

Frequently Asked Questions (FAQs) on blood steroid Markers in the Steroidal Module of the Athlete Biological Passport (ABP)

What Sample types are used for the Steroidal Module?

Since 2014, all steroid profiles from anti-doping urine Samples analyzed in WADA-accredited Laboratories have been included in the Steroidal Module of the ABP. From August 2023, it is also possible to include blood (serum) Samples in this module to generate data complementing that of the longitudinal Marker data from urine Samples.

The specific tubes required to collect blood Samples are defined in the International Standard for Testing and Investigation (ISTI) Guidelines for Sample Collection and are identical to those already used when collecting blood (serum) for other analyses (for example, the hGH Isoforms Differential Immunoassay). More specifically, blood Samples shall be collected in tubes containing an inert polymeric serum separator gel as well as a clotting activation factor and allow the collection of up to 5 milliliters (mL) of blood.

Is blood Sample volume a limiting factor when deciding to proceed with analysis for the Steroidal Module?

Yes. Where the maximum volume (5 mL) of blood is collected, the corresponding volume of serum after centrifugation is approximately 2 mL. The table below provides an estimation of the volume of serum needed for different analyses that can be performed in this sample type, including the scenario of when confirmation procedures are performed following atypical Initial Testing Procedure (ITP) results (note: 1 mL = 1000 microliters (µL)):

Analysis	Initial (est.)	Confirmation (est.)	
IGF-I (TD LC-MS)	100 µL (2x50 µL)	100 µL (2x50 µL)	
P-III-NP (Centaur)	250 µL (duplicate)	250 µL (duplicate)	
hGH Isoforms	200 µL REC and PIT from 1 kit	600 µL REC and PIT from 2 kits	hGH doping scenario → ~ 1.8 mL
Endogenous steroids	100 µL	100 µL	Testosterone doping scenario → ~ 800 µL
Steroid esters	200 µL	200 µL	
AAE (EPO)	500 µL	500 µL	Blood doping scenario → ~ 1.2 mL <i>Possible in Blood ABP (whole blood) sample</i>
Total (incl. dead vol)	~ 1.8 mL	~ 2.0 mL	

Depending on the requested test menu and the ITP results, it is possible that the available serum volume is a limiting factor of the process. Therefore, it is advisable that the Anti-Doping Organization (ADO) sets the order of priorities of analyses according to their risk assessment and communicates them with the Laboratory.

What are the Markers measured in blood for the Steroidal Module?

The Steroidal Module in blood consists of one primary Marker, the **Testosterone/Androstenedione (T/A4) ratio** calculated by dividing the serum concentrations reported for T by A4. Recent studies demonstrated that T/A4 is sensitive to transdermal testosterone administration, specially in Athletes with low levels of steroids in urine. Because T concentration alone can also be a valuable Marker of testosterone doping, this target compound is considered as a secondary Marker while A4 data alone are not processed by the Adaptive Model within the ABP.

How are blood steroid Markers measured in Laboratories?

For the sensitivity of the ABP profiles, the use of a harmonized Analytical Testing Procedure is essential to generate individual *Athlete* longitudinal data that are comparable between Laboratories. WADA-accredited Laboratories quantify T and A4 using a harmonized Analytical Testing Procedure consisting of Liquid-Chromatography coupled to Mass Spectrometry (LC-MS).

Laboratories report both T and A4 concentrations in ADAMS in nanograms per milliliter (ng/mL) and the primary Marker, T/A4, is then automatically calculated in ADAMS. Both T/A4 and T Markers are then displayed in two different graphs on the Passport page representing the longitudinal variations of the Markers.

Do all Laboratories have the capacity to analyze blood Samples for the Steroidal Module?

No. Because the Analytical Testing Procedure mentioned above is not a mandatory method to implement for a WADA-accredited Laboratory, not all Laboratories are involved in analyzing blood Samples for the Steroidal Module. The WADA Laboratory Guidelines for the Quantification of Endogenous Steroids in Blood for the Athlete Biological Passport set the framework and objectives of the quantitative method, and after development and validation of the Analytical Testing Procedure, the Laboratory shall include the method within its ISO 17025 scope of accreditation before applying the method to the analysis of anti-doping Samples.

The list of Laboratories having the analytical capacity to analyze blood Samples for the Steroidal Module is constantly evolving and ADOs are advised, in advance of the Samples collection and transport, to consult the ADAMS details of the Laboratory or to contact the Laboratory regarding the availability of the specific Analytical Testing Procedure.

What are the requirements for blood Sample collection and transport for the Steroidal Module?

