Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

DRIED BLOOD SPOTS (DBS) FOR *DOPING CONTROL*Requirements and Procedures for Collection, Transport, <u>Analytical</u> *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* collection, transport, <u>Analytical *Testing*</u> 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 *Testing* and Investigations (ISTI) is applicable to the general principles of DBS *Sample* collection and transport, whereas the *International Standard* for <u>Laboratories</u> (ISL) sets out the general requirements to be followed by <u>Laboratories</u> for the <u>Analytical Testing</u> and storage of DBS *Samples*. However, this *TD* describes specific technical requirements for DBS *Sample* collection, transport, <u>Analytical Testing</u> and storage and hence supersedes the ISTI, the ISL and other relevant *TDs*, where applicable.

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

2.0 Conducting the DBS Sample Collection Session

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 <u>Sample Collection Session</u> and allowed to dry on an absorbent Sample support.

The conduct of the DBS <u>Sample Collection Session</u> shall follow the requirements of Articles 7.1 to 7.4.7 of the ISTI, where applicable to blood <u>Samples</u> ².

TD2021DBS Page 1 of 12

.

¹ In this context, the term "DBS" refers to a blood *Sample* that is collected and allowed to dry on an absorbent *Sample* support, including *Samples* collected by "spotting" blood directly onto a cellulose-based card or other absorbent *Sample* support made of cellulose, as well as those collected via a specific device with integrated microneedle(s)/microlancet(s).

² Articles 7.3.3, 7.3.6 c), 7.4.1 a) and 7.4.5 p) of the ISTI only apply to the collection of urine *Samples* and Articles 7.4.1 c) and 7.4.5 s) of the ISTI refer to requirements for the collection of *Blood Athlete Biological Passport (ABP) Samples* and hence are not relevant to the collection of DBS *Samples*.

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

The following additional information shall be recorded in relation to the DBS <u>Sample Collection</u> Session in addition to the requirements set by Article 7.4.5 of the ISTI:

In addition to the reference to the equipment manufacturer (ISTI, Article 7.4.5 k), detailed information on the model of the DBS <u>Sample Collection Equipment</u> (e.g., catalogue number) shall be recorded if the equipment manufacturer commercializes several DBS <u>Sample</u> collection kits.

The <u>Doping Control Officer</u> (<u>DCO</u>)/<u>Blood Collection Officer</u> (<u>BCO</u>) shall collect the <u>Sample</u> from the <u>Athlete</u> according to the protocol described in the ISTI, Annex D: Collection of Blood <u>Samples</u>, with the exceptions contained in Articles 2.1-2.4 of this <u>TD</u>.

2.1 Responsibility

Due to the absence of venipuncture during DBS collection, in many jurisdictions, DBS Samples may be collected by a trained <u>DCO</u> without the need for a specialized <u>BCO</u> if standard precautions in healthcare settings are followed and the <u>DCO</u> is properly and sufficiently trained. Procedures for DBS collection shall be consistent with local standards and regulatory requirements. The <u>DCO</u> and/or the <u>BCO</u> have the responsibility for:

- a) Collecting the DBS Sample;
- b) Ensuring that each Sample is properly identified and sealed;
- c) Answering relevant questions during the provision of the Sample; and
- d) Properly disposing of DBS sampling equipment that is opened but not used, or used pieces of equipment not sealed with the absorbent *Sample* support.

2.2 Requirements for DBS Sample Collection Equipment

For DBS Sample collection, the <u>Sample Collection Authority</u> (<u>SCA</u>) shall only use <u>Sample Collection Equipment</u> which meets the minimum requirements of ISTI Article $6.3.4 \, a) - n$), including the following amendments:

- a) Can withstand temperatures of -20 °C. Tests conducted to determine integrity under freezing conditions shall use the material that will be stored in the Sample containers, i.e., capillary blood applied on a DBS absorbent Sample support (superseding ISTI 6.3.4 f) (ii)); and
- b) Comply with the local and international regulations for the transport of DBS, if applicable (superseding ISTI 6.3.4 j).

