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Editorial

Broadening the Horizon of Antidoping Analytical Approaches Using Dried Blood Spots

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Advances in analytical instrumentation and, accordingly, analytical sensitivity and accuracy have been a major impetus for exploring new avenues and strategies in various arenas, including antidoping testing. The challenges and opportunities in using minimally invasively produced dried blood spots (DBS) in sports drug testing have been assessed for >2 decades (1), but the potential of DBS in doping control been recognized only in the past 10 years. This potential was fueled by specific needs, existing sampling and testing limitations, and newly introduced options for sample collection, processing, and analysis (2–9). The spectrum of analytes that can be qualitatively and/or quantitatively determined from DBS has continuously grown to include representative target analytes from all classes of prohibited substances and methods of doping as defined in the World Anti-Doping Agency's Prohibited List (10). Among this spectrum are erythropoiesis-stimulating agents and blood transfusion practices, which have presented 2 of the most complicated analytical challenges in sports drug testing. Recently, the door to detecting autologous blood transfusion was opened further, as shown by Cox et al. (11) in this issue of *Clinical Chemistry*. This method complements earlier approaches based on either venous blood testing (12) or DBS tests of alternative markers indicative of blood transfusions or stimulated erythropoiesis (13, 14).

At present, the central parameters of the hematological module of the Athlete Biological Passport are the hemoglobin concentration and the reticulocyte percentage (RET%), measured in venous blood samples that are collected, shipped, and analyzed using automated hematology analyzers under highly standardized and controlled conditions (15). The CD71 protein, which has been used to detect autologous blood transfusions, generally requires flow cytometry. The sampling and transport requirements, in addition to the limited shelf life of blood samples, create logistical burdens that can negatively affect testing frequency and/or flexibility. It is in this respect that the report by Cox et al. contributes to the detection of blood doping practices. These authors took 2 new approaches—the use of DBS and the measurement of 3 proteins (CD71, ferrochelatase [FECH], and coproporphyrinogen oxidase [CPOX])—that reflect different stages of reticulocyte maturation.

In brief, DBS (prepared from capillary blood sampled with 2 different collection devices and spotted on cellulose-based DBS cards) was subjected to a sequence of washing steps to eliminate soluble proteins while retaining target membrane proteins. Subsequently, stable isotope-labeled peptide internal standards with trypsin-cleavable extensions were added, and trypsinization yielded proteotypical peptides of CD71, FECH, CPOX, and band 3 plus corresponding isotopically labeled analogs, allowing for quantification of the target compounds by means of liquid chromatography-high resolution/high accuracy tandem mass spectrometry. The test method was optimized and extensively validated, demonstrating the assay's fitness for purpose regarding intra- and interassay precision, limits of quantification, digestion efficiency, matrix effects, and interferences.

This study demonstrates several benefits of DBS sampling and analysis strategy in providing information on erythropoiesis-stimulated responses of proteins from both immature reticulocytes (IRCs) and reticulocytes without the need for venipuncture-based sample collection and temperature-controlled and time-sensitive shipments. First, over a period of 29 days, the target proteins in DBS were found to be stable when DBS cards were kept under dry conditions at room temperature, enabling conventional mailing of DBS cards. Second, the obtained data on DBS-derived CD71 concentrations correlated well with IRC counts determined using the World Anti-Doping Agency–mandated hematology analyzer. Third, longitudinal intraindividual variation of CD71/band 3, FECH/band 3, and CPOX/band 3 from DBS over a period of 12 weeks demonstrated superior mean CV% compared with the mean CV% for venous blood measurement of IRCs, RET%, and immature reticulocyte fraction (IRF%) from venous blood analyzed by the hematology analyzer.

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The authors also assessed the potential presence of differences between capillary and venous blood samples. Although the capillary blood–collection devices that were used occasionally failed in producing the desired specimen because of blood clotting, using capillary instead of venous blood did not add variability to the obtained test results. Nevertheless, minor but statistically significant differences between the 2 types of sample collection (venous vs capillary) were found in case of one of the 2capillary blood collection devices.

It is important to note that this was a proof-of-principle study that showed the potential of the testing strategy by analyzing CD71, FECH, and CPOX from DBS or RET% and IRF% from venous blood samples that were collected from anemic patients, not athletes. IRCs respond particularly quickly to alterations in stimulating or suppressing erythropoietic signaling that are caused by, for example, increased blood concentrations of natural or endogenous (but also recombinant) erythropoietins or blood transfusions. Consequently, the chosen target proteins yielded significantly altered values in the patient cohort, exhibiting a response even larger than that determined by RET% and IRF%. However, as the authors indicated, further research is required to fully assess the effects of other potentially confounding factors on the identified markers (e.g., altitude training sessions commonly utilized by athletes, legitimate iron supplementation). Furthermore, authentic drug administration studies will eventually be required to probe for the assay's sensitivity and detection window, presumably in combination with additional biomarkers of altered erythropoiesis such as delta-aminolevulinate synthase 2, which was recently shown to be readily determined from DBS (14).

This study adds to the value of DBS in antidoping, showing how DBS-based doping controls offer options extending beyond target testing of doping agents and doping practices by complementing routine doping controls based on urine or blood sampling. This approach could be especially important when DBS is considered as a complementary test matrix, allowing an antidoping organization to provide an enhanced level of analytical information, for example, when urine sample–derived adverse analytical findings occur concerning drugs that are prohibited in competition only. DBS collected at the same time as the routine doping control urine specimen could be analyzed and might add information on the blood drug concentration that prevailed in competition in support of the subsequent decision-making processes (6). Furthermore, the simple sample collection and cost-effective transport and storage of DBS can be exploited to maintain (7) or increase (8) testing frequencies, potentially reducing time periods during which athletes are not subjected to drug tests and, thus, enhancing both deterrence and options for managing adverse analytical findings in which scenarios other than doping are argued.

DBS cannot and should not substitute for routine urine and blood samples because urine and blood offer superior detection capability for selected classes of doping agents. Balancing testing schemes and test matrices when considering sport discipline–related needs and making higher testing frequencies available in support of protecting the honest athlete could improve antidoping efforts, facilitated by DBS and newly established analytical approaches.

Nonstandard Abbreviations: DBS, dried blood spot; RET%, reticulocyte percentage; FECH, ferrochelatase; CPOX, coproporphyrinogen oxidate; IRC, immature reticulocytes; IRF%, immature reticulocyte fraction.

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