In order to ensure valid results and to control of potential confounding factors, the collection and transport of blood Samples for the purpose of the Steroidal Module shall respect the following criteria at a minimum:

- Collection of Samples
 - a. A blood Sample shall be collected at least 1 hour (60 minutes) after exercise (training or competition).
- Samples shipment
 - a. All uncentrifuged blood Samples shall be transported under refrigerated conditions (not frozen).
 - b. Centrifuged blood Samples may be transported either frozen or refrigerated.
 - c. Blood Samples shall be transported in a device that maintains the integrity of Samples over time, in a cool and constant environment, monitored by a temperature data logger.
 - d. The maximum time between Sample collection and analysis at a Laboratory shall not be greater than 6 days (144 hours). Note that some other analyses with shorter permitted collection-to-analysis times (ex. for the Endocrine Module, or the hGH Isoform Differential Immunoassay) may no longer be possible on blood Samples with extended transport times (see Article 2.3.2 of the ABP Operating Guidelines).

Which Athletes should be prioritized for testing for the blood Steroidal Module?

Testosterone and related compounds are known to be beneficial for strength and power-based sports, but also for endurance sports, in particular for recovery. Therefore, both females and males across all sports and disciplines should be considered by an ADO as part of their risk assessment for their blood Testing strategy related to the Steroidal Module. In addition, the following criteria should be accounted:

- Athletes with low urinary steroid profile concentrations; and
- Athletes with highly variable urine steroidal passport.

ADOs are encouraged to contact their Athlete Passport Management Unit (APMU) in order to identify such male athlete profiles.

Should a urine be collected at the same time as a blood Sample for the Steroidal Passport?

Yes. The integration of steroid Marker data from both urine and blood Samples is valuable and complementary when evaluating an Athlete's steroidal Passport. In addition, information related to confounding factors impacting both urine and blood steroid Markers are identified to a greater extent in urine. Also, further analyses might be requested in urine (e.g., Gas Chromatography / Combustion / Isotope Ratio Mass Spectrometry (GC/C/IRMS)) or blood (e.g., steroid esters) to support Passport interpretation or to identify an Adverse Analytical Finding. Consequently, it is highly recommended that whenever an ADO collects a blood Sample for the Steroidal Module, a urine Sample is collected as well.

How many blood tests should an ADO plan for as a minimum per Athlete for the Steroidal Module and when should these Samples be collected?

When developing a test distribution plan for the blood Steroidal Module, an annual minimum of three tests per Athlete should be considered as a starting point for ADOs.

As T can be used at different times of the year/season for different purposes, it is recommended to distribute the tests throughout the year with, ideally, a Sample collected during the off-season/preparation phase, in-competition and post-competition period. In some circumstances, where there is a suspicion of doping, ADOs may consider targeting the Athlete with more than one blood Sample over a period of few days to profile the biological response to doping.

Can stored Samples collected prior to the launch of the blood Steroidal Module be analyzed and included in the Athletes' Passports?

Possibly. Blood Samples collected before the launch of the blood Steroidal Module in August 2023 may potentially be analyzed and included in steroidal Passport of Athletes. However, ADOs shall contact the analyzing Laboratory to ensure that pre-analytical conditions and analytical requirements are guaranteed to report appropriate quantification of the Markers.

Can Samples already analyzed with the validated Analytical Testing Procedure be included in an Athlete's blood steroidal Passport?

Yes. T and A4 values of a blood Sample that has been analyzed with the harmonized, validated and accredited Analytical Testing Procedure before the launch of the blood Steroidal Module could be integrated in the steroidal Passport. ADOs should coordinate with the analyzing Laboratory to request the upload of data in the ADAMS Test Report related to these blood Samples.

Does an ADO need to request analysis for the Steroidal Module when sending a blood Sample to a Laboratory?

Yes. There is no standard test menu for blood samples and consequently, the ADOs shall indicate this specific analysis in the documents accompanying the Sample to inform the Laboratory to perform the analyses of blood steroid Markers for the Steroidal Module.

How long should a blood Sample analyzed for the Steroidal Module be stored after analysis?

In compliance with the International Standard for Laboratories (ISL) the default minimum storage time of serum samples is three (3) months after reporting, which could be extended up to ten years upon request of

the Testing Authority. Given that blood Samples have a higher cost of collection and transportation, a more targeted collection compared to urine, and the possibility of retroactive analysis for the blood Steroidal Module, it is recommended to consider storing such blood Samples systematically for a longer period than is required by the ISL (for example, greater than 12 months). ADOs should contact the analyzing Laboratories to agree on the best strategy and timeframe to store such Samples.

Will the blood and urine steroidal Passports be managed by the same APMU?

Both sample types (urine and blood) are displayed in the same ADAMS Passport page giving access to blood and urine data to the same WADA-approved APMU assigned to the Passport Custodian (PC). This shared view allows the APMU to simultaneously evaluate blood and urine steroidal Markers, which are complementary, and to provide global recommendations in the same APMU Report in ADAMS. To ensure appropriate management of Passports, it is important for ADOs to inform their APMU about their intention to collect blood Samples for the Steroidal Module.

Does the blood Steroidal Module require a new panel of Experts?

