specific binding. Moreover, DBS-based DNA analysis showed sequencing-based resolution, including systematic evaluation of DNA yield thresholds in the analysis of urine and DBS.[85,86] These approaches shift DBS from a replacement matrix to a problem-solving matrix that can accelerate decision-making when atypical patterns appear in analyses. Similarly, DBS has also been assessed for high-throughput sample authentication using single nucleotide polymorphism panels with matrix-assisted laser desorption Ionization-time-of-flight mass spectrometry. The risk of substitution or swapping was evaluated, and the guidelines for analysis of a routine steroid profile were established.[87] Such identity and interpretation tools will be essential if DBS expands into remotely supervised or decentralized collection models.

Emerging blood-doping surveillance likewise benefits from DBS but increasingly relies on longitudinal biomarker detection rather than single detection. As shown in Figure 4, complementary work using sodium *N*-lauroylsarcosinate polyacrylamide gel electrophoresis (SAR-PAGE) indicated that mRNA biomarkers are promising targets for micro-dose EPO treatment by generating passport-like monitoring even under altitude exposure, which causes variations in hematological status.[88] Additionally, mass-spectrometry-based targeted proteomics

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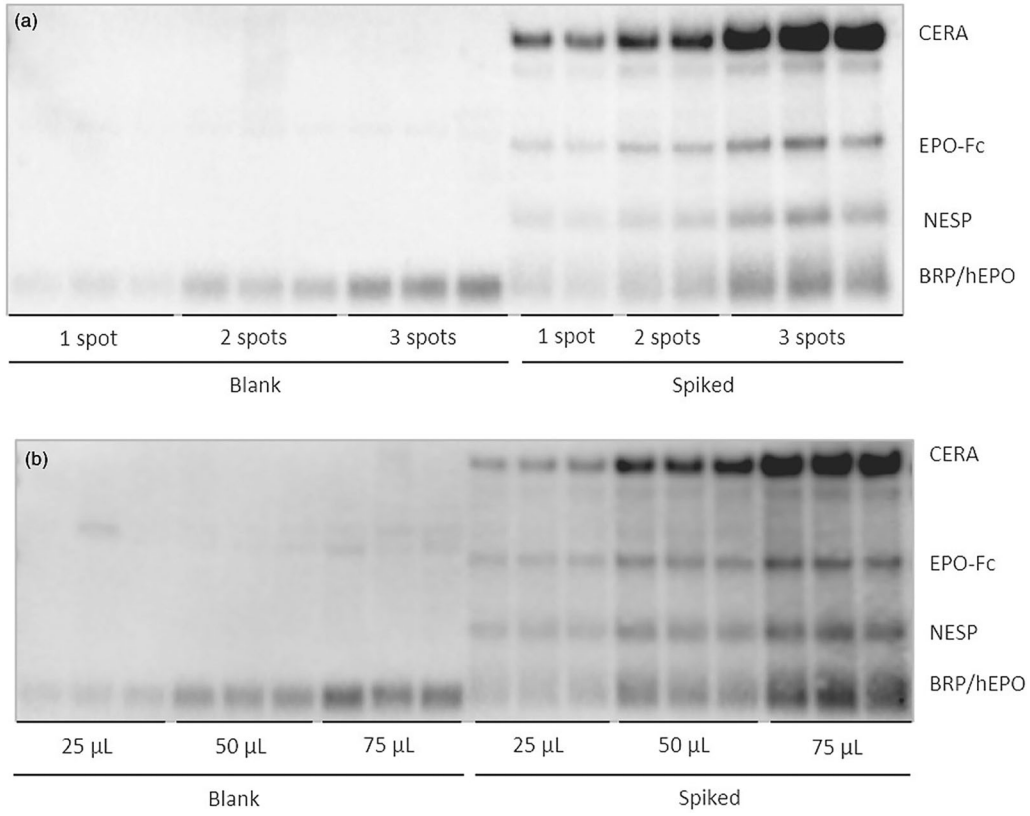

FIGURE 4 Detection of erythropoietin receptor agonists (ERAs) and endogenous erythropoietin (hEPO) using sodium *N*-lauroylsarcosinate polyacrylamide gel electrophoresis (SAR-PAGE) and immunodetection. (a) ERAs and hEPO signals vs. the number of DBS spots from 60 µL whole blood. (b) ERAs and hEPO signals vs. volume (from 20 µL to 75 µL) of whole blood on a single dried blood spot (DBS) (reprinted from Reference 87, Copyright (2022), with permission from the Wiley-VCH).

