Blood Testing Protocol
IAAF Blood Testing Protocol

Two main focus areas:

- **Blood sampling** for the detection of Prohibited Substances and Prohibited Methods, e.g. CERA or other ESAs, hGH, blood transfusion, HBOCs

- **Blood sample screening** for the measurement of individual Athlete blood variables within the framework of the Athlete Biological Passport.
  - Target Testing
  - Support an anti-doping rule violation
IAAF Blood Testing Protocol

• Samples can be used for either detection of Prohibited Substances/Methods or Athlete Biological Passport or both

• Whenever a blood sample is collected for the Athlete Biological Passport, IAAF Blood Testing Protocol provisions must be applied
The Protocol

- Part I - Blood Collection
- Part II - Blood Storage and Transport
- Part III - Blood Analysis
- Part IV - Results Management

Provisions must be followed to ensure both legal fortitude and scientific certainty.

Include mandatory processes set by WADA for operating an Athlete Biological Passport (IST and ISL).
Part I. - Blood Collection

2. Preparations for a Blood Sampling Session
   - Qualified Staff
   - Adequate Blood Sampling Facilities
   - Blood Sampling Materials
   - Blood Sampling Documentation

3. Sample Collection Session
   - Timing, and Notification of Athlete
   - Blood Sampling Documentation
   - Selection of material – Venipuncture
   - Post-withdrawal procedures
   - Trouble shooting
Part I. - Blood Collection
2. Preparations for a Blood Sampling Session

• Qualified Staff
  – Medically qualified personnel or phlebotomists; the BCO must be able to provide evidence to the Athlete of his qualification to collect blood samples
  – If qualified, a DCO may perform the duties of a BCO
  – BCOs/other responsible officials must be adequately trained in blood sampling procedures set out in the IAAF Anti-Doping Rules and Regulations
Part I. - Blood Collection
2. Preparations for a Blood Sampling Session

• Adequate Blood Sampling Facilities (IAAF)
  – A room or facility equipped for blood sampling may be set up at the Doping Control Station and/or at any other site at which the Athletes are to be located for Testing (hotel, medical centre, training centre, competition venue etc.)
  – The size of the room, the material, equipment, furniture, hygiene and temperature conditions for an optimal blood collection is under the responsibility of the BCO
Part I. - Blood Collection
2. Preparations for a Blood Sampling Session

• The Blood Collection Facility (WADA)
  – Be solely reserved for Doping Control purposes
  – Maintain Athlete privacy and confidentiality
  – Provide a high standard of cleanliness
  – Be well-lit and well-ventilated
  – Be accessible only to authorized personnel
  – Be secure enough to store sample collection equipment
  – Contain a table and chairs for administration and completion of paperwork
  – Contain a comfortable chair or bed for sample provision and any after-care that may be required
Part I. - Blood Collection
2. Preparations for a Blood Sampling Session

• The Blood Collection Facility (WADA)
  – Contain a refrigerator or cool-box
  – Be large enough to accommodate the number of Athletes, Athlete Representative and Sample Collection Personnel who will occupy the area
  – Be suitably located in relation to the field of play or other location where Athletes will be notified
Part I. - Blood Collection

2. Preparations for a Blood Sampling Session

- Blood Sampling Materials
  - The kit or the combination of kits depends on the analysis required

For analysis of the variables of the Athlete Biological Passport:
  - Number of Samples: 1 (no “B” Sample required)
  - Volume required: 1 x 3mL (or as specified by relevant Laboratory).
  - The tube used contains solid EDTA as anticoagulant
  - Tubes should be gently inverted 8 to 10 times soon after collection
Part I. - Blood Collection
2. Preparations for a Blood Sampling Session

• Blood Sampling Materials

For analysis of Prohibited Substances and Methods in whole blood (e.g. detection of blood transfusion) or in plasma (e.g. HBOCs and CERA):

- Number of Samples: 2 ("A" Sample and "B" Sample)
- Volume required: 2 x 3mL
- Contains solid EDTA as anti-coagulant.
- Tubes should be gently inverted 8 to 10 times soon after collection
Part I. - Blood Collection
2. Preparations for a Blood Sampling Session

• Blood Sampling Materials

For analysis of Prohibited Substances and Methods in serum (e.g. detection of hGH, HBOCs and CERA):

– Number of Samples: 2 (“A” Sample and “B” Sample)
– Volume required: 2 x 5mL
– Tube has an inert polymeric serum separator gel and a clotting activation factor
– Tubes should be gently inverted min 5 times soon after collection

BD Vacutainer® SST II
Part I. - Blood Collection

2. Preparations for a Blood Sampling Session

Blood Sampling Materials – Kits from Berlinger

94-1098 BEREG-KIT small single 'Basic'

