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Blood Analytical Requirements
for the *Athlete Biological Passport*

1. Introduction

This Technical Document (TD) has been established to harmonize the analysis of blood *Sample*s collected, both *In-Competition* and *Out-of-Competition*, for the measurement of individual *Athlete* blood *Markers* within the framework of the *Athlete Biological Passport* (*ABP*).

The *International Standard* for Laboratories (ISL) is applicable to the analysis of blood *Sample*s carried out in connection with the measurement of individual *Athlete* blood *Markers* within the framework of the *ABP*. This TD describes certain specificities of blood analysis related to the *ABP*.

To standardize analytical results in the *ABP* framework, blood *Sample*s shall only be analyzed in the dedicated network of Laboratories (i.e. *WADA*-accredited or *WADA*-Approved Laboratories for the *ABP*) which are accredited or approved by WADA to perform the analysis and with analyzers of comparable technical characteristics. The instrumentation and test shall by validated and ISO/IEC (17025 or 15189) accredited and the Laboratories shall participate in the *WADA* External Quality Assessment Scheme (EQAS) for blood samples prior to analysis of *Doping Control Sample*s.

If not reasonably possible for blood *Sample*s to be analyzed in a Laboratory or *WADA*-Approved Laboratory for the *ABP* for technical and/or geographical reasons, blood *Sample*s can be analyzed at a satellite facility of a Laboratory or using mobile units operated under applicable ISO/IEC accreditation (17025 or 15189) by a Laboratory. Satellite facilities and mobile units shall also be validated, ISO/IEC (17025 or 15189) accredited and participate in the *WADA* EQAS for blood samples prior to analysis of *Doping Control Sample*s. *Sample* handling shall be conducted in compliance with the Technical Document on Laboratory Internal Chain of Custody (TD LCOC).

2. Sample Reception and Timing

The blood *Sample* shall be analyzed as soon as possible upon reception and no later than 12 hours of *Sample* reception unless the *Sample* Collection Authority provides specific information regarding the *Sample* collection and transportation conditions

which would allow the Laboratory to extend the time window of the analysis of the *Sample* without affecting blood stability.

In cases when the Laboratory or *WADA*-Approved Laboratory for the *ABP* is unable to immediately analyze the *Sample* after reception, the Laboratory or *WADA*-Approved Laboratory for the *ABP* is responsible for maintaining the *Sample* at a cool temperature (approximately 4°C) between reception and the start of the analytical procedure. The temperature data logger shall accompany the *Sample* until Sample homogeneization. The blood *Sample* shall not be aliquoted before analysis[1].

If there is a Laboratory or *WADA*-Approved Laboratory for the *ABP* deviation from the aforementioned procedure, the Laboratory or *WADA*-Approved Laboratory for the *ABP* shall proceed with the analysis and report the results into *ADAMS* with a detailed description of the deviation.

3. Instrument Check

Before performing any blood analyses, all reagents must be verified to ensure that they are within their expiration dates, and that they comply with the reagent manufacturer's recommendations. Operational parameters of the instrument must be properly controlled (background level, temperature of the incubation chambers, pressure, etc.) and fall within the manufacturer's specifications.

All internal quality controls (levels 1, 2 and 3) shall be analyzed twice consecutively following the specifications provided by the manufacturer prior to the analysis of *Samples*. All results shall be in agreement with the reference value ranges provided by the manufacturer. These internal quality controls shall be furnished exclusively by the manufacturer of the instrument and handled in strict accordance with the specifications provided by the manufacturer (e.g. expiration dates, storage conditions). The internal quality controls shall be monitored via quality control charts with appropriate control limits.

At least one internal quality control from the manufacturer (either level 1, 2 or 3) shall be analyzed after every 30 to 50 blood *Samples*. At the end of each analysis session and after all blood *Sample* analyses are completed, one internal quality control (either level 1, 2 or 3) shall be analyzed once again to demonstrate the continuous stability of the instrument and the quality of the analyses done.

On a regular basis (as determined by the head of the Laboratory or *WADA*-Approved Laboratory for the *ABP*), one fresh blood *Sample* shall be homogenized for a minimum period of 15 minutes on an appropriate mixer (e.g. roller mixer) and then analyzed seven consecutive times. Coefficients of variation shall be below 1.5% for

[1] It is possible to aliquot the *Sample* after analysis for the *ABP*, when appropriate.

