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Written by:	*WADA*	Approved by:	*WADA* Executive Committee
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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 with analyzers of comparable technical characteristics in the dedicated network of laboratories (*i.e. WADA*-accredited or WADA-Approved Laboratories for the ABP). The Analytical Method shall be included within the laboratory's ISO/IEC (17025 or 15189) scope of accreditation, and the laboratories shall participate in the *WADA* External Quality Assessment Scheme (EQAS) for blood samples prior to analysis of *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 by a Laboratory under applicable ISO/IEC accreditation (17025 or 15189). Satellite facilities and mobile units shall also be ISO/IEC (17025 or 15189) accredited and participate in the *WADA* EQAS for blood samples prior to analysis of *Sample*s. *Sample* handling shall be conducted in compliance with the TD on Laboratory Internal Chain of Custody (TD LCOC).

2. *Sample* Reception and Timing of Analysis

The blood *Sample* shall be analyzed as soon as possible upon reception and no later than 12 hours after *Sample* reception unless the Sample Collection Authority (SCA) provides specific information regarding the *Sample* collection and transportation conditions (for example, the SCA provides a projected time window for analysis during which the projected Blood Stability Score would remain acceptable) that would allow the Laboratory or WADA-Approved Laboratory for the ABP to extend the time window of the analysis of the *Sample* without compromising *Sample* validity.

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In cases when the Laboratory or WADA-Approved Laboratory for the ABP is unable to analyze the *Sample* immediately 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 Testing procedure. The temperature data logger shall accompany the *Sample* until *Sample* homogenization. The blood *Sample* shall not be aliquoted before *ABP* analysis, however the *Sample* may be aliquoted after the *ABP* analysis has been satisfactorily completed, when appropriate (*e.g.* for the performance of other analyses such as for homologous blood transfusion or agents affecting erythropoiesis).

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. If the *Sample* cannot be analyzed, the Laboratory or WADA-Approved Laboratory for the ABP shall report the *Sample* as "Not Analyzed" and provide a description of why it could not be analyzed in *ADAMS*.

3. Instrument Check

The Laboratory or WADA-Approved Laboratory for the ABP shall maintain an instrument maintenance schedule to ensure proper performance; particularly if an analysis has not been recently conducted and the instrument remains idle for an extended period of time.

The analyst shall ensure that all reagents are within their expiration dates and comply with the reagent manufacturer's recommendations before performing an analysis. Operational parameters of the instrument (background level, temperature of the incubation chambers, pressure, etc.) shall be verified as compliant with manufacturer's specifications.

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

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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.

At least once a month, following the satisfactory analysis of all internal quality controls (levels 1, 2 and 3) as described above, one fresh blood *Sample* shall be homogenized for a minimum period of 15 minutes on an appropriate mixer (*e.g.* roller mixer) and analyzed at least seven (7) consecutive times under Repeatability conditions. The Repeatability of the determinations, expressed as coefficients of variation (CV %), shall be below 1.5 % for Haemoglobin (HGB) and Haematocrit (HCT), and below 15 % for Reticulocyte percentage (RET%).

4. External Quality Assessment Scheme (EQAS)

The Laboratories or WADA-Approved Laboratories for the ABP shall participate in and meet the requirements of *WADA's* EQAS for blood *Markers* for the *ABP*. *WADA's* EQAS program is the only EQAS relevant to the Laboratory's or WADA-Approved Laboratory for the ABP's compliance with the requirements for the analysis of blood *Markers* within the framework of the *ABP* (in case of discrepancy with other blood EQAS programs).

All internal quality controls (levels 1, 2 and 3) shall be analyzed twice following the specifications provided by the manufacturer prior to the analysis of EQAS samples. All results relevant to the *ABP* shall be in agreement with the reference value ranges of the manufacturer. The EQAS sample shall be homogenized for a minimum period of 15 minutes using an appropriate mixer (*e.g.* roller mixer) prior to analysis. The external quality controls shall be analyzed multiple times consecutively (based on the EQAS rules), and 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%

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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 *Samples*

5.1 The temperature data logger shall be stopped before *Sample* homogenization, upon removal of the blood *Samples* from the cooling device or refrigerator. The blood *Sample* shall be homogenized for a minimum period of 15 minutes using an appropriate mixer (*e.g.* roller mixer) prior to analysis.

In cases when the temperature data logger accompanies multiple *Samples*, and 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.

5.2 The blood *Sample* shall be analyzed twice and the Laboratory's procedure should minimize the delay between the two analyses. Absolute differences between the two (2) analyses shall be equal or less (≤) than each of the following criteria in order to accept the results:

- 0.1 g/dL for HGB;
- 0.15 % for RET% 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 absolute differences between the results of the two analyses are greater than those defined above, then the blood *Sample* shall be analyzed twice again in accordance with section 5.2. In cases of repeated analysis of a *Sample*, the *Sample* shall be mixed prior to re-analysis using the automated mixing feature of the blood analyzer or by appropriate manual inversion. This reanalysis procedure shall be repeated until the absolute differences between the results of the two (2) most recent analyses are within the criteria specified 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*.

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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 report the following into *ADAMS*:

- Status ("Submitted" or "Not Analyzed");
- *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

- Include a comment describing any relevant deviation as part of the *Sample*'s *ADAMS* record.