SUPPORTING DOCUMENT

FREQUENTLY ASKED QUESTIONS (FAQs) on the use of Dried Blood Spots (DBS) for doping control

1. In which documents are the requirements and procedures for DBS testing described?

The mandatory requirements related to the collection, initial storage and transport of DBS samples are described in Annex J of the ISTI "Collection, Storage and Transport of Dried Blood Spot Samples", while some requirements around DBS sample collection equipment are included in Article 6.3.4 of the ISTI. Additional guidance related to sample collection and transport procedures are also presented in the Guidelines for Sample Collection and WADA's template Doping Control Officer manual.

DBS Samples are, by definition, blood samples and thus an approved biological material in anti-doping testing. Therefore, relevant requirements for the analytical testing and storage of blood samples as described in the International Standard for Laboratories (ISL) shall be followed unless otherwise specified in the Technical Document on DBS for doping control (TD DBS).

This FAQ document is a supporting document to assist Anti-Doping Organizations (ADOs) and Laboratories with the implementation of Annex J of the ISTI and the TD2023DBS. Where the interpretation of any text within the FAQ is in contradiction with the ISTI and/or TD2023DBS, the ISTI and/or TD2023DBS shall prevail.

2. What is a DBS sample?

A DBS sample is a series of small volumes (drops) of capillary blood, which is collected by a puncture/incision of the skin to access capillaries (small blood vessels), applied onto an absorbent sample support and allowed to dry.

Similar sampling techniques that collect capillary blood for analysis without a drying step, often simply called capillary microsampling, are not within the scope of Annex J of the ISTI and of the TD DBS.

3. Is DBS sampling new?

DBS sampling and analysis is a method that was introduced in 1963 for the screening of phenylketonuria in newborn babies and its use has since then been extended to the screening of other metabolic disorders, as well as to other fields of application such as therapeutic drug monitoring and pharmacokinetic studies.

In the field of anti-doping, DBS testing has been researched by WADA-accredited Laboratories since 2000 and more recently by several Anti-Doping Organizations (ADOs), which have assisted in developing a strong basis for the use of DBS for doping control.

4. What are the advantages of DBS samples for doping control analyses?

- The collection of DBS samples is less intrusive than urine and less invasive than then current blood collection procedures involving venipuncture;

- The collection of DBS samples can be done within a few minutes[1], thereby decreasing the time an athlete spends in the doping control station;
- Depending on the type of collection device chosen and the local regulations, the collection of DBS samples does not require a phlebotomist;
- DBS samples are more stable than traditional blood and urine samples under the conditions of sample transportation and storage used in anti-doping.
    - Refrigeration during transport is not required. Compared with the current transport requirements for traditional blood samples, DBS samples can thus be inexpensively transported. This also brings the advantage of testing in remote locations, where collecting and transporting a traditional blood sample may be challenging;
    - Specific analysis may be performed for some Prohibited Substances or subclasses of Prohibited Substances, e.g. steroid esters, that may otherwise be more rapidly degraded in other types of samples;
- The storage of DBS samples only requires a limited space and may therefore decrease the cost of long-term storage compared to the other types of samples.

5. Which Prohibited Substances are within the scope of the TD DBS?

The TD2023DBS specifically covers the requirements for analytical testing procedures to be applied on DBS samples for the detection of Non-Threshold Substances with no Minimum Reporting Levels (MRL) only.

The application of DBS sample collection for the analysis of Non-Threshold Substances subject to an MRL or of Threshold Substances is expected to be addressed in further versions of the TD DBS (or other relevant documents) following the definition of pre-analytical and analytical requirements for quantitative analyses (including definition of method performance and substance reporting requirements). For instance, the effect of the hematocrit level (i.e. the proportion of red blood cells in blood) remains one of the main issues to overcome. Indeed, variations in hematocrit are known to impact the spot surface area (i.e. blood with a low hematocrit level will generally spread more over the DBS absorbent sample support than blood with a high hematocrit level), but can also lead to variations in analyte recovery, among other factors. This factor should be well controlled for an accurate quantification of Threshold Substances.