Haemoglobin (HGB) and Haematocrit (HCT), and below 15% for Reticulocyte percentage (RET%) to confirm the appropriate precision of the instrument.

4. External Quality Assessment Scheme

The Laboratories (or as otherwise approved by *WADA*) shall participate in and meet the requirements of *WADA's* EQAS for blood variables. The external quality controls shall be analyzed multiple times consecutively (based on the EQAS rules), and then the mean results of the following blood variables (full blood count) shall be returned:

Red Blood Cell (Erythrocyte) Count	RBC
Mean Corpuscular Volume	MCV
Haematocrit	HCT
Haemoglobin	HGB
Mean Corpuscular Haemoglobin	MCH
Mean Corpuscular Haemoglobin Concentration	MCHC
White Blood Cell (Leukocyte) Count	WBC
Platelet (Thrombocyte) Count	PLT
Reticulocytes Percentage	RET%

Laboratories or *WADA*-Approved Laboratory for the *ABP* may also participate in ring tests between laboratories (hospitals, clinics, etc.) using the same technology and the same procedure.

5. Analysis of Blood *Sample*

The temperature data logger shall be stopped before *Sample* homogenization[2]. The blood *Sample* shall be homogenized for a minimum period of 15 minutes using an appropriate mixer (e.g. roller mixer) prior to analysis.

The blood *Sample* shall be analyzed twice consecutively.

Absolute differences between the two consecutive analyses shall be equal or less than each of the following criteria in order to accept the results:

- 0.1g/dL for HGB analysis;
- 0.15 absolute difference for RET% analysis if either the first or second measurement is lower or equal to 1.00%; otherwise 0.25 absolute difference.

The data from the second injection is used to confirm the first injection data. Therefore, if the absolute differences between the results of the analyses are within the criteria above, then only the first injection data is reported into *ADAMS*. If the

[2] In case the temperature data logger accompanies multiple *Samples*, and that these *Samples* are analyzed in the same batch by the Laboratory, the temperature data logger shall be stopped before the homogenization of the first *Sample*. The Laboratory shall proceed with the analysis of all *Samples* associated to the temperature data logger without delay.

absolute differences between the results of the two analyses are greater than those defined above, the analysis shall be started again in accordance with section 5 above.

The requirements for an Initial *Testing* Procedure, an "A" *Sample* Confirmation Procedure and a "B" *Sample* Confirmation Procedure, as defined in the ISL, shall not be applicable to blood *Samples* analyzed for the purposes of the *ABP*.

6. Reporting

The Laboratory or *WADA*-Approved Laboratory for the *ABP* shall promptly report into ADAMS the raw temperature profile recorded by the temperature data logger. The filename shall consist in the concatenation of the data logger ID with the date of *Sample* reception by the lab ("YYYY-MM-DD" in local time) separated by an underscore. For example, for a data logger ID "KG34V10" and a date of sample reception "2015-03-25", the Laboratory or *WADA*-Approved Laboratory for the *ABP* shall report the temperature profile under the filename "KG34V10_2015-03-25.txt". The Laboratory or *WADA*-Approved Laboratory for the *ABP* shall report the temperature profile before the test results of the *Sample*.

The Laboratory or *WADA*-Approved Laboratory for the *ABP* shall then report the following into *ADAMS*:

- *Sample* code;
- Type of test (*Out of Competition*/*In-Competition*);
- Sport and discipline;
- Date and time of receipt of the *Sample*;
- Date and time of analysis of the *Sample*;
- The name of the *Testing* Authority;
- The name of the Sample Collection Authority;
- Type of *Sample* (blood Passport);
- Type of analyzer;
- Test results (other variables may be included for quality purposes):

Blood Variable		**Unit(s)**
Haemoglobin	HGB	g/dL
Hematocrit	HCT	%
Immature Reticulocyte Fraction	IRF	%
Mean Corpuscular Haemoglobin	MCH	pg
Mean Corpuscular Haemoglobin Concentration	MCHC	g/dL
Mean Corpuscular Volume	MCV	fL
OFF-Score	-	-
Platelets	PLT	10^3/uL
Red Blood Cell Distribution Width	RDW-SD	fL
Red Blood Cells	RBC	10^6/uL
Reticulocytes – in absolute number	RET	10^6/uL

Reticulocytes Percentage	RET%	%
White Blood Cells	WBC	10^3/uL