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PERSPECTIVE

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Do dried blood spots have the potential to support result management processes in routine sports drug testing?—Part 2: Proactive sampling for follow-up investigations concerning atypical or adverse analytical findings

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Abstract

Capillary blood sampled as dried blood spot (DBS) has shown substantial potential as test matrix in sports drug testing in various different settings, enabling the analysis of numerous different drugs and/or their respective metabolites. In addition to established beneficial aspects of DBS specimens in general (such as the minimally invasive and non-intrusive nature, and simplified sample transport), a yet unexplored advantage of DBS in the anti-doping context could be the opportunity of preserving a source of information complementary to routine doping controls performed in urine or venous blood. Whenever follow-up investigations are warranted or required, frequently collected and stored (but yet not analyzed) DBS samples could be target-tested for the compound(s) in question, in order to contribute to results management and decision-making processes.

KEYWORDS
adverse analytical finding, atypical finding, doping, dried blood spot, sport

1 | CURRENT STATUS

Anti-doping laboratories aim at sustainably optimizing and refining test methods to accommodate the constantly increasing demands in routine doping controls, particularly concerning analytical sensitivity and, accordingly, retrospectivity[1] especially for substances prohibited at-all-times as documented in the World Anti-Doping Agency's (WADA's) prohibited list.[2] The considerable advances in knowledge concerning drug metabolism and renal elimination[3–6] combined with substantial improvements in analytical instrumentation have allowed for lowering detection limits to sub nanograms per milliliter levels for numerous urinary target analytes over the past decade. This is, on the one hand, a major accomplishment that has demonstrated its value and utility for instance in comprehensive re-testing programs such as those conducted on long-term stored doping control samples collected at different Olympic Games.[7] More than 100 adverse analytical findings (AAFs) not detected at the time of the respective games were revealed by meanwhile developed analytical strategies. On the other hand, the advances in instrument technologies and analytical sensitivity have also resulted in the detection of minute amounts of doping agents that an athlete might have been exposed to unknowingly and unintentionally.[8,9] This severely affects and complicates the decision-making processes of result management authorities and requires utmost vigilance and caution from the athletes to avoid the inadvertent intake even of trace amounts of prohibited substances.[10–12] Furthermore, the clarification of an unintentionally caused AAF ex post is in most instances extremely challenging[13] and, evidently, associated with immense procedural costs. As a matter of fact, reports on

AAFs presumed, substantiated, or unsubstantiated to originate from contamination scenarios are seen with increasing frequency.[14–19]

Anti-doping laboratories accredited by WADA operate under the regulations of the World Anti-Doping Code (WADC)[20] and corresponding international standards with its related documents. A central aspect of these documents is the definition of AAFs with "A report from a *WADA*-accredited laboratory or other *WADA*-approved laboratory that, consistent with the *International Standard for Laboratories*, establishes in a *Sample* the presence of a *Prohibited Substance* or its *Metabolites* or *Markers* or evidence of the *Use of a Prohibited Method*" and atypical findings (ATFs) with "A report from a *WADA*-accredited laboratory or other *WADA*-approved laboratory which requires further investigation as provided by the *International Standard for Laboratories* or related *Technical Documents* prior to the determination of an *Adverse Analytical Finding*".[20–22]

From the analytical perspective, criteria that characterize an AAF are unambiguously formulated and likewise is the definition of ATFs. With the implementation of the 2021 WADC, ATFs can also be issued for exogenous substances, which was previously limited to endogenous compounds only. For xenobiotics such as clenbuterol, the reporting of an ATF required an exemption notice that was installed in 2019, which contains instructions to laboratories and anti-doping organizations (ADOs) concerning the aforementioned "further investigation."[23] For clenbuterol in particular, there is an apparently incessant illicit use in selected breeding industries,[18,24–28] which has been a purported as well as proven source of clenbuterol findings in routine doping controls over the past decade.[29,30] However, providing evidence for the source of clenbuterol detected in an athlete's doping control sample based solely on analytical data from a single specimen has remained a yet unresolved challenge.[31–36]

2 | POTENTIAL STRATEGY IN SUPPORT OF IMPROVED RESULTS MANAGEMENT

For the case management, various situations may require attention including (but not limited to) the follow-up investigation concerning a (potentially) inadvertent exposure to a doping substance and the necessity of conducting further investigations into a reported ATF. A most desirable scenario for a fair and objective conduct of both inquiries is the availability of additional analytical data. Such data would be particularly informative if originating from samples collected regularly and frequently prior to and/or immediately after the routine doping control sample that produced the AAF or ATF, in order to support/verify or rebut/disprove presented arguments and potential situations that could have caused the AAF or ATF result under investigation (Figure 1).