6. Can DBS samples completely replace the collection and analysis of urine and other blood samples?

No. DBS samples, at this time, can only be applied to Analytical Testing Procedures for the detection of Non-Threshold Substances with no MRL, limiting the number and type of analyses. Traditional urine and blood samples are still required to be collected for a comprehensive analysis of the full menu of Prohibited Substances and Prohibited Methods.

[1] Solheim SA, Ringsted TK, Nordsborg NB, Dehnes Y, Levernaes MCS, Mørkeberg J. No pain, just gain: Painless, easy, and fast dried blood spot collection from fingertip and upper arm in doping control. Drug Test Anal. 2021 Oct;13(10):1783-1790. https://doi.org/10.1002/dta.3135.

7. Which laboratories can analyze DBS samples?

The analysis of DBS Samples is performed in WADA-accredited Laboratories. However, it is currently not mandatory for all WADA-accredited Laboratories to conduct analysis of DBS samples. The list of the relevant WADA-accredited Laboratories will be maintained in ADAMS. Testing Authorities (TAs) are encouraged to contact the WADA-accredited Laboratories for guidance.

ABP Laboratories perform only ABP blood sample analysis, and as such do not have specific expertise or are not equipped for performing analysis of DBS samples (e.g. for chromatographic-mass spectrometric based analytical methods).

8. Can the Laboratories apply a Flexible Scope of ISO/IEC 17025 Accreditation to the analysis of DBS samples?

No, analytical testing procedures validated for a certain sample matrix (e.g. urine, plasma) shall be revalidated when used for capillary blood DBS samples. However, once an analytical testing procedure applied to DBS samples has been included in the Laboratory's Scope of ISO/IEC 17025 Accreditation, flexibility may be applied in line with the principles outlined in the ISL and the ILAC-G29/06:2020 *Guidelines for harmonization of scopes of ISO/IEC 17025 accreditation of WADA anti-doping laboratories*.

9. How should the results of DBS analytical testing be used?

Results of DBS analytical testing shall be reported in ADAMS as described in ISL 5.3.8.4 and managed in the same manner as the analytical findings coming from other types of samples. The Results Management Authority (RMA) would conduct Results Management for a potential Anti-Doping Rule Violation (ADRV) if the DBS sample result is reported as an Adverse Analytical Finding (AAF). No supportive evidence based on the analysis of urine and/or whole blood/serum/plasma samples is needed to confirm the results.

Results of DBS analytical testing may also assist in targeting specific types of analysis for certain Prohibited Substances and/or Prohibited Methods; be used for target testing and other testing strategies and intelligence; support other anti-doping rule violations; and/or support requests for the further analysis of stored samples.

10. Is it mandatory for TAs to conduct DBS testing?

No, DBS testing is not mandatory. At present, the goal is to have a harmonized adoption of DBS testing, not a universal adoption. With time and when the DBS sample analytical menu is extended, experience from the TAs will help optimize the planning of DBS testing and define further DBS testing strategies at a global level.

11. Who is responsible for collecting the DBS samples?

Due to the absence of venipuncture during DBS collection, in many jurisdictions, DBS samples may be collected by a Doping Control Officer (DCO) without the need for a Blood Collection Officer (BCO), if standard precautions from healthcare settings are followed and the DCO is suitably trained. Procedures for DBS sample collection and qualification of the personnel collecting DBS samples shall be consistent with the ISTI as well as with local standards and regulatory requirements.

Due to the specific training requirements for the successful collection of DBS samples, athletes are not authorized to collect their own samples, but may assist in the collection e.g. by initiating the button when using a device with integrated microneedle(s)/microlancet(s) after instructions from the DCO/BCO.

12. How are DBS samples collected and what type of DBS Sample Collection devices can be used?

While Annex J of the ISTI outlines the DBS sample collection procedures, based on the equipment used, the procedures will vary. In short, there are two main categories of DBS Sample Collection equipment, which impacts how the blood is collected:

1) Cellulose-based cards (or other absorbent sample support made of cellulose or of another material), used in conjunction with lancets, are used to collect the DBS sampled from the fingertip.

