WADA Technical Document – TD2026DBS

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Written by: Reviewed by:	*WADA* Science / DBS Working Group *WADA* Laboratory Expert Advisory Group	Approved by:	*WADA* Executive Committee
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Dried Blood Spots (DBS) for *Doping Control* Requirements and Procedures for Analytical *Testing* and *Sample* Storage

1.0 Introduction and Scope

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

The term "DBS" refers to a capillary blood sample that is collected by puncture/incision of the skin and then deposited directly onto an absorbent sample support and allowed to dry. DBS *Samples* may be collected by "spotting" capillary blood directly onto an absorbent sample support, either untreated cellulose or a synthetic polymer, or using a specific device with an integrated microneedle(s)/microlancet(s).

This version of the *TD* DBS specifically covers the requirements for Analytical *Testing* Procedures which are applied to DBS *Samples* for the detection of Non-Threshold Substances without a *Minimum Reporting Level* (*MRL*) only [1].

2.0 Analytical T*esting* of DBS *Samples*

Any aspects of the Analytical *Testing* of DBS *Samples* shall be done in accordance with Article 5.0 of the *International Standard* for Laboratories (ISL) [2] and its related relevant *TD*s, Technical Letters (TLs) and Laboratory Guidelines (LGs), unless otherwise specified in this *TD*.

2.1 DBS Collection Devices Requirements

The Laboratory shall receive DBS *Samples* collected by an *Anti-Doping Organization* (*ADO*) or *Delegated Third Party* (*DTP*) for which the DBS Collection Device meets the following criteria:

a) Has a sample support made of either untreated cellulose or synthetic polymer.

b) Collects a minimum (≥) of 15 µL capillary blood in each spot/pebble, with a minimum total of 3 spots/pebbles for the "A" *Sample* and 1 spot/pebble for the "B" *Sample*, and

[Comment to Article 2.1 b): The use of DBS Collection Devices, which collect low capillary blood volume per spot/pebble, may limit the application of different Analytical Testing Procedures. This applies, in particular, for Analytical Testing Procedures that differ in their Sample preparation/extraction protocols or require the whole spot/pebble to achieve the necessary detection sensitivity.

With a minimum three (3) "A" spots/pebbles, the Testing Authority (TA) may choose to request the application of one (1) or two (2) different Initial Testing Procedures (ITPs), while considering that if both procedures produce Presumptive Adverse Analytical Finding(s) (PAAF), only one (1) may be confirmed using the remaining "A" spot/pebble and, if needed, the one

available "B" spot/pebble. In those cases, the selection of the PAAF(s) to be confirmed shall be made in consultation with the TA.

Notwithstanding the above, a single spot/pebble may be used for different Analytical Testing Procedure(s) if i) they share the same Sample preparation/extraction protocols, and ii) the Analytical Testing Procedure has been validated to meet Test Method performance criteria in a volume less than a whole spot/pebble. Conversely, if available, the Laboratory may combine two (2) or more spots/pebbles from the same DBS Sample, or from different DBS Samples[1] linked to a single Sample Collection Session (SCS) from the Athlete, to have sufficient volume to perform the required Analytical Testing Procedure(s).

Whichever the DBS Collection Device used, the Laboratory's Analytical Testing Procedure shall meet the expected Test Method performance requirements [i.e., Limit of Detection (LOD) of ITPs or Limit of Identification (LOI) of Confirmation Procedures (CPs), where applicable] by using a single spot/pebble, as determined by the availability of only one (1) "B" spot/pebble.]

c) Collects "A" and "B" spots/pebbles that can be sealed separately.

[Comment to Article 2.1 c). While the ADOs may select for use any of the DBS Collection Devices that meet the criteria listed in Articles 2.1.a)-c), it is recommended that the Laboratory consults with their customers regarding their plans and capacity for selection and use of DBS Collection Devices before proceeding to the development and validation of the Analytical Testing Procedure(s).]