[Comment: Unlike traditional urine and blood Samples, the shipment of DBS Samples is not subject to the regulations published by the International Air Transport Association (IATA).]

TD2021DBS Page **2** of **12**

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

In addition, the <u>Sample Collection Equipment</u> for DBS Samples shall:

- c) Comply with local regulatory requirements for medical devices where necessary, as well as any other applicable law or regulation;
- d) Contain unique labels (*Sample* code number) for containers and DBS absorbent *Sample* support (*e.g.*, DBS cellulose card); and
- e) Allow the collection, storage and secure transportation of DBS on absorbent *Sample* support that can be sealed as distinct "A" and "B" *Samples* (<u>Tamper Evident</u> kit consisting of "A" and "B" containers/sub-containers and/or storage sleeves/packages/receptacles).

[Comment: Due to logistical reasons at the <u>Laboratory</u>, it is recommended to seal the "A" and "B" Samples in separate containers. Transporting and/or storing "A" and "B" Samples in the same container is however acceptable, provided that they are sealed as distinct "A" and "B" Samples.]

More specifically, the DBS <u>Sample Collection Equipment</u> shall fulfill the following criteria:

Related to the equipment for DBS Sample collection specifically:

- f) Contain a single-use Sample collection device (e.g., disposable lancets to be used in conjunction with cellulose cards, devices with integrated microneedle(s)/microlancet(s)) for the puncture/incision and collection of capillary blood at the fingertip and/or from the upper arm (alternative sites of punctures may be authorized for Athletes with physical impairments, if required); and
- g) 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 approximately 40 μL of capillary blood in the "A" spot(s) and with at least a total of approximately 20 μL of capillary blood in the "B" spot(s).

[Comment: The volumes of 40 μ L for the "A" spot(s) and 20 μ L of capillary blood in the "B" spot(s) are the minimum volumes required for a <u>Laboratory</u> to perform chromatography-mass spectrometric <u>Analytical Methods</u>. However, it is recommended to collect a total of approximately 60 μ L of capillary blood in the "A" spot(s) and a total of approximately 40 μ L of capillary blood in the "B" spot(s) when possible.]

[Comment: Depending on the DBS <u>Sample Collection Equipment</u> used, the volume and number of spots may vary. If a spot has a small volume (e.g., less than 20 µL), several spots may be combined to perform the required <u>Analytical Testing Procedure(s)</u>. If a spot has a large volume, a sub-punch may be taken to perform the required <u>Analytical Testing Procedure(s)</u>.]

[Comment: Specific analyses require minimum volumes of dried blood; therefore, this should be taken into account when requesting multiple analyses from a single Sample. Additional Samples may be necessary, or analyses should be prioritized.]

TD2021DBS Page 3 of 12

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

In addition, the DBS <u>Sample Collection Equipment</u> should meet the following criteria:

- h) The absorbent Sample support should be made of untreated cellulose paper or alternative absorbent material (e.g., synthetic polymer). To use an alternative absorbent material other than the one initially validated, a method verification (e.g., Selectivity, Limit of Detection [LOD]/Limit of Identification [LOI]) at a minimum, shall be performed to demonstrate that the absorbent Sample support is Fit-for-Purpose;
 - [Comment: If specific absorbent Sample supports have been indicated in an applicable WADA International Standard, TD or Guidelines, then the use of an alternative Sample support shall be validated with the involvement of the relevant <u>Laboratory</u>(ies) and approved by WADA prior to use for Sample collection.]
- i) Allow a reliable and consistent DBS Sample collection (e.g., no clotting of blood before DBS is deposited onto the absorbent Sample support); data from the equipment manufacturer and/or results of testing by a testing institution that is independent of the manufacturer may be used for the assessment of product reliability (e.g., expected failure rate of DBS collection);
- j) Allow the collection of a known volume of capillary blood and its application on an absorbent *Sample* support and/or allow hematocrit correction/measurement;
- k) Have a built-in indicator or similar visual cues showing that an acceptable volume of Sample has been collected; and
- I) DBS Sample collection devices with integrated microneedle(s)/microlancet(s) should allow collection and direct depositing on the absorbent Sample support without physical manipulation by the <u>Sample Collection Personnel (e.g.,</u> does not require on-site pipetting at the <u>Doping Control Station</u>, thus avoiding risk of contamination of the DBS Sample).