from capillary DBS established individualized blood protein reference using dense sampling methods.[89] It confirmed that DBS could be a suitable matrix for high-frequency omics with a moderate workload for quantitative analysis. Related proteomic and peptide-centric strategies also extended to manage longitudinal DBS workflows with estimation in the time since sampling using time-dependent targeted peptide ratios.[90]

DBS also provides a meaningful insight into gene-doping detection, where the analytical target is nucleic acid rather than a conventional small molecule. Previous work showed that EPO transgene targets can be detected from DBS using real-time polymerase chain reaction (PCR), which enables reanalysis with simple sample handling.[91] More recent method development focused on improving sensitivity, highlighting the better performance of polymeric volumetric devices than cellulose cards regarding transgene recovery and detection robustness, even at low concentrations.[92] Therefore, DBS has been recognized as an alternative matrix to fresh blood for illicit genetic targets. Related studies applied gene-doping concepts into animal and model systems, such as equine DBS testing using quantitative PCR and digital PCR, and mouse models coupled to next-generation sequencing workflows in automated laboratory settings.[93,94]

Extending analyses of protein and peptide, DBS was reported for biomarkers of recombinant human growth hormone exposure and for protein targets such as human chorionic gonadotropin using smart sampling, which is the integration of sample preparation into the sampler itself to simplifyworkflows.[95,96] Meanwhile, mass spectrometric workflows were introduced for complex biological targets, including myostatin-targeting monoclonal antibodies using affinity purification and field-asymmetric ion mobility spectrometry to mitigate interferences, expanding the range of molecular weight of target analytes.[97] Followed by the expansion of targets, toxicology in DBS, including simultaneous detection of cocaine and opiates by tandem MS and routine screening of exercise mimetics such as adiponectin receptor agonists in urine and DBS, further underscores that DBS method design hinges on optimizing sensitivity, extraction recovery, and matrix effects under realistic constraints.[98]

Blood doping detection using DBS-based biomarkers and multi-omics approaches

Blood doping is commonly understood as the illicit manipulation of an athlete's blood to increase red blood

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cell mass and thereby enhance oxygen delivery to working muscle, improving endurance performance. In practice, this is most often achieved through autologous or homologous blood transfusions. The detection of these molecules is challenging since the performance advantage can persist, while direct analytical traces are limited, and the detection window depends on timing, individual physiology, and hydration status. A DBS-based LC–MS/MS assay that quantifies the CD71/Band3 ratio showed improved sensitivity over conventional reticulocyte percentage to identify autologous transfusion effects for DBS-compatible longitudinal blood-doping surveillance.[99]

As it can enhance oxygen transport while leaving limited direct chemical traces, making biomarker-based strategies is vital. Building on DBS microsampling workflows, DBS offers a practical bridge between field collection and laboratory confirmation with a strong chain-of-custody. Recent approaches aimed at analysis of erythropoiesis stimulation through transcriptomic signatures from DBS using RNA panels extracted in an automated system, which is suitable for routine screening.[100] In particular, the RNA-based 5′-aminolevulinate synthase 2 has been proposed as a distinguished DBS biomarker for longitudinal monitoring of stimulated erythropoiesis.[101] Complementary to RNA markers, protein-level assays were studied to identify physiology related to blood doping in DBS, including mass-spectrometric analysis of membrane proteins regarding erythroid maturation and doping-related adaptations.[102] Targeted evaluation of homologous blood transfusion detection *in vivo* further reported how analysis of DBS would be employed for confirmatory testing beyond conventional matrices.[103] A genetic approach comparing mitochondrial and nuclear markers has also been explored to strengthen individual identification from the collected sample.[22] Finally, quantitative analysis of immature reticulocyte–related features in DBS by mass spectrometry offers an additional orthogonal axis for detecting erythropoietic manipulation.[104]

Evolution of DBS implementation at the Olympic Games: From pilot validation to operational integration

The Tokyo 2020 Olympic Games marked the first structured application of DBS analysis within the Olympic antidoping environment. Although capillary DBS sampling was not yet performed on site, 69 steroid ester analyses were conducted using EDTA whole blood that was sampled by DBS cards in the laboratory. To evaluate the analytical sensitivity and specificity under major event conditions, this method served as a transitional validation phase. Rather than an independent matrix, DBS serves as a complementary tool for conventional blood testing workflows, and has established the technical foundation for future implementation.[105,106]

A significant milestone was achieved at the Beijing 2022 Winter Olympic Games, where DBS was officially introduced as an independent analytical matrix within the antidoping program. Unlike ABP, a total of 105 DBS samples were analyzed under a testing menu that focused on six testosterone esters. HRMS (LC–HRMS, Q Exactive HF) was employed, and results were provided within a 24–48 h reporting framework. This implementation demonstrated that capillary dried blood sampling was not only analytically reliable but also operationally feasible for a mega sporting event.[107,108]