94-1094 BEREG-KIT small
Part I. - Blood Collection
2. Preparations for a Blood Sampling Session

Blood Sampling Materials – Kits from Berlinger

Accessory packages (tubes, needle, holder) for single whole blood sample

Transportation to lab:
94-1098 + 94-1093 BEREG-KIT small single 'Easy'
+ 94-1099 BEREG-KIT small single 'Top'

Sample analyzed on site:
94-1082 Mini-Kit
Part I. - Blood Collection

2. Preparations for a Blood Sampling Session

Blood Sampling Materials – Kits from Berlinger

Accessory packages for two samples:

Two whole blood/plasma samples
- 94-1095 (with needle)
- 94-1086 (with butterfly)

Two serum samples
- 94-1096 (with needle) or 94-1087 (with butterfly)
Part I. - Blood Collection

2. Preparations for a Blood Sampling Session

Necessary equipment for Blood Sample Collection
(WADA - Guidelines for blood sample collection v2.3 2011)

- Sterile needles
- Butterfly needles
- Disposable plastic syringes
- Vacutainer collection tubes (3 or 5 mL) (serum separator tubes and/or EDTA (anti-coagulant) tubes, as required)
- Sterile disinfectant pads
- Gloves providing barrier protection
- Tourniquets
- A disposal container for bio-hazardous waste
- A bio-hazard spill kit
- Adhesive bandage and gauze
Part I. - Blood Collection
2. Preparations for a Blood Sampling Session

Necessary equipment for Blood Sample Collection
(WADA - Guidelines for blood sample collection v2.3 2011)

- A cold-box
- Sealed, tamper evident Sample transport kits
- Secure transport bags and seals
- Transport temperature monitoring device
- All doping control documentation, including doping control forms, Athlete notification forms, supplementary report forms, chain of custody forms, etc
Part I. - Blood Collection
3. Sample Collection Session

- **Timing and notification:**
  - DCO/chaperone should plan notification approach according to competition/training schedules
    - No-Advance-Notice notification
  - No blood collection for 2 hours following training session or competition.
  - If already notified, the athlete shall be monitored in the meantime (nature and duration of exertion shall be recorded)
The schedule of blood tests Pre-Competition is a difficult issue, based on:
- late arrivals of the teams (1-2 days before in Area competitions)
- LOC organized training sessions or competition venue visits
- accreditation, meals, meetings, etc.

Best schedule:
- Early morning (i.e. 7:30-8:00) or Mealtimes
- Arrival time (except after long travel)

Be sure to have enough number of BCOs, DCOs and chaperones for the time consuming administrative work
Part I. - Blood Collection
3. Sample Collection Session

- Verification of Athlete identity
- Provide Athlete proof of BCOs blood sampling qualification
- Inform Athlete about sampling procedure
- Written consent for blood sample collection from Athlete

- The athlete is requested to sit for at least 10 minutes before collection (waiting time, or time-out practically needed to fill in the form with the athlete)

- NOTE: always write the arrival time of the athlete to the anti-doping station
6.2.1 The DCO/Chaperone shall ensure that the Athlete is escorted from the place of notification to the Blood Collection Facility under constant supervision.

6.2.2 The DCO/Chaperone cannot prevent the Athlete eating or drinking products of their choice, but shall recommend that the Athlete chooses from a selection of individually sealed, non-caffeinated and non-alcoholic beverages in order to hydrate. The DCO/Chaperone shall not handle food or drink items for the Athlete.
Part I. - Blood Collection

3. Sample Collection Session

Blood Sampling Forms

- Chain of Custody Form:
  - Sample ID linked to analysis
  - Storage/Transportation from DCO to Laboratory
Part I. - Blood Collection

3. Sample Collection Session

- Blood Sampling Documentation
- Blood sampling Form:
  - Athlete Notification/Consent
  - Sample ID linked to athlete information for analysis

**Blood Sampling Form**

**Athlete Notification/Consent**

- Sample ID linked to athlete information for analysis
Part I. - Blood Collection
3. Sample Collection Session

Information for analysis

- IC/OOC testing
- # samples: 1 or 2; EDTA and/or serum
- 10 min seating prior to collection?
- Strenuous physical exercise past 2 hours?
- Previous altitude training in the last 2 weeks? (specify)
- Previous altitude simulation in the last 2 weeks? (specify)
- Lost or donated blood in the last 3 months
- Received blood transfusion the last 6 months
- Declaration of medication (last 7 days)
Consent or Refusal

No blood sample shall be taken from an athlete, unless he has first given his written consent to such sampling (i.e. on blood sampling form or notification form).