[Comment: When a DBS Sample is collected by finger-pricking, the use of capillary tubes to transfer blood from the finger-prick to the absorbent Sample support 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.]

Related to the equipment for the sealing, transport, storage and <u>Laboratory</u> handling of the DBS *Samples*:

- m) The Sample container and/or storage sleeves/packages/receptacles shall contain a desiccant to allow the spots to dry expeditiously when already sealed (without having to wait before sealing) and offering protection against possible premature degradation or contamination of the Sample;
- n) The Sample container should be designed to prevent the absorbent Sample support from adhering to the Sample container (e.g., spacer); and

TD2021DBS Page 4 of 12

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

o) The "A" and "B" *Samples* should be noticeably and easily separable without physical manipulation of the absorbent *Sample* support after collection (*e.g.*, no cutting a DBS card with scissors).

2.3 Facilities

The <u>SCA</u> shall only use a <u>Doping Control Station</u> for DBS <u>Samples</u> which meets the minimum requirements of ISTI Article 6.3.2.

The *Doping Control* Station should:

- a) Contain a comfortable chair for Sample provision and any aftercare that may be required;
- b) Contain a clean and sanitized table or surface for the processing of the Samples; and
- c) Include adjacent hand-washing facilities.

2.4 DBS Sample Provision

Procedures involving blood collection shall be consistent with the local standards and regulatory requirements regarding precautions in healthcare settings where those standards and requirements exceed the requirements set out below.

- 2.4.1 The <u>DCO/BCO</u> shall wear gloves during the *Sample* collection process and until the *Sample* is sealed;
- 2.4.2 The <u>DCO/Chaperone</u> shall, where practicable, ensure the *Athlete* thoroughly washes their hands with water only prior to the provision of the *Sample* and until the *Sample* is sealed:
 - [Comment: Any traces of talcum powder, resin, or other products that Athletes use should be thoroughly cleaned, and disinfectant pads or swabs may be used if needed.]
- 2.4.3 The <u>DCO</u> and/or <u>BCO</u> shall ensure that the *Athlete* is offered comfortable conditions for the provision of the *Sample* ³;
- 2.4.4 The <u>DCO</u> and/or <u>BCO</u> shall assess the most suitable location for puncture that is unlikely to adversely affect the *Athlete* or their sporting performance (e.g., non-dominant hand/arm). This should be a site of puncture that is free of any calluses, cuts, scars and tattoos. The <u>DCO</u> and/or <u>BCO</u> should select an alternative suitable site of puncture for *Athletes* with physical impairments if applicable;

TD2021DBS Page **5** of **12**

³ The requirement for the *Athlete* to remain in a normal seated position with feet on the floor for at least 10 minutes prior to providing a *Sample* does not apply before the provision of a DBS *Sample*.

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

[Comment: The <u>DCO</u> and/or <u>BCO</u> should decide whether the DBS Sample be collected from the right or left hand/arm. However, they may not be given the choice of the collection between the hand or arm, as this is dependent on the Sample Collection Equipment used by the SCA.]

- 2.4.5 As part of the *Sample* collection, the *Athlete* shall warm the *Sample* collection site by, for example, washing the hands in warm water, shaking the hand/arm, massaging the puncture site, or placing the hand/arm in a warm blanket or equivalent;
- 2.4.6 The <u>DCO</u> and/or <u>BCO</u> shall clean the skin with a sterile disinfectant pad or swab. Disinfectant gels shall not be used. Once the disinfectant is completely dried, the <u>DCO</u> and/or <u>BCO</u> shall take the capillary blood *Sample* from the fingertip or an area on the upper arm using the DBS collection device in accordance with the instructions provided by the equipment manufacturers;

