| Document Number: | TD2021BAR | Version Number: | 2.0 |
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| Date: | 20 May 2021 | Effective Date: | 01 June 2021 |

# ANALYTICAL REQUIREMENTS FOR THE HEMATOLOGICAL MODULE OF THE *ATHLETE BIOLOGICAL PASSPORT*

## 1.0 Introduction

The purpose of this *Technical Document* (*TD*) is to harmonize the analysis of *ABP* blood *Sample*s collected, both *In-Competition* and *Out-of-Competition*, for the measurement and reporting of individual *Athlete* blood *Markers* within the framework of the hematological module of the *Athlete Biological Passport* (*ABP*).

The *International Standard* for Laboratories (ISL) [1] is applicable to the analysis of *ABP* 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*.

In order to standardize analytical results in the *ABP*, *ABP* blood *Sample*s shall only be analyzed with analyzers of comparable technical characteristics in the dedicated network of laboratories (*i.e. WADA*-accredited laboratories or *ABP* Laboratories). The Analytical Method for measuring *ABP* blood variables shall be included within the Laboratory or *ABP* Laboratory's Scope of ISO/IEC (17025 or 15189) Accreditation, and the Laboratory or *ABP* Laboratory shall satisfactorily participate in the relevant *WADA* External Quality Assessment Scheme (EQAS), as determined by *WADA*, prior to applying the Analytical Method to *ABP* blood *Sample*s.

*Sample* handling shall be conducted in compliance with the *TD* on Laboratory Internal Chain of Custody (TD LCOC) [2].

If not reasonably possible for *ABP* blood *Sample*s to be analyzed in a Laboratory or *ABP* Laboratory for technical and/or geographical reasons, *ABP* blood *Sample*s can be analyzed at a satellite facility of a Laboratory or using mobile units operated by a Laboratory under their 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 *Markers* for the *ABP* prior to analysis of *ABP* blood *Sample*s.

## 2.0 *ABP* blood *Sample* Reception and Timing of Analysis

Upon reception at the Laboratory or *ABP* Laboratory, the *ABP* blood *Sample* shall be analyzed as soon as possible and no later than twelve (12) hours after 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 (BSS) should remain acceptable) that would allow the Laboratory or *ABP* Laboratory to analyze the *Sample* beyond twelve (12) hours after reception without compromising the *ABP* blood *Sample* validity.

In cases when the Laboratory or *ABP* Laboratory is unable to analyze the *ABP* blood *Sample* immediately after reception, the Laboratory or *ABP* Laboratory is responsible for maintaining the *ABP* blood *Sample(s)* at a cool temperature (approximately 4°C) between reception and the start of the analysis. The temperature data logger shall accompany the *ABP* blood *Sample(s)* until homogenization.

The *ABP* blood *Sample* shall not be aliquoted before the *ABP* analysis is satisfactorily conducted. Only after the analysis for the *ABP* has been satisfactorily completed may the Laboratory or *ABP* Laboratory aliquot the *ABP* blood *Sample* for the performance of other Analytical *Testing* Procedures (*e.g.* test for homologous blood transfusion, EPO and agents affecting erythropoiesis).

If there is a Laboratory or *ABP* Laboratory deviation from the aforementioned procedure, the Laboratory or *ABP* Laboratory shall proceed with the analysis and report the results into *ADAMS* with a detailed description of the deviation. If the *ABP* blood *Sample* cannot be analyzed, the Laboratory or *ABP* Laboratory shall report the *Sample* as "Not Analyzed" and provide a description of why it could not be analyzed in *ADAMS*.

## 3.0 Instrument Check

The Laboratory or *ABP* Laboratory 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.

In each analysis session:

- All internal quality controls (QC levels 1, 2 and 3) shall be analyzed twice, following the specifications provided by the manufacturer, prior to the analysis of *Samples*.
- If more than 30 *Samples* are analyzed, at least one internal QC from the manufacturer (either level 1, 2 or 3) shall be analyzed in the middle of the analytical session, and every 30 - 50 *Samples* for larger batches.
- At the end of each analysis session and after all blood *Sample* analyses are completed, one internal QC (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.

All results relevant to the *ABP* shall be in agreement with the reference value ranges of the manufacturer. These internal QCs 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 QCs shall be monitored via QC-charts with appropriate control limits.