2.2 Acceptance of DBS *Samples* for Analysis

The Laboratory shall accept for analysis DBS *Samples* collected by an *ADO* or *DTP*, unless the *Sample* meets any of the following conditions.

a) The Laboratory does not have an Analytical *Testing* Procedure validated for the DBS Collection Device received.

b) In cases where the Laboratory receives two (2) or more DBS *Samples*, which are linked to a single SCS from the *Athlete* according to the *Doping Control* Form (DCF), and if a single analysis has been requested, the Laboratory shall analyze only one (1) of the DBS *Samples* collected, unless otherwise instructed by the TA and/or required for the performance of the Analytical *Testing* Procedure. The second DBS *Sample* shall be stored frozen to allow future analysis on request (see Article 6.1.c).

[Comment to Article 2.2 b): However, if multiple Testing menus have been requested, the Laboratory shall use as many of the collected DBS Samples as needed to perform the applicable Analytical Testing Procedures.]

c) In cases where the Laboratory receives DBS *Samples* with irregularities (see Article 2.2.1).

[1] "A" and "B" spots/pebbles from different DBS *Samples* shall not be combined.

2.2.1 DBS *Samples* with Irregularities

The Laboratory shall observe and document conditions that exist at the time of DBS *Sample* reception or registration [2] that may adversely impact on the integrity of a *Sample* or the performance of Analytical *Testing* Procedure(s). For DBS *Samples* specifically, examples of irregularities to be noted include, but are not limited to:

a) Insufficient number of spots/pebbles to perform the required Analytical *Testing* Procedure(s).

b) Non-filled spots/pebbles.

c) Low spot volume.

d) Double spots, *i.e.*, the *Sample* does not form a single clear spot but instead appears as two (2) overlapping or separate spots.

e) Absence of desiccant in the *Sample* container.

f) *Sample* not dry.

g) *Sample* adhered to the container.

h) Spots *not* visible for inspection.

[Comment to Article 2.2.1: Unlike for other blood Samples (e.g., ABP blood Samples), the freezing of DBS Samples shall not be considered an irregularity, because it would not impact the later performance of the Analytical Testing Procedures.]

In these cases, the Laboratory shall immediately inform the TA on the possible impact of the noted irregularity(-ies) on the requested analysis and shall include the noted irregularity(-ies) in the *Sample* record.

The Laboratory may analyze *Samples* with irregularities if the irregularity does not impact the *Sample*'s chain of custody/unique identification or the suitability of the *Sample* to be analyzed with the requested *Testing* menu.

For those *Sample* irregularities that affect the *Sample*'s chain of custody/unique identification or its analytical suitability, the Laboratory shall seek instructions from the TA, in writing, on the performance of Analytical *Testing* on the *Sample*.

[2] For some DBS Collection Devices, irregularities might be detected only after the opening of the *Sample*.

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3.0 Initial Storage of DBS *Samples* and Aliquoting for Analysis

The initial storage of DBS *Sample*s shall adopt the following requirements to ensure the stability and integrity of the *Sample* and minimize storage time at room temperatures.

a) The Laboratory shall maintain the DBS "A" *Sample* refrigerated or frozen and protected from light and moisture until analysis. Before aliquoting, the *Sample* shall be allowed to approach room temperature in an airtight and dry container (*e.g.*, desiccator, plastic box containing desiccant) to avoid condensation.

b) The Laboratory shall store the DBS "B" *Sample* frozen with desiccant after reception and until analysis (if applicable) or until disposal.

c) However, if the DBS "A" and "B" *Samples* are in the same container, the "B" *Sample* can remain refrigerated and protected from light and moisture until the ITP(s) and the "A" CP, if applicable, have been completed.

[Comment 1 to Article 3.0: In all circumstances, the Laboratory shall take appropriate steps to ensure the integrity of the Sample(s).]