If the DBS Sample is collected from the fingertip:

- a) The middle or ring finger should be selected if possible. The little finger may also be selected but the collection may be more painful;
- b) The puncture should be done with a lancet, slightly lateral to the pad of the finger, on the last phalanx of the finger;
- Blood flow can be increased by gently massaging the proximal portion of the finger in a distal direction. However, squeezing or milking the finger should be avoided as it may cause hemolysis and dilution of the Sample;
- d) The first drop of blood shall be wiped away with a dry sterile compress/gauze pad;
- e) Only the drop of blood shall enter into contact with the DBS absorbent *Sample* support, while the finger shall not touch it. The drop of blood should not be smeared onto the absorbent *Sample* support; and
- f) Only one drop of blood shall be applied per spot, because the dipping of several drops onto the same spot would cause an inhomogeneous *Sample*.

If the DBS Sample is collected from the upper arm with a device with integrated microneedle(s)/microlancet(s):

The *Athlete* is permitted to press the button to engage the microneedle(s)/microlancet(s) after having received the necessary instructions from the <u>DCO/BCO</u>. The <u>DCO/BCO</u> is responsible for applying and removing the device from the *Athlete*'s arm;

2.4.7 The volume of capillary blood removed shall be adequate to satisfy the relevant analytical requirements for the *Sample* analysis to be performed, *i.e.*, at least a total of approximately 40 µL of capillary blood in the "A" spot(s) and at least a total of approximately 20 µL of capillary blood in the "B" spot(s) for chromatography-mass

TD2021DBS Page **6** of **12**

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

- spectrometric <u>Analytical Methods</u>. Other special analyses may require additional *Samples* and/or increased *Sample* volume;
- 2.4.8 The <u>DCO/BCO</u> shall verify that capillary blood is well deposited on the absorbent Sample support and that a sufficient number of spots in the "A" and "B" Samples (to produce a sufficient amount of capillary blood, as described above) are saturated with blood:
- 2.4.9 If the amount of capillary blood that can be collected from the *Athlete* at the first attempt is insufficient, the <u>DCO/BCO</u> shall repeat the procedure up to a maximum of three (3) attempts in total. Should all three (3) attempts fail to produce a sufficient amount of capillary blood, the <u>DCO</u> shall terminate the <u>Sample Collection Session</u> and record the reasons for its termination:
 - [Comment: An attempt is defined as the act of puncturing the skin, i.e., only if the lancet or microneedle(s)/microlancet(s) has(ve) been engaged and punctured the skin.]
- 2.4.10 If more than one attempt is needed, another site of puncture shall be selected by the <u>DCO/BCO</u>. The skin shall be cleaned and a new lancet/Sample Collection device shall be used for the puncture of the skin;
- 2.4.11 After collection, the <u>DCO/BCO</u> shall apply pressure to the puncture site(s) and shall then apply a dressing(s);
- 2.4.12 The <u>DCO</u> and/or <u>BCO</u> shall dispose of used pieces of equipment that are not sealed with the absorbent *Sample* support in accordance with the required local standards for handling blood;
- 2.4.13 The <u>DCO</u> and/or <u>BCO</u> shall be responsible if the *Sample* requires further minimal on-site processing, such as removal of the absorbent *Sample* support (*e.g.*, cellulose paper, cartridge) from the collection device and transfer into the <u>Tamper Evident</u> kit. The *Athlete* shall remain in the collection area and observe their *Sample* until it is sealed in a <u>Tamper Evident</u> kit;
- 2.4.14 The *Athlete* shall seal their *Sample* in the <u>Tamper Evident</u> kit as directed by the <u>DCO</u> and/or <u>BCO</u>. In full view of the *Athlete*, the <u>DCO</u> and/or <u>BCO</u> shall check that the sealing is satisfactory. The *Athlete* and the <u>DCO</u> and/or <u>BCO</u> shall sign the *Doping Control* form (DCF); and
- 2.4.15 The sealed Sample can be stored at room temperature in the sealed secure <u>Tamper Evident</u> kit until transport, but in a manner which minimizes the potential for Sample degradation due to factors such as time delays, exposure to light and extreme temperature variations.