No, as the fields of expertise are the same for urine and blood Passports, the same steroidal Experts have the knowledge to evaluate Marker data originating from both Sample types and provide guidance to APMUs and recommendations to ADOs for additional actions, if required.

What types of recommendations should ADOs expect from their APMU evaluating blood steroid Marker data?

As with the other modules of the ABP, whenever a Laboratory result is matched with a Doping Control Form (DCF) in ADAMS, the Athlete's Passport is updated in ADAMS with the following possible outcomes:

Passport status	Reasons	APMU/Expert review
Normal	<ul style="list-style-type: none"> All Markers are within the individual limits. Markers' sequences are normal. 	Not mandatory but might be reviewed by APMU according to agreement with Passport Custodian
Flagged (<i>new status</i>)	<ul style="list-style-type: none"> Secondary Marker (Testosterone) is above or below the individual limits. Sequence of secondary Marker (Testosterone) deviates from expected values. 	<p>APMU review is highly recommended.</p> <p>Expert review could be initiated upon APMU's request.</p>

Atypical Passport Finding (ATPF)	<ul style="list-style-type: none"> • Primary Marker, T/A4, is above or below the individual limits. • Sequence of primary Marker, T/A4 deviates from expected values. 	APMU and 1 st Expert review (internal or external from the APMU) are mandatory.
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Based on APMU and/or Expert(s) evaluation of a blood steroidal Passport, the following recommendations, indicated in the APMU Report in ADAMS, would require further ADOs actions:

- Confirmation analysis of Markers in one or several blood and/or urine Samples. These Samples could either have generated an ATPF or a flag (i.e., outside the individual limits of the Marker) or be part of the Athlete’s baseline level of the Markers.
- Collection of one or more follow-up blood Samples (for example, to confirm an Athlete’s baseline levels or establish a potential doping scenario).
- Storage of one or more blood and/or urine Samples for a period greater than the default time frame of three (3) months.
- Analysis of one or several blood Samples for the detection of steroid esters. This Analytical Testing Procedure is not mandatory to implement for WADA-accredited Laboratories and ADOs are advised to contact the Laboratories to be informed about the availability of this method.
- Analysis of one or several urine Samples from the Athlete to detect the presence of synthetic forms of testosterone and related compounds by GC/C/IRMS.

What are the possible outcomes following an APMU/Expert evaluation of a blood steroidal Passport?

An APMU/Expert evaluation of a Passport will result in one of the following possible opinions:

- Normal
- Suspicious
- Likely Medical Condition
- Likely doping

Should the panel of Experts agree on a Likely Doping opinion, the administrative steps, described in the Annex C of the International Standard for Results Management (ISRM), will be accompanied by the following recommendations:

- Unanimous likely doping
- APF (Athlete Passport Finding)
- Athlete’s explanation provided to Expert panel
- APF confirmed

ADOs should also be aware that three additional steroid APMU recommendations could be provided in ADAMS related to indicate a potential urine exchange:

- Suspicion of Urine Exchange
- Multiple DNA Profiles Identified
- Urine Exchange Confirmed

These recommendations are mentioned by an APMU when a urine Sample steroid profile is not consistent with other Sample(s) from the Athlete's Passport. Consequently, the ADOs should initiate, in collaboration with their APMU, further investigations to identify such potential urine exchange.

Can blood Samples be invalidated in the Steroidal Module?

Yes. While there is no automatic rule in ADAMS to invalidate a Sample after analysis for the Steroidal Module, an APMU can reassess the validity of a Sample based on their interpretation and additional information reported by the Laboratories (e.g., hemolysis or degradation of the Sample).

What is the expected timeline from the collection of a Sample to the entry of an APMU recommendation in ADAMS for blood steroid Markers?

In order to ensure cost effectiveness, Laboratories analyze Samples for the blood Steroidal Module in batches. It is expected that Laboratories will report the results in ADAMS within a maximum of twenty (20) days after the Sample reception date. Therefore, ADOs should expect to receive APMU/Experts recommendations within a period of one month after collection of a Sample. This timeline should be considered by ADOs when developing their testing strategy (e.g., to collect multiple Samples during a short period of time when doping may be suspected).

Specific agreements between ADOs, Laboratories and/or APMU are possible to make the whole process more responsive (e.g., during main events) and it is recommended that ADOs contact their APMU and the Laboratories to agree on the terms for such specific needs or situations.

What happens in case of a steroidal Passport resetting?

As indicated in ISRM, Annex C, article C.7, a Passport shall be reset in the two following scenarios:

1. When the Athlete has been found to have committed an anti-doping rule violation based on the Passport; or
2. When an Athlete is found to have committed an anti-doping rule violation based on a Prohibited Substance or Prohibited Method which may affect the Markers.

Because the same Passport identification number (BPID) is attributed for the three modules of the ABP (Hematological, Steroidal and Endocrine) for each Athlete, whenever a module is reset, the three modules will be affected. Passports that are reset are still available in ADAMS for ADOs having the custodianship or a sharing agreement.