DBS *Sample* aliquoting [3] shall follow the general requirements described in the ISL [2], with the following specifications for DBS *Samples*.

a) The Laboratory shall aliquot, *i.e.*, take spots/pebbles, from the DBS *Sample* container by using clean tools (*e.g.*, hole puncher, tweezers) to avoid contamination.

b) After spots/pebbles have been taken for extraction and analysis, and any ITP(s) and/or CP(s) have been completed, any spots/pebbles remaining in the "A" *Sample* shall be stored frozen, unless otherwise specified in a *WADA TD*, TL or LGs.

[Comment 2 to Article 3.0: In DBS automated analysis, spots/pebbles are not physically punched out from the DBS Collection Device. Therefore, the entire DBS Collection Device remains at room temperature until the ITP(s) and CP(s), if applicable, have been completed.]

[3] For DBS *Samples*, Aliquots are intended as separate spots/pebbles removed from the *Sample* support.

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4.0 Selection and Validation of Analytical *Testing* Procedures

The selection and validation of Analytical *Testing* Procedures for DBS analysis shall be in accordance with the ISL [2], as applicable to Non-Threshold Substances without an *MRL*[1], with the following specifications:

a) The Laboratory should validate and implement the Analytical *Testing* Procedures included in the Minimum DBS *Testing* Menus (see Annex A of this *TD*). The Laboratory may implement additional Test Methods or target Analytes for the analysis of particular Non-Threshold Substances without *MRL*[4].

b) When available, DBS samples shall be used as Quality Control (QC) samples (for example, DBS samples obtained prior to and after a controlled drug administration may serve as Negative (NQC) and Positive (PQC) QC samples, respectively). However, if there are no available DBS samples, QC samples can be generated, for example, from whole venous or capillary blood containing EDTA as anticoagulant by spotting them and allowing them to dry on the same DBS *Sample* Collection Device/absorbent support that will be used for *Sample* analysis.

c) For the preparation of PQC, the Laboratory should avoid spiking the Reference Materials (RMs) directly on the *Sample* absorbent support; it is preferable to spike/fortify whole venous or capillary blood before spotting it on the adsorbent support.

d) All validation parameters (as per the ISL[2]) shall be evaluated with representative samples, collected from different individuals, using the same DBS *Sample* Collection Device/absorbent support and analytical protocol as the one that will be used for the *Samples*. Whenever possible, it is recommended to use capillary blood for the evaluation of Selectivity.

e) Analytical *Testing* Procedures validated for a 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 [2]) does not apply when changing to another *Sample* matrix (*e.g.*, from plasma to DBS).

f) For Analytical *Testing* Procedure validated in DBS, any of the following conditions requires at least a partial re-validation:

   i. Any significant changes to the DBS *Sample* Collection Device and/or to the absorbent sample support (*e.g.*, change from untreated cellulose to synthetic polymer, or change between two synthetic polymers) shall trigger the reassessment of Selectivity and LOD of ITPs and/or LOI of CPs.

[4] Laboratories are not required to validate the Test Methods on multiple DBS Collection Devices/sample supports. The Laboratory should select the most appropriate DBS Collection Device(s)/sample support(s) taking into consideration geographical availability and *Testing* needs, in consultation with its customers. See also Comment to Article 2.1 c).

ii. Any significant changes to the DBS *Sample* preparation workflow (*e.g.,* punching and automation specific steps) shall trigger the reevaluation of carryover.

5.0 *Sample* Analysis

DBS *Sample* analysis of Non-Threshold Substances without an *MRL* shall be done in accordance with the relevant provisions of the ISL [2] and applicable *TDs* (*e.g.*, *TD* IDCR [3], *TD* EPO [4]), TL*s* or LGs, with the following specifications for DBS *Sample* analysis.

5.1 "A" CP

A new "A" Aliquot shall be used for the "A" CP. Therefore, the "A" CP Aliquot shall not be obtained (punched or cut out) from the same spot/pebble as the one used for the ITP.