TD2021DBS Page 7 of 12

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

3.0 Requirements for Transport of DBS Samples and Documentation

The transport of DBS *Samples* shall be done in accordance with Articles 9.1 to 9.3 of the ISTI, with the following specifications:

- a) DBS Samples can be shipped as non-hazardous materials using regular mail or courier services, subject to any applicable regulations;
- b) While the Sample containers shall be transparent, it is recommended to transport DBS Samples in a non-transparent transport box/bag to protect the Samples from light exposure; and
- c) DBS Samples can be transported at ambient temperature. If collecting other blood Samples (e.g., ABP Samples), they can also be shipped refrigerated. However, extreme temperature variations shall be avoided.

4.0 Analytical Testing of DBS Samples

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

4.1 Acceptance of DBS Samples for Analysis

The <u>Laboratory</u> 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 <u>Laboratory</u> receives two (2) or more DBS <u>Samples</u>, which are linked to a single <u>Sample Collection Session</u> from the same <u>Athlete</u> according to the DCF, the <u>Laboratory</u> shall analyze only one (1) of the <u>Samples</u> collected, unless otherwise instructed by the <u>Testing Authority</u> (<u>TA</u>);

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

4.1.1 Samples with Irregularities

As per Article 5.3.3.1 of the ISL 2021, the <u>Laboratory</u> 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 <u>Analytical Testing Procedures</u>. For DBS *Samples* specifically, additional examples of irregularities to be noted below include, but are not limited to:

a) Absence of desiccant in the Sample container:

TD2021DBS Page **8** of **12**

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

- b) Samples not dry; and
- c) 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.]

4.2 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*.

4.2.1 The <u>Laboratory</u> should maintain the DBS "A" *Sample* refrigerated and protected from light until analysis. Before aliquoting, the *Samples* should be allowed to reach room temperature in an airtight and dry container (e.g., desiccator, plastic box containing desiccant) to avoid condensation. The <u>Laboratory</u> shall obtain <u>Aliquot(s)</u> from the DBS *Sample* container by using clean tools (e.g., hole puncher, tweezers) to avoid contamination. The <u>Aliquots</u> taken should be saturated with blood (e.g., the back of a cellulose card may be visually inspected and <u>Aliquots</u> taken preferentially from areas of the spots that are fully saturated). After <u>Aliquots</u> have been taken for analysis, the "A" *Sample* should be returned to refrigerated storage until the <u>Initial Testing Procedure(s)</u> (<u>ITP</u>) and the <u>Confirmation Procedure(s)</u> (<u>CP</u>) (if applicable) have been completed, and shall then be stored frozen (approximately at -20 °C) unless otherwise specified in a *WADA TD*, <u>Technical Letter</u> or <u>Laboratory Guidelines</u>; 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); and

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

4.2.2 If the DBS "A" and "B" *Samples* are in the same container, the "B" *Sample* can remain refrigerated until the <u>ITP</u>s and the <u>CP</u>s (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 <u>Laboratory</u>.]

4.3 Selection and Validation of Analytical *Testing* Procedures

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

TD2021DBS Page **9** of **12**

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

- 4.3.1 The <u>Laboratory</u> shall have criteria to determine if any significant changes to the DBS Sample collection procedure 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;
- 4.3.2 <u>Analytical Testing Procedures</u> validated for a certain specific Sample matrix (e.g., urine, plasma) shall be revalidated when used for capillary blood DBS Samples. A <u>Flexible Scope of ISO/IEC 17025 Accreditation</u> (see ISL 4.4.2.2) does not apply when changing to another Sample matrix (e.g., from urine to DBS);
- 4.3.3 <u>Analytical Testing Procedures</u> applied to DBS <u>Samples</u> may present additional risks of carryover (e.g., punching step, automated workflow) and the appropriate conditions required to mitigate carryover of the <u>Analyte</u> from <u>Sample</u> to <u>Sample</u> during processing or instrumental analysis shall be determined during method development and validated to demonstrate <u>Fitness-for-Purpose</u>;
- 4.3.4 All validation parameters (e.g., <u>Selectivity</u>, carryover, <u>LOD</u> for the <u>ITP</u>, <u>LOI</u> for the <u>CP</u>) 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*; and
- 4.3.5 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 onto the appropriate *Sample* collection device/absorbent *Sample* support to be used for the analysis.