By the time of the Paris 2024 Olympic Games, DBS had transitioned from innovation to consolidation. A total of 196 DBS samples were analyzed, focusing on the presence of steroid esters, including testosterone, boldenone, and nandrolone esters. With fully integrated traditional blood and urine testing strategies, DBS analyses were conducted without significant reporting delays. This development, starting from laboratory-based validation in Tokyo, to official field implementation in Beijing, and ultimately to operational consolidation in Paris, revealed the maturation of DBS as a sustainable matrix within the Olympic antidoping framework.[109]

The operational viability of DBS-based strategies has also been demonstrated outside of the Olympic context. Large-scale events such as the FIFA World Cup reported successful blood-based antidoping testing under limited turnaround timelines, indicating the scalability and robustness of advanced blood-derived analytical approaches in major sporting events.[110]

Global implementation of DBS testing based on WADA annual reports (2022–2024)

According to the WADA Testing Figures, the implementation of DBS analysis has significantly increased over the past 3 years. In 2022, a total of 2748 DBS samples were analyzed worldwide. In 2023 and 2024, this number increased to 4242 and 5214, respectively. The 54% increase between 2022 and 2023 was followed by an additional 23% rise in 2024, resulting in a twofold expansion over the course of 3 years. These data suggest that DBS testing is progressively being integrated into the global antidoping testing framework.[111–113]

Although the absolute number of adverse analytical findings (AAFs) from DBS increased from 4 cases in 2022 to 11 in 2024, the overall AAF rate remained relatively low. This should be considered in the context of the current analytical scope of DBS testing. Compared with urine analysis, DBS currently covers a limited range of prohibited substances, which inherently reduces the total number of probable AAFs. Nevertheless, the AAF rate increased gradually from 0.15% in 2022 to 0.21% in 2024. This expanding trend implies that laboratories are progressively broadening the

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range of target analytes in DBS workflows to improve their analytical coverage and practical relevance.

The number of WADA-accredited laboratories that perform DBS analysis also evolved during this time period. In 2022, only 5 laboratories reported DBS testing, but this number doubled to 10 in 2023. In 2024, nine laboratories conducted DBS analyses. Notably, apart from the participation of the laboratory in the Olympic host country, no additional structural expansion was observed in 2024, which implies that broader laboratory adoption remains technically challenging. Method development, compliance with WADA TDs, and ISO/IEC 17025 accreditation requirements remain major barriers to wider implementation.

Overall, DBS testing has evolved from an exploratory initiative to a complementary matrix in antidoping practice, and further standardization and infrastructure development will be critical for its continued expansion.

CONCLUSION

Analysis of DBS has evolved from a clinical microsampling tool into an increasingly operational matrix for antidoping testing. Even though DBS enables minimally invasive collection and simplified transport with blood-based information on a large scale, it is not a simple substitution for liquid blood or urine. Inherent sources of variability, including Hct effect, volume uncertainty, substrate effects, and whole-blood matrix effects, must be managed. Recent literature reported that these limitations can be mitigated through strategies such as sampling, automated extraction, internal standardization, and sensitive LC–MS workflows tailored to small sample mass. Improved DBS-based analytical methods may confirm the possibility not only for antidoping analysis but also for diverse biomedical and analytical contexts. In forensic toxicology, DBS sampling has shown utility in the analysis of post-mortem specimens, where analyte stability in the dried matrix is a practical advantage over conventional liquid samples.[114] Environmental pollutants monitoring can also employ DBS. The DBS-platform combined with the LC–MS/MS method was validated for the analysis of pesticide monitoring in rat and human blood samples[115] (environmental pollutants). For therapeutic drug monitoring, LC–MS/MS-based simultaneous quantification of seven antiepileptic drugs from DBS and dried plasma spots was validated and applied clinically, indicating that home-based sampling is analytically feasible.[116]

Importantly, analytical progress has been made with regulatory clarity. WADA's TDDBS framework strengthens consistency in data interpretation, and method development is increasingly aligned with these requirements through reproducible, standardized workflows. Analytical improvements are evident for challenging targets such as steroid esters. Furthermore, applications of DBS beyond current technical coverage, such as ERAs-related testing and biomarker approaches, emphasize broader future utility. DBS is recognized as a complementary matrix for next generation antidoping and depends on continued harmonization of devices, automated workflows, and interlaboratory standardization.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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