In case of refusal:
- Immediate report to IAAF (disciplinary procedure)
- Immediate urine sample to be analyzed for substances and methods included in the banned list, including rh-EPO
Part I. - Blood Collection
3. Sample Collection Session

- **Selection of material**
  - Athlete should choose sampling material from a selection
  - Athlete should check if material is intact.

- **Venipuncture**
  - Preferable on not dominant arm
  - If required, tourniquet is placed approx. 10 cm above the puncture location (not tightened)
  - Disinfection of skin
  - If used, tourniquet is tightened (enough for vein dilation, ensuring arterial circulation)
  - Equipment enabled and tube(s) labelled with bar code
  - Insertion of needle, connect tube to holder
  - Release tourniquet as the blood begins entering into the tube
Part I. - Blood Collection
3. Sample Collection Session

- **Venipuncture, cont’d**
  - Once the tube is removed from the holder, gently homogenize the blood in the tube manually by inverting the tube gently at least 3 times (WADA: 5 or 8-10 times)

- **Post-withdrawal procedure**
  - BCO/other responsible official checks label(s) of tube(s) of blood with the number recorded on the Blood Sampling Form
  - BCO applies dressing to puncture location, if necessary
  - Tube(s) of blood is placed and sealed in the sample collection container in the presence of the Athlete. Athlete is asked to check it is secure.
  - Once all is completed, the Blood Sampling Form is signed by Athlete and BCO other responsible official.
Part I. - Blood Collection
3. Sample Collection Session

- **Trouble shooting**
  - **No blood enters into the tube:**
    If needle has failed to enter vein, retry delicately once. If not successful, remove and discard needle and select another kit and another collection location (other arm). The BCO shall not perform more than three attempts to collect blood.
  
  - **The blood stops flowing:**
    If blood flow ceases before the tube is filled, the vein may have collapsed. Remove tube from holder, and see if vein recovers. If not, remove and discard needle and select another collection location.
  
  - **The DCO shall record the reasons for terminating the collection attempt.**
Part II. - Blood Storage and transport

- Considerations:
  - Time of storage (on-site analysis, transport to off-site lab?)
  - Number of samples
  - Environmental conditions (hot or cold temperatures?)

- A suitable storage device may be:
  - a refrigerator
  - an insulated cool box
  - an isotherm bag

  - Should maintain blood samples at a cool and constant temperature (2 to 12º C) during storage and transport.
  - A temperature data logger is recommended for documentation.
  - Blood samples must not be allowed to freeze.
  - Be kept under secure storage and transport conditions.

- Chain of custody forms
Part II. - Blood Storage and transport
Data Logger
Part III. – Blood analysis

- Sample analysis in a WADA accredited or IAAF approved laboratory, or satellite facility of WADA/IAAF approved lab.

- The blood sample shall be analyzed **within 36 hrs of collection**
  (this does not necessarily mean 34 hours for storage/transport and 2 hrs for analysis...)

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[European Athletics logo]
The World Anti-Doping Code  

ATHLETE BIOLOGICAL PASSPORT OPERATING GUIDELINES  

AND COMPILATION OF REQUIRED ELEMENTS  

January 2012  
Version 3.0  

The Code, § 2.2 from 2009:  

“Use or Attempted Use may also be established by other reliable means such as admissions by the Athlete, witness statements, documentary evidence, conclusions drawn from longitudinal profiling, or other analytical information which does not otherwise satisfy all the requirements to establish “Presence” of a Prohibited Substance under Article 2.1”.
Blood Analytical Requirements for the **Athlete Biological Passport**

- **Analytical procedure:**
  - Instrument check (reagents, operational parameters)
  - 3 QCs (low, normal, high) from Sysmex measured 2x before samples
  - 1 QC measured after every 30 samples

  - Participate in WADAs monthly **External Quality Assessment Scheme (EQAS)**
  - Monthly precision test with one fresh blood sample (7x)
• **Analysis of blood samples:**
  - Homogenized minimum 15 minutes prior to analysis.
  - Analyze each sample twice
  - To be accepted, the absolute difference between the two results shall be equal or less than:

    - 0.1g/dL for HGB analysis;
    - 0.15 absolute difference for % Reti analysis (if first measurement lower or equal to 1.00%);
    - 0.25 absolute difference for % Reti analysis (if first measurement higher than 1.00%).