5.2 "B" CP

For the performance of the "B" CP, a new Aliquot(s) shall be obtained from the designated "B" spot(s)/pebble(s).

6.0 Storage of DBS *Samples*

6.1 Short-term Storage of DBS *Samples*

a) All DBS *Samples* retained for storage in the Laboratory shall be stored frozen and protected from light with desiccant.

b) DBS *Samples* shall be stored, at a minimum, for six (6) months after reporting all results ("A" and "B" *Samples*, if applicable) in *ADAMS,* unless the *Sample* has been identified for long-term storage (see Article 6.2) or for secondary use (in which case direct DBS *Sample* identifiers shall be removed - see ISL [2]).

c) Not analyzed DBS *Samples* (including *Samples* with irregularities) shall also be stored, at a minimum, for six (6) months after *Sample* reception, or for the storage period requested by the TA (if longer).

6.2 Long-term Storage of DBS *Samples*

At the direction of the TA or *Results Management* Authority (RMA, if different) 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 the ISL [2]. The *ADO* requesting the long-term storage shall be responsible for any costs associated with an extended DBS *Sample* storage beyond the minimum six (6) months established for the short-term storage.

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7.0 References

[1] *TD* MRPL: *Technical Document* Minimum Required Performance Levels and Applicable *Minimum Reporting Levels* for Non-Threshold Substances Analyzed by Chromatographic-Mass Spectrometric Analytical Methods.

[2] *ISL*: The World Anti-Doping *Code International Standard* for Laboratories.

[3] *TD* IDCR*: Technical Document* on Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for *Doping Control* Purposes.

[4] *TD* EPO *Technical Document* on Harmonization of Analysis and Reporting of Erythropoietin (EPO) and other EPO-Receptor Agonists (ERAs) by Polyacrylamide Gel Electrophoretic (PAGE) Analytical Methods.

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Annex A – Minimum DBS *Testing* Menus

Table A.1 Multi Class Menu [5]

Prohibited Class	**MRPL** (ng/mL)
S1.1 Anabolic Androgenic Steroids (AAS)	10
S1.2 Other Anabolic Agents - SARMs	2
S2.1.2 HIF Activating Agents	200
S3. Beta-2 Agonists	10
S4.1. Aromatase Inhibitors	10
S4.2. Anti-Estrogenic Substances	10
S4.4. Metabolic Modulators	10
S5. Diuretics And Masking Agents[6]	10

Table A.2 Steroid Esters Menu

Prohibited Class (Specific Examples)	MRPL (ng/mL)
S1.1 Anabolic Androgenic Steroids (AAS) - Steroids Esters	
- Boldenone	2
- Nandrolone	2
- Testosterone	1

[5] For each class of *Prohibited Substances* listed in Table A.1, this excludes Threshold Substances (*e.g.,* salbutamol, formoterol) and Non-Threshold Substances with *MRL* (*e.g.*, higenamine, salmeterol, tetroquinol, clomifene, meldonium or those diuretics associated with contaminations – see TL24).

[6] Brinzolamide and dorzolamide shall not be included as part of the Analytes in the DBS Multi Class Menu.

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Table A.3 Small Peptides Menu

Prohibited Class	**MRPL** (ng/mL)
S2.2.1 Testosterone-stimulating Peptides in Males	20
S2.2.3 Growth Hormone (GH) Fragments	20
S2.2.4 Growth Hormone Releasing Factors - GH Secretagogues (GHS) - GH Releasing Peptides (GHRPs)	20
S2.3. Growth Factors and Growth Factor Modulators - TB-500	20
S5. Diuretics and Masking Agents - Desmopressin, Vasopressin	20

Table A.4 ERAs Menu

Prohibited Class (Specific Examples)	**MRPL**[7]
S2.1.1 Erythropoietin Receptor Agonists	
- rEPO	30 IU/L
- dEPO	30 pg/mL
- CERA	150 pg/mL
- EPO-Fc	150 pg/mL

[7] See also TD EPO [4]