   The drop of blood from the puncture/incision can then be directly dropped onto the cellulose card or untreated capillaries can be used for the transfer of capillary blood onto the absorbent sample support. The use of capillary tubes is permitted but should not be encouraged. In any case, it is important to only use capillary tubes that are untreated and do not contain anticoagulants.

2) Devices with integrated microneedle(s)/microlancet(s) are used to collect the DBS samples from the upper arm.

   DBS sample collection devices with integrated microneedle(s)/microlancet(s) should allow the collection and direct depositing of the capillary blood on the absorbent sample support without physical manipulation (i.e. without a pipette or other specialized device for liquid handling).

Alternative suitable sites of puncture, such as earlobes or the abdomen, may be used for athletes with physical impairments, if needed. However, it is recommended to contact the equipment manufacturers for information on the performance of the available devices on alternative puncture sites.

The ADOs are responsible for choosing the type of Sample Collection Equipment after consultation with the WADA-accredited Laboratories. However, due to variations regarding the laws and regulations for the import and the use of DBS collection devices, the Sample Collection Authority (SCA) shall verify that the Sample Collection Equipment used is compliant with local regulatory requirements for medical devices where necessary, as well as any other applicable law or regulation.

13. What is the minimum volume of blood that should be collected?

The “A” and “B” absorbent sample support shall allow the collection of distinct “A” and “B” spots (or equivalent) with a minimum total of approximately 60 µL, including 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). Ideally, a minimum total of approximately 100 µL (3 “A” and 2 “B”) should be collected.

A volume of 20 µL is in principle sufficient for a chromatography-mass spectrometric analytical testing procedure. (Affinity-binding assays and electrophoretic methods are currently not within the scope of the TD2023DBS.) Therefore, a minimum volume of 40 µL for the “A” Sample allows to perform the Initial Testing Procedure (ITP) and the Confirmation Procedure (CP), if applicable, while 20 µL for the “B” Sample is the minimum required volume for performing the “B” CP.

When DBS samples are collected by finger-pricking and the drop of blood is directly applied onto the cellulose card, the exact volume deposited is not known. Typically, a spot volume of 20-70 µL is generated if free falling drops of capillary blood are collected, while the volume collected is 15-50 µL if a hanging drop is directly brought

into contact with the cellulose card [2]. The finger shall not touch the cellulose card and successive drops shall not be layered on top of each other, but each new drop shall be deposited in a new circle predefined on the card [3].

14. How should TAs advise the Laboratories of the type of analysis they require on a DBS sample?

TAs shall ensure that the type(s) of analysis required for each DBS sample is/are recorded at a minimum on the chain of custody documentation (or equivalent) shipped with the samples to the Laboratory or via another system that the TA has agreed with the Laboratory. This requires that clear instructions are provided to the DCO who is authorized to collect the DBS sample(s).

15. How should the DBS samples be transported?

As DBS samples are generally stable, they can be transported at ambient temperature and refrigeration is not necessary. However, if the SCA collects venous blood samples during the same sample collection session, DBS samples can be shipped refrigerated with the venous blood samples.

Similar to the urine and venous blood samples, DBS samples are sensitive to light exposure and should therefore be transported in a non-transparent transport box/bag.

16. Can a TA store a DBS sample in long-term storage and not analyze the sample collected until a future point in time?

No. As per the ISL, all samples received at the Laboratory must be analyzed (in the case of DBS samples, for at least one Prohibited Substance or Prohibited Method) unless there are documented valid irregularities for their rejection. Then, samples can be stored for further analysis for up to 10 years.

[2] Capiau S, Veenhof H, Koster RA, et al. Official International Association for Therapeutic Drug Monitoring and Clinical Toxicology Guideline: Development and Validation of Dried Blood Spot-Based Methods for Therapeutic Drug Monitoring. *Ther Drug Monit*. 2019;41(4):409-430. https://doi.org/10.1097/FTD.0000000000000643.

[3] WHO manual for HIV drug resistance testing using dried blood spot specimens, third edition. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO. https://www.who.int/publications/i/item/9789240009424.