A conceivable strategy addressing these demands would consist of offering a testing scheme based on the collection of dried blood spot (DBS) samples. As explored and successfully applied during the COVID-19 pandemic in research and pilot study settings, athletes can be subjected to remotely supervised test missions.[37,38] Such tests, sampled, for example, as DBS regularly every 14 days, could be collected and stored in a *WADA*-accredited facility (e.g., an anti-doping laboratory), and only in case of an AAF or ATF that necessitates and justifies further investigations, relevant DBS samples are analyzed. This approach offers

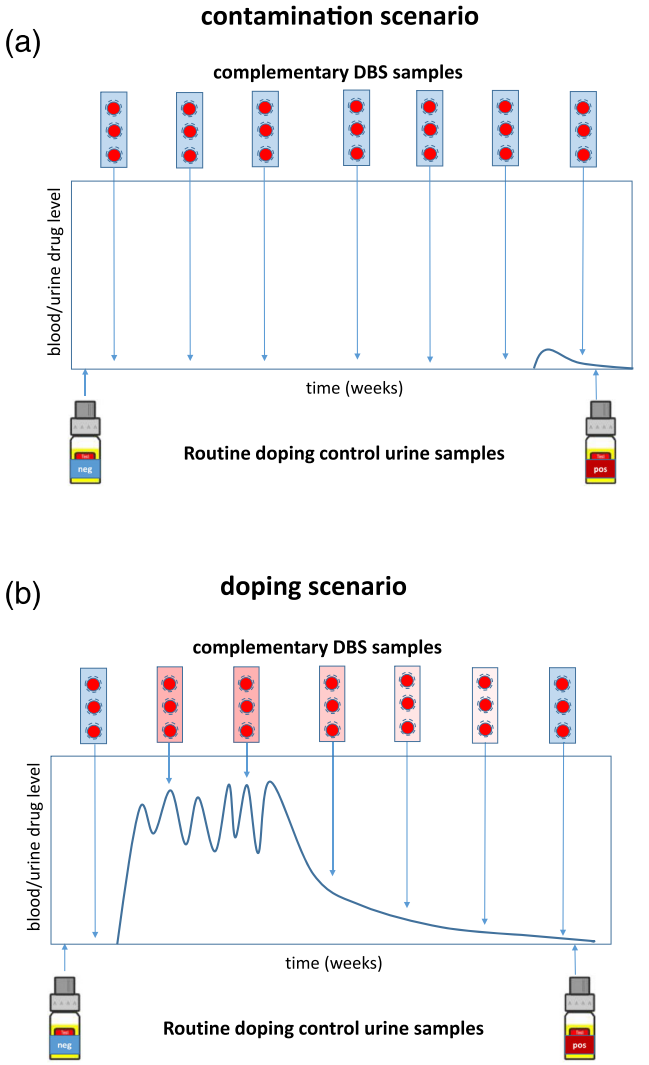

FIGURE 1 Cartoon of two hypothetical scenarios leading to an atypical finding/adverse analytical finding (ATF/AAF) in urine, where complementarily collected dried blood spot (DBS) samples analyzed "after the fact" of the urine sample test result contribute to decision-making processes. (a) Contamination scenario: DBS samples collected between a negative and a positive doping control sample suggest no repeated use of pharmacologically relevant amounts of a doping agent; (b) doping scenario: DBS samples collected between a negative and a positive doping control sample demonstrate the repeated use/exposure to pharmacologically relevant amounts of a doping agent

1. low-cost/low-effort and minimally invasive sample collections;
2. low-cost sample storage;
3. the asservation of test matrix to support follow-up investigations, if required;

4. the production of analytical data/evidence complementing routine doping control test results;
5. the potential of longitudinal monitoring of an athlete's exposure to doping agents.

This "stockpiling" of additional test samples will be advantageous to both the athletes and the ADOs whenever desirable or mandatory further investigations (e.g., in case of obligatory follow-up requests with ATFs) are to be conducted. Due to the fact that analyses of these additional samples are suggested to be only performed if regular routine doping controls produce an AAF or an unexplained ATF warrants in-depth investigations, extra costs are reduced to a minimum.