At least once a month, following the satisfactory analysis of all internal QCs (levels 1, 2 and 3) as described above, one fresh blood sample shall be homogenized for a minimum period of fifteen (15) minutes on an appropriate mixer (*e.g.* roller mixer). The fresh blood sample shall be 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%).

*[Comment: Samples from Athletes shall not be used as a fresh blood sample to conduct the Repeatability analysis.]*

## 4.0 External Quality Assessment Scheme (EQAS)

The Laboratories or *ABP* Laboratories 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 *ABP* Laboratory's compliance with the requirements for the analysis of blood *Markers* within the framework of the hematological module of the *ABP* (in case of discrepancy with other blood EQAS programs).

All internal QCs (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 fifteen (15) minutes using an appropriate mixer (*e.g.* roller mixer) prior to analysis. The external QCs 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% |

Laboratories or *ABP* Laboratories may also participate in ring tests with other laboratories (hospitals, clinics, etc.) using the same technology and the same procedure.

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## 5.0 Analysis of *ABP* Blood *Samples*

### 5.1 Temperature Data Logger

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

In cases when the temperature data logger accompanies multiple *ABP* blood *Samples*, and these *ABP* blood *Samples* are analyzed in the same batch by the Laboratory or *ABP* Laboratory, the temperature data logger shall be stopped before the homogenization of the first *ABP* blood *Sample*. The Laboratory shall proceed with the analysis of all *ABP* blood *Samples* associated with the same temperature data logger without delay.

### 5.2 *ABP* Blood *Sample* Analysis

The *ABP* blood *Sample* shall be analyzed twice. The Laboratory's or *ABP* 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 *ABP* blood *Sample* shall be analyzed twice again in accordance with Article 5.2. In cases of repeated analysis, the *ABP* blood *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 (ITP), an "A" *Sample* Confirmation Procedure (CP) and a "B" *Sample* CP, as defined in the ISL [1], shall not be applicable to *ABP* blood *Samples* analyzed for the purposes of the *ABP*.

## 6.0 Reporting

### 6.1 Temperature Report

The Laboratory or *ABP* Laboratory shall promptly submit into *ADAMS* the raw temperature profile report 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 Laboratory or *ABP* Laboratory ("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 *ABP* Laboratory shall report the temperature profile under the filename "KG34V10_2015-03-25.txt". The Laboratory or *ABP* Laboratory shall report the temperature profile into *ADAMS* before the test results of the *Sample*, when temperature data can be retrieved from the logger.

> *[Comment: Where the Sample meets the requirements of the ISTI Annex I, Article I.2.7, and is analyzed at the Sample collection site without delay, a temperature data logger is not necessary and the Laboratory shall proceed to reporting the test results of the Sample.*
>
> *In cases that the Laboratory is unable to upload a suitable temperature profile report from the temperature data logger into ADAMS, the Laboratory shall proceed to upload the test results of the relevant Sample(s).]*

### 6.2 Reporting *ABP* Blood *Sample* Test Results

The Laboratory or *ABP* Laboratory should report the *ABP* blood *Sample* test results as soon as possible and within three (3) days after *Sample* reception. The following shall be reported into *ADAMS*:

- Status ("Submitted" or "Not Analyzed");
- *ABP* blood *Sample* code;
- Type of test (*Out-of-Competition* / *In-Competition*);
- Sport and discipline;
- Date and time of receipt of the *ABP* blood *Sample*;
- Date and time of analysis of the *ABP* blood *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 | $10^3/\mu L$ |
| Red Blood Cell Distribution Width | RDW-SD | fL |
| Red Blood Cells | RBC | $10^6/\mu L$ |
| Reticulocytes – in absolute number | RET | $10^6/\mu L$ |
| Reticulocytes Percentage | RET% | % |
| White Blood Cells | WBC | $10^3/\mu L$ |

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

## 7.0 References

[1] The World Anti-Doping *Code International Standard* for Laboratories (ISL).

[2] *WADA Technical Document* TD LCOC: Laboratory Internal Chain of Custody.

*[Comment: Current versions of WADA ISL and Technical Documents may be found at https://www.wada-ama.org/en/what-we-do/science-medical/laboratories ]*