  - If not accepted, the analysis procedure must be repeated
  - Only the first measurement is reported
  - Results are reported into ADAMS
Part IV. – Result Management

9. Management of Athlete Biological Passport programme

9.1 The IAAF Medical and Anti-Doping Department shall be responsible for administering and managing the Athlete Biological Passport programme within and on behalf of the IAAF

9.2 The IAAF shall establish a mechanism which allows for all Athlete Biological Passports to be distributed to experts for review in accordance with this protocol(........).
Part IV. – Result Management
ABP Operating Guidelines and Compilation of Required Elements (WADA)

- Roles and Responsibilities of the Partners
- ABP Administration
- Implementation of Haematological Module

- Appendix A: TD2010BSCR (Blood Sample Collection Requirements)
- Appendix B: TD2010BSTR (Blood Sample Transport Requirements)
- Appendix C: TD2010BAR (Blood Analytical Requirements)
- Appendix D: TD2010RMR (Results Management Requirements)
APPENDIX D

Results Management Requirements for the *Athlete Biological Passport*

WADA Technical Document – TD2010RMR

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<th>2.0</th>
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<td>Approved by:</td>
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1. Administrative management (APMU, Expert Panel)

2. **Review by the Adaptive Model**

3-7. Expert-, APMU-, ADO review process
Part IV. – Result Management
The Adaptive module

- Mathematical probability model based on Bayesian statistics
- Takes into account individual and preanalytical factors – calculates an expected normal range for each athlete
- Predicts the probability for doping
- Is used for the haematological module in the ABP
Part IV. – Result Management

Haematological Module

Longitudinal profiles of haematological Markers, with which an athlete becomes his/her own point of reference:

RBC: Red Blood Cell (Erythrocyte) count
HGB: Haemoglobin
HCT: Hematocrit
RET%: Reticulocytes percentage
RET#: Reticulocyte count
WBC: White Blood Cell (Leukocyte) count
MCV: Mean Corpuscular Volume
MCH: Mean Corpuscular Hemoglobin
MCHC: Mean Corpuscular Hemoglobin Concentration

- Calculated Markers:
  - OFF-score\(^1\) (HGB and RET%)
  - Abnormal Blood Profile Score\(^2\) (ABPS) (HCT, HGB, RBC, RET%, MCV, MCH, MCHC)

\(^1\) Gore et al., 2003; \(^2\) Sottas et al., 2006
Part IV. – Result Management

A normal haemoglobin profile
Part IV. – Result Management

An abnormal blood profile
Part IV. – Result Management
Guidelines for Passport Operation
- Roles and Responsibilities of the Partners

- The Athlete Passport Management Unit (APMU):
  - Person(s) appointed by the ADO to administer Athlete Biological Passports (ABPs)
  - Should ensure independence between planning, interpretation and results management of an ABP
  - Could be associated with a WADA accredited lab, or an ADO

- The Responsibilities of the APMU:
  - Update passports, evaluate results and profiles
  - Provide the ADO necessary recommendations for effective and targeted testing
  - Contact the Expert Panel when sample or profiles (Hgb, Off-score, ABPS) are outside expected ranges
Part IV. – Result Management
Guidelines for Passport Operation
- Roles and Responsibilities of the Partners

- **The Expert Panel:**
  - Should consist of experts in clinical hematology, sports medicine and/or exercise physiology
  - Chosen by the ADO and/or APMU

- **The responsibilities of the Expert Panel:**
  - Determine whether the atypical values/profiles can be the result of a normal physiological or pathological condition, or if doping is likely.
  - Should outline a possible doping scenario
  - Must have an *unanimous opinion* for a case to proceed (Adverse Passport Finding)
M2. CHEMICAL AND PHYSICAL MANIPULATION

1. **Tampering**, or attempting to tamper, in order to alter the integrity and validity of *Samples* collected during *Doping Controls* is prohibited. These include but are not limited to catheterisation, urine substitution and/or alteration.

S5. DIURETICS AND OTHER MASKING AGENTS

Masking agents are prohibited. They include: **Diuretics, probenecid, plasma expanders** (e.g. intravenous administration of albumin, dextran, hydroxyethyl starch and mannitol) and other substances with similar biological effect(s).
Analytical issues
Incubation of urine samples with detergent and protease

Urine samples containing natural erythropoietin (EPO) and recombinant EPO (BRP+NESP) incubated 2 hrs @ RT with washing powder (image A) or the protease Subtilisin A (image B)

3 mg detergent and 10 µg pure protease was sufficient to break down all protein in the urine samples!

Dehnes et al., 2008
Analytical issues
Protein deterioration by proteases

IAAF

ANTI-DOPING REGULATIONS

3.30 The DCO/Chaperone should, where practicable, ensure the Athlete washes his hands thoroughly prior to the provision of the Sample.

- Proteins contain amino acid chains
- Proteases are enzymes that cleave such chains
- Proteases are used to remove protein residues
- Proteases are important ingredients in washing powder and dishwashing tabs
In order to ensure good and reliable final results – all steps from test planning to sample analysis must be done professionally and according to prevailing protocols.
Blood Testing Protocol