SUPPORTING DOCUMENT

FREQUENTLY ASKED QUESTIONS (FAQs) on the Technical Document on Dried Blood Spots (DBS) for Doping Control (TD2021DBS)

1. What is a DBS Sample?

A DBS Sample is a blood sample that is collected by a puncture/incision of the skin to access capillaries (small blood vessels) and the collection of small volumes of capillary blood onto an absorbent Sample support which then are 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 the TDDBS.

2. 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 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 has assisted in in developing a strong basis for the use of DBS for Doping Control.

3. 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;
- The collection of DBS Samples can be done within a few minutes, usually in less than 5 minutes from the time the collection kit is selected;
- Due to the improved stability of DBS Samples,
  - Refrigeration during transport is not needed (unless Samples are exposed to extreme temperature variations). Compared with the current transport requirements for whole blood, DBS Samples can thus be inexpensively transported. This also brings the advantage of Testing in remote locations;

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1 The FAQs on the TD2021DBS is a supporting document to assist Laboratories and ADOs with the implementation of the TD2021DBS. Where the interpretation of any text within the FAQ is in contradiction with the TD2021DBS, the TD2021DBS shall prevail.

2 Unpublished data: Solheim SA, Ringsted TK, Andersen AB, Nordsborg N, Dehnes Y, Mørkeberg JS. What is the athletes’ preferred Dried Blood Spot (DBS) sampling site? Athletes’ and DCOs’ feedback.
- Special 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.

4. What is the goal of the TDDBS?

The TDDBS aims to harmonize DBS Testing by providing specific requirements and procedures for DBS Sample collection, transport, Analytical Testing and storage. The TDDBS is set to come into force on 1 September 2021.

DBS Samples are, by definition, blood Samples. Therefore, requirements for the collection, transport, Analytical Testing and storage of blood Samples as described in the International Standard for Testing and Investigations (ISTI) and the International Standard for Laboratories (ISL) shall be followed unless otherwise mentioned in the TDDBS.

5. Which Prohibited Substances are within the scope of the TDDBS?

This TD specifically covers the requirements for the validation of Analytical Testing Procedures to be applied on DBS Samples for the detection of Non-Threshold Substances only. The requirements for the analysis of Threshold Substances will be the scope of future documents or of revised versions of the relevant TD(s).

The quantitative determination of Prohibited Substances is expected to be addressed in further method development/validation. 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 recovery, among others factors. This factor should be well controlled for an accurate quantification of Prohibited Substances.

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

No. DBS Samples can only be applied to Analytical Testing Procedures for the detection of Non-Threshold Substances due to the small volume collected; this limits the number of and type of analyses. Urine and blood Samples (ABP blood and plasma/serum Samples) are still required to be collected for a comprehensive analysis of the full menu of Prohibited Substances and Prohibited methods.

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, often within a clinical setting, and as such do not necessarily 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. A Flexible Scope of ISO/IEC 17025 Accreditation (see ISL 4.4.2.2) does not apply when changing to another Sample matrix (e.g. from urine to DBS). However, once an Analytical Testing Procedure has been included in the Laboratory’s Scope of ISO/IEC 17025 Accreditation, flexibility may apply to add Analytes to the same Analytical Testing Procedure.

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 would conduct Results Management for a potential anti-doping rule violation if the DBS result is reported as an Adverse Analytical Finding. No supportive evidence based on the analysis of urine and/or plasma/serum Samples is needed to confirm the results. Results of DBS Analytical Testing may also assist in identifying the targeting of specific types of analysis for certain Prohibited Substances and/or follow up Sample urine or blood collections.

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 DCO without the need for a BCO as long as standard precautions from healthcare settings are followed and the DCO is properly and sufficiently trained. Procedures for DBS collection and qualification of the personnel collecting DBS Samples shall be consistent with TD2021DBS as well as 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.

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

For consistency with the other blood collection procedures, The Doping Control Officer (DCO)/Blood Collection Officer (BCO) shall ask the Athlete to remain seated for at least 10 minutes with their feet on the floor prior to the DBS collection.

There are two main categories of DBS Sample Collection devices:

1) Cellulose-based cards, used in conjunction with lancets, collects the DBS Sample from the fingertip.

   The drop of blood from the puncture/incision can then be directly dropped onto the cellulose card or untreated glass capillaries can be used for transfer of capillary blood onto the absorbent Sample support.

2) Devices with integrated microneedle(s)/microlancet(s) collects the DBS Sample 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 site 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.

Due to variations regarding the laws and regulations for the import and the use of DBS collection devices, the Sample Collection Authority 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 at least a total of 60 µL, including approximately 40 µL of capillary blood in the “A” spot(s) and with at least approximately 20 µL of capillary blood in the “B” spot(s). Ideally, at least a total of approximately 80 µL (3 “A” and 1 “B” or 2 “A” and 2 “B”) should be collected.

A volume of 20 µL is in principle sufficient for a chromatography-mass spectrometric Analytical Testing Procedures. (Affinity-binding assays and electrophoretic methods are currently not within the scope of the TD2021DBS.) Therefore, a volume of at least 40 µL
for the “A” Sample allows to perform the Initial Testing Procedure and the Confirmation Procedure (if applicable), while 20 µL for the “B” Sample is the minimum required volume for performing the “B” Confirmation Procedure.

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 of a volume of 20-70 µL is generated if free falling drops of capillary blood are collected, while the volume collected is of 15-50 µL if a hanging drop is directly brought into contact with the cellulose card³. 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 card⁴.

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 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 Doping Control Officer who is authorized to collect the Sample(s).