3 | HYPOTHETICAL SCENARIOS

1. An athlete's urine is tested positive for a low concentration of the selective androgen receptor modulator ligandrol. The closest doping control test prior to that particular sample had been collected 4 months before, with a negative result. The later positive test result may represent either the tail-end of an intentional doping scenario that took place weeks before sample collection or a situation of a much more recent (hours/days) inadvertent contamination (e.g., via food/food supplement), but the low testing frequency does not allow to establish one or another scenario. The athlete claims inadvertent exposure to the doping substance, but cannot substantiate this claim with evidence, and an anti-doping rule violation with a 4-year ban is likely.
2. An athlete with a very short haircut tests positive for clenbuterol (2 ng/ml) and an ATF is reported in accordance to WADA regulations, and follow-up investigations are required for results management. The closest doping control test prior to that particular sample had been performed 4 months before, with a negative result. Whether or not this is the result of an intentional intake of clenbuterol weeks before or represents a recent inadvertent contamination scenario (e.g., via consumption of contaminated meat) remains unclear due to the low testing frequency. The ATF follow-up is limited to largely (if not exclusively) non-analytical aspects such as the review of whereabouts and alimentary habits as in here, for example, hair sampling is not an option due to the above mentioned very short haircut.

For both scenarios, provided that the proposed (voluntary) fortnightly anti-doping-compliant DBS sampling would have existed, up to eight additional specimens could be used in support of the result management and decision-making process.

4 | ASPECTS TO ELABORATE

The proposed option of complementing routine doping controls by additional "contingency-plan" DBS tests will require the consideration of various aspects in order to create a strategy that contributes significantly to anti-doping procedures where information adding to an ATF or AAF is needed.

Such aspects would include at least

1. the coordination and organization of the DBS sampling
   a. managed, for example, by ADOs and testing authorities, including, for example, remotely observed sample collection by athletes or by visit of future test centers with dedicated (automated) DBS sampling option
   b. if required, sample authenticity can be corroborated by DNA test
2. the coordination of the transfer to and storage at WADA-accredited test facilities
   a. some ADOs and testing authorities offer these services
3. the management of incurring costs for DBS test samples
   a. incurring costs include DBS sampling, shipping, and storage
   b. coverage of expenses, for example, by ADOs/sponsors/athletes
4. the management of sample storage duration, documentation and chain of custody data
   a. for example, in accordance with International Standards for urine and blood sample storage
5. the definition of the analytical requirements and role of the provided additional data in accordance with the International Standard for Laboratories[22]
6. the definition of the status of the DBS sample (matrix; anti-doping sample), ownership, rights, and permissions regarding the additional test samples (e.g., who can request further investigations, under which circumstances, etc.).

5 | CONCLUSIONS

A growing number of cases have been reported where a presumably unintentional administration or exposure to doping agents, especially anabolic agents, had to be explored. Lengthy (and supposedly costly) proceedings followed, also because scientific evidence complementary to the AAFs obtained from a routine doping control sample were scarce. Frequent collections of additional test specimens covering especially those periods of reduced routine doping control sampling would offer the opportunity of producing further analytical data, facilitating the complex result management process and, eventually, supporting decisions on grounds of best possible information.

Also, if future analytical sensitivity and anti-doping regulations would introduce reporting processes, which increase the prevalence of ATFs in sports drug testing, strategies for efficient follow-up investigations are of essential benefit. One such strategy could exploit the extensive analytical experience with DBS matrix accumulated over more than a decade concerning analytes relevant in doping controls,[39–49] if as above frequently sampled and stored DBS (complementing the monitoring of drug use or drug exposure) are available.

The portfolio of testing options applicable to DBS samples in sports drug testing is constantly growing.[50] This, in combination with a substantially increased sampling frequency, provides not only the ADO with an efficient tool for result management purposes but also the athlete with a more comprehensive doping control record and general anti-doping programs with an additional tool for deterrence from doping. Due to the extremely diverse nature of doping agents and methods of doping, the above-presented thought experiment will not provide the desired information in all possibly occurring situations. Neither might the strategy of sample collection followed by analytical testing only in case of follow-up investigations be applicable to many ATFs or AAFs, nor might the proposed options to consider DBS in anti-doping be covered by currently enforced anti-doping regulations. Yet, taking potential applications of DBS into consideration for further developing the use of alternative matrices in anti-doping appears to be a worthwhile endeavor.

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