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 Samples 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 Samples 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 Samples 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 Samples.

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 Samples to be analyzed in a Laboratory or ABP Laboratory for technical and/or geographical reasons, ABP blood Samples 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 Samples.

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:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cell (Erythrocyte) Count</td>
<td>RBC</td>
</tr>
<tr>
<td>Mean Corpuscular Volume</td>
<td>MCV</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>HCT</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>HGB</td>
</tr>
<tr>
<td>Mean Corpuscular Haemoglobin</td>
<td>MCH</td>
</tr>
<tr>
<td>Mean Corpuscular Haemoglobin Concentration</td>
<td>MCHC</td>
</tr>
<tr>
<td>White Blood Cell (Leukocyte) Count</td>
<td>WBC</td>
</tr>
<tr>
<td>Platelet (Thrombocyte) Count</td>
<td>PLT</td>
</tr>
<tr>
<td>Reticulocytes Percentage</td>
<td>RET%</td>
</tr>
</tbody>
</table>

Laboratories or ABP Laboratories may also participate in ring tests with other laboratories (hospitals, clinics, etc.) using the same technology and the same procedure.
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 K, Article K.2.4, 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/µL
Red Blood Cell Distribution Width | RDW-SD | fL
Red Blood Cells | RBC | 10^6/µL
Reticulocytes – in absolute number | RET | 10^6/µL
Reticulocytes Percentage | RET% | %
White Blood Cells | WBC | 10^3/µL

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

7.0 References


[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 ]