# WADA Technical Document – TD2023DBS

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| Written by:<br><br>Reviewed by: | Collaborative DBS Working Groups / *WADA* Science<br><br>*WADA* Laboratory Expert Advisory Group | Approved by: | *WADA* Executive Committee |
| Date: | 17 November 2022 | Effective date: | 1 January 2023 |

# DRIED BLOOD SPOTS (DBS) FOR *DOPING CONTROL*
# Requirements and Procedures for Analytical *Testing* and Storage

## 1.0 Introduction and Scope

This *Technical Document* (*TD*) has been established to harmonize Dried Blood Spot (DBS) *Testing* by providing specific requirements and procedures for DBS *Sample* Analytical *Testing* and storage.

DBS *Samples* are, by definition, blood *Samples*, and shall be considered as such unless otherwise mentioned in this *TD*.

The *International Standard* for Laboratories (ISL) sets out the general requirements to be followed by Laboratories for the Analytical *Testing* and storage of blood *Samples*, and therefore, of DBS *Samples*. However, this *TD* describes specific technical requirements for DBS *Sample* Analytical *Testing* and storage and hence supersedes the ISL and other relevant *TD*s, where applicable.

This version of the TD DBS specifically covers the requirements for Analytical *Testing* Procedures to be applied on DBS *Samples* for the detection of Non-Threshold Substances without *Minimum Reporting Levels* (*MRL*) only.

DBS *Samples*[1] are collected by puncture/incision of the skin to access capillary vessels (small blood vessels). One DBS *Sample* consists of a series of small volumes of capillary blood, which are collected within the same *Sample* Collection Session and allowed to dry on an absorbent Sample support.

## 2.0 Analytical *Testing* of DBS *Samples*

Any aspects of the Analytical *Testing* of DBS *Samples* shall be done in accordance with Section 5 of the ISL and its related relevant *TD*s, Technical Letters and Laboratory Guidelines, unless otherwise specified in this *TD*.

[1] In this context, the term "DBS" refers to a capillary blood *Sample* that is collected and allowed to dry on an absorbent *Sample* support, including *Samples* collected by "spotting" capillary blood directly onto a cellulose-based card or other absorbent *Sample* support made of cellulose or of another material, as well as those collected via a specific device with integrated microneedle(s)/microlancet(s).
The collection of a venous blood *Sample* and its spotting onto an absorbent *Sample* support (e.g., cellulose paper), where the Sample is allowed to dry, is not considered a DBS *Sample*.

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## 2.1 Acceptance of DBS *Samples* for Analysis

The Laboratory shall analyze each DBS *Sample* received, unless the *Sample* meets any of the conditions listed in Article 5.3.3 of the ISL 2021 or the following condition:

In cases where the Laboratory receives two (2) or more DBS *Samples*, which are linked to a single *Sample* Collection Session from the same *Athlete* according to the *Doping Control* form, the Laboratory shall analyze only one (1) of the *Samples* collected, unless otherwise instructed by the *Testing* Authority (TA) and/or required by the Analytical *Testing* Procedure(s) as per the Comment below.

*[Comment: It is recommended that the Laboratory uses the Sample with the greater number of fully saturated blood spots (or equivalent). If necessary, the Laboratory may combine spots from two (2) or more DBS Samples, which are linked to a single Sample Collection Session from the same Athlete, in order to have sufficient volume to perform the required Analytical Testing Procedure(s) for both the Initial Testing Procedure [ITP] and Confirmation Procedure [CP].]*

### 2.1.1 *Samples* with Irregularities

As per Article 5.3.3.1 of the ISL 2021, the Laboratory shall observe and document conditions that exist at the time of *Sample* reception or registration that may adversely impact on the integrity of a *Sample* or on the performance of Analytical *Testing* Procedures. For DBS *Samples* specifically, additional examples of irregularities to be noted below include, but are not limited to:

- Absence of desiccant in the *Sample* container;
- *Samples* not dry; and
- *Sample* adhered to the container.

*[Comment: Unlike for other blood Samples (e.g., ABP blood Samples), the freezing of DBS Samples should not be considered an irregularity, because it would not impact the later performance of the Analytical Testing Procedures.]*

## 2.2 Initial Storage and DBS *Sample* Aliquoting for Analysis

DBS *Sample* aliquoting shall follow the general requirements described in the ISL (see ISL Article 5.3.4), with the following specifications for DBS *Samples*.

2.2.1 The Laboratory should maintain the DBS "A" *Sample* refrigerated and protected from light until analysis. Before aliquoting, the *Samples* should be allowed to approach room temperature in an airtight and dry container (e.g., desiccator, plastic box containing desiccant) to avoid condensation. The Laboratory shall obtain Aliquot(s) from the DBS *Sample* container by using clean tools (e.g., hole puncher, tweezers) to avoid contamination. The Aliquots taken should be saturated with blood. After Aliquots have been taken for analysis, if spots still remain in the "A" *Sample*, it should be returned to refrigerated storage until the ITP and the CP (if applicable) have been completed, and shall then be stored frozen (approximately at -20 °C) unless otherwise specified in a *WADA TD*, Technical Letter or Laboratory Guidelines. The "B" *Sample* shall be stored frozen

(approximately -20 °C) after reception until analysis (if applicable) or until disposal according to the ISL or other relevant *TD*(s).

*[Comment: In DBS automated analysis, Aliquots are not physically punched out from the DBS card. Therefore, the entire DBS card remains at room temperature until the ITP or CP has been completed.]*

2.2.2 If the DBS "A" and "B" *Samples* are in the same container, the "B" *Sample* can remain refrigerated until the ITPs and the CPs (if applicable) of the "A" *Sample* have been completed.