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

4.4 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:

4.4.1 "A" Confirmation Procedure

Aliquots

If the Sample collection device/absorbent Sample support used collects a volume greater than (>) 20 μ L 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.

TD2021DBS Page **10** of **12**

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

5.0 Storage of DBS Samples

5.1 Initial Storage of DBS Samples

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

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

The <u>Laboratory</u> 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 <u>TA</u> or *WADA* ⁴.

b) DBS Samples with Irregularities:

The <u>Laboratory</u> shall retain the "A" and "B" DBS <u>Sample(s)</u> with irregularities for a minimum of three (3) months after reporting the final analytical result in <u>ADAMS</u>, or for a longer period as determined by the <u>TA</u>, <u>Results Management Authority</u> (<u>RMA</u>) or WADA ⁴.

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

The <u>Laboratory</u> shall retain the "A" and "B" DBS <u>Sample(s)</u> with an <u>Adverse Analytical Finding</u> or <u>Atypical Finding</u> for a minimum of six (6) months after reporting the final analytical result (for the "A" or the "B" <u>Sample</u>, as applicable) in <u>ADAMS</u> ^{5,6} or for a longer period as informed to the <u>Laboratory</u>, in writing, by the relevant <u>TA</u>, <u>RMA</u> or WADA ⁴.

d) DBS Samples under challenge, dispute or investigation:

TD2021DBS Page **11** of **12**

_

⁴ The <u>Laboratory</u> may charge storage costs to the <u>TA</u> or *WADA*, as applicable, for the storage of *Samples* for periods longer than the stated minimum storage times. However, the <u>Laboratory</u> may store *Samples* beyond the applicable minimum storage times at their own discretion and expense. In such cases, the <u>Laboratory</u> shall inform the responsible <u>TA</u>. Any <u>Further Analysis</u> on these *Samples* will require the approval of the <u>TA</u> or *WADA*.

⁵ If the "B" Sample <u>CP</u> is not performed, the <u>Laboratory</u> may dispose of both the "A" and "B" Samples six (6) months after reporting the "A" Sample analytical result. However, if the "B" Sample <u>CP</u> is performed, then the <u>Laboratory</u> shall retain both the "A" and "B" Sample(s) for a minimum of six (6) months after reporting the "B" Sample analytical result.

⁶ Nevertheless, the <u>Laboratory</u> shall contact and inform the relevant <u>TA</u> and *WADA* before disposing of any Samples with Adverse Analytical Findings for which the <u>TA</u> or <u>RMA</u> (if different) has not provided instructions about the performance or not of the "B" <u>CP</u> (see ISL Article 5.3.6.2.3).

Document Number:	TD2021DBS	Version Number:	1.0
Written by:	Collaborative DBS Working Groups, WADA	Approved by:	WADA Executive Committee
Reviewed by:	WADA Laboratory Expert Group		
Date:	20 May 2021	Effective Date:	1 September 2021

If the <u>Laboratory</u> has been informed by the <u>TA</u>, the <u>RMA</u> or *WADA* (in writing and within the applicable storage period as defined in this Article 5.1) that the analysis of a DBS *Sample* is challenged, disputed or under investigation, the <u>Laboratory</u> shall retain both the "A" and "B" *Samples* until further notice by the TA, the RMA or *WADA*, as applicable ⁴.

5.2 Long-term Storage of DBS Samples

At the direction of the <u>TA</u> 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 <u>Further Analysis</u>, subject to the conditions set out in Articles 5.3.6.3 of the ISL, and 5.1 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*.

TD2021DBS Page **12** of **12**