*[Comment: In all circumstances, appropriate steps to ensure the integrity of the Sample(s) shall be taken by the Laboratory.]*

2.3 Selection and Validation of Analytical *Testing* Procedures

The selection and validation of Analytical *Testing* Procedures shall be in accordance with Article 5.3.5 of the ISL, as applicable to Non-Threshold Substances without an *MRL*, with the following specifications.

2.3.1 The Laboratory shall have criteria (including a risk assessment) to determine if any significant changes to the DBS *Sample* collection device and/or to the absorbent *Sample* support (e.g., change from cellulose to another material) are conditions that should trigger a full or, at a minimum, a partial re-validation of the *Analytical Testing* Procedure.

2.3.2 *Analytical Testing* Procedures validated for a certain specific *Sample* matrix (e.g., urine, plasma) shall be revalidated when used for 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 plasma to DBS).

2.3.3 Analytical *Testing* Procedures applied to DBS *Samples* may present additional risks of carryover (e.g., punching step, automated workflow) and the appropriate conditions required to mitigate carryover of the Analyte from *Sample* to *Sample* during processing or instrumental analysis shall be determined during method development and validated to demonstrate Fitness-for-Purpose.

2.3.4 All validation parameters (e.g., Selectivity, carryover, LOD for the ITP, LOI for the CP) shall be evaluated with representative samples, using the same or similar *Sample* collection device/absorbent *Sample* support as the one that will be used for the *Samples*.

2.3.5 Calibrators, quality control (QC) and other types of reference samples can be generated from venous whole blood containing EDTA as anticoagulant. However, the venous blood sample shall be deposited and allowed to dry on the appropriate *Sample* collection device/absorbent *Sample* support to be used for the analysis.

*[Comment: The reference samples can also be DBS blood samples from controlled administration studies for positive QCs or negative (non-spiked) QC samples.]*

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2.4 Sample Analysis

DBS *Sample* analysis of Non-Threshold Substances without an *MRL* shall be done in accordance with the relevant provisions of Article 5.3.6 of the ISL, with the following specification for DBS *Sample* analysis:

2.4.1 "A" Confirmation Procedure

Aliquots

If the *Sample* collection device/absorbent *Sample* support used collects a volume greater than (>) 20 µL per spot and the spot is homogenous and saturated with blood, the new "A" Aliquot needed for the CP can be punched from the same spot as the one used for the ITP if no other spot is available.

3.0 Storage of DBS *Samples*

3.1 Initial Storage of DBS *Samples*

All DBS *Samples* retained for storage in the Laboratory shall be stored frozen with desiccant in a secure location under continuous chain of custody. The Laboratory shall keep all chain of custody and other records (either as hard-copy or in digital format) pertaining to those *Samples*.

a) DBS *Sample*(s) without an *Adverse Analytical Finding* or *Atypical Finding*:

The Laboratory shall retain the "A" and "B" *Sample*(s) without an *Adverse Analytical Finding* or *Atypical Finding* for a minimum of three (3) months after reporting the final analytical result in *ADAMS*, or for a maximum of ten (10) years after the *Sample* collection date, if the long-term storage of the *Sample*(s) has been requested, in writing, by the relevant TA or *WADA*[2].

b) DBS Samples with Irregularities:

The Laboratory shall retain the "A" and "B" DBS *Sample*(s) with irregularities for a minimum of three (3) months after reporting the final analytical result in *ADAMS*, or for a longer period as determined by the TA, *Results Management* Authority (RMA) or *WADA*[2].

c) DBS *Sample*(s) with an *Adverse Analytical Finding* or *Atypical Finding*:

The Laboratory shall retain the "A" and "B" DBS *Sample*(s) with an Adverse Analytical Finding or *Atypical Finding* for a minimum of six (6) months after reporting the final analytical result (for the "A" or the "B"

[2] The Laboratory may charge storage costs to the TA or *WADA*, as applicable, for the storage of *Samples* for periods longer than the stated minimum storage times. However, the Laboratory may store *Samples* beyond the applicable minimum storage times at their own discretion and expense. In such cases, the Laboratory shall inform the responsible TA. Any Further Analysis on these *Samples* will require the approval of the TA or *WADA*.

*Sample*, as applicable) in *ADAMS*[3,4], or for a longer period as informed to the Laboratory, in writing, by the relevant TA, RMA or *WADA*[2].

d) DBS *Samples* under challenge, dispute or investigation:

If the Laboratory has been informed by the TA, the RMA or *WADA* (in writing and within the applicable storage period as defined in this Article 3.1) that the analysis of a DBS *Sample* is challenged, disputed or under investigation, the Laboratory shall retain both the "A" and "B" *Samples* until further notice by the TA, the RMA or *WADA*, as applicable[2].

3.2 Long-term Storage of DBS *Samples*

At the direction of the TA or *WADA*, any DBS *Sample* may be placed in long-term storage for up to ten (10) years after the *Sample* collection date for the purpose of Further Analysis, subject to the conditions set out in Articles 5.3.6.3 of the ISL, and 3.1 of this *TD*. All requirements detailed in Article 5.3.11.3 of the ISL also apply to the long-term storage of DBS *Samples*.

[3] If the "B" *Sample* CP is not performed, the Laboratory may dispose of both the "A" and "B" *Samples* six (6) months after reporting the "A" *Sample* analytical result. However, if the "B" *Sample* CP is performed, then the Laboratory shall retain both the "A" and "B" *Sample*(s) for a minimum of six (6) months after reporting the "B" *Sample* analytical result.

[4] Nevertheless, the Laboratory shall contact and inform the relevant TA and *WADA* before disposing of any *Samples* with *Adverse Analytical Findings* for which the TA or RMA (if different) has not provided instructions about the performance or not of the "B" CP (see ISL Article 5.3.6.2.3).