Laboratory Guidelines

Version 2.0

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Analytical Requirements for the Endocrine Module of the *Athlete Biological Passport*

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Objective

These Laboratory Guidelines have been developed to ensure a harmonized application of Analytical *Testing* Procedures for the measurement of

Analytical and Reporting Requirements for the Blood *Markers* of the Endocrine Module of the *Athlete Biological Passport*

1.0 Introduction

The purpose of this *Technical Document* (*TD*), which constitutes an integral part of the *International Standard* for Laboratories (ISL)[1] is to harmonize the analysis and reporting of the blood (serum) *Markers* of human Growth Hormone (hGH) as part of the Endocrine Module of the *Athlete Biological Passport* (*ABP*). **The document provides guidance on the pre-analytical details, *Sample* preparation procedure, the performance of the analyses and the reporting of the test results.**) to uncover the *Use* of *Prohibited Substances* associated with hGH biological activity, namely recombinant hGH preparations, hGH secretagogues, hGH analogs and releasing factors, and Insulin-like Growth Factor-I (IGF-I).

Scope

These Laboratory Guidelines contain requirements for the implementation of the Analytical *Testing* Procedures for the quantification of hGH *Markers* as part of the Endocrine Module of the *ABP*, which allows the detection of hGH doping and may also have utility in detecting GH secretagogues and IGF-I abuse in sport [1,2]. These Laboratory Guidelines follow the rules established in the *WADA International Standard for* Laboratories (ISL) [3] and relevant *Technical Documents* (*TDs*) regarding the Analytical *Testing* of blood *Samples*.

Introduction to the Analytical *Testing* Procedures

The Analytical *Testing* Procedures for the Endocrine Module involve the measurement of two (2) *Markers* of hGH biological activity, namely Insulin-like Growth Factor-I (IGF-I) and N-terminal Pro-peptide of Type III Collagen (P-III-NP), which are naturally present in blood and whose concentrations are increased following hGH administration [4–11]. The measured concentrations of these two (2) *Markers* are then combined in a discriminant function formulae to calculate a GH-2000 score, which is gender-specific and includes an adjustment for age to reflect the age-related decline in hGH and *Marker* concentrations [4].

In order to generate individual *Athlete* longitudinal data that are comparable between Laboratories, a specific IGF-I / P-III-NP assay pairing is applied for the measurement of concentrations of IGF-I and P-III-NP in blood (serum) for the purposes of the *ABP*. The assays used for the Endocrine Module of the *ABP* are limited to:

- Intact IGF-I quantification by top-down Liquid Chromatography (tandem) Mass Spectrometry (LC-MS^n; $n \geq 1$) [12], as detailed in Table 2 below.
- P-III-NP quantification using Siemens ADVIA Centaur P-III-NP chemiluminescence immunoassay (Siemens Healthcare Laboratory Diagnostics, Camberley, UK). The Siemens ADVIA Centaur P-III-NP assay is an automated, two-site sandwich, chemiluminescent immunoassay [13]. The assay uses two (2)

monoclonal mouse antibodies: the first antibody is an acridinium ester-labeled anti-P-III-NP antibody. The second antibody is a biotin-labeled anti-P-III-NP antibody. The solid phase contains streptavidin-coated paramagnetic particles and during the reaction, the light emitted by the acridinium label is directly proportional to the concentration of P-III-NP in the sample. The Siemens P-III-NP assay is calibrated by the manufacturer using a standard derived from bovine P-III-NP.

For the purposes of the *ABP*, an initial quantification of the "A" *Sample* is performed. When requested, a confirmatory quantification of the "A" *Sample* may additionally be performed using the same assay pairing (see, Article 6.2) to confirm the concentrations and to perform identification of IGF-I (as per TD IDCR[14]).

The concentrations of IGF-I and P-III-NP reported by the Laboratories, as well as the GH-2000 score automatically calculated in *ADAMS*, are integrated in the Endocrine Module of *ADAMS* using a similar Bayesian approach to that applied in the Steroidal and Hematological Modules of the *ABP*.[15]

1.1 Procedure for Analysis of the Blood *Markers* of the Endocrine Module

The Analytical *Testing* Procedure (ATP) involves the measurement of the serum concentrations of two *Markers* of hGH biological activity, namely IGF-I and N-terminal Pro-peptide of Type III Collagen (PIIINP), which are naturally present in blood and whose concentrations are increased following hGH administration. The measured concentrations of these two *Markers* are then combined in a score calculated automatically in *ADAMS* (*i.e.*, the GH-2000 score), which is sex-specific and includes an adjustment for age to reflect the age-related decline in hGH and *Marker* concentrations.

a) The ATP for the blood endocrine *Markers* is not a mandatory ATP (see ISL *TD* ATP [2]) and is not applied to all serum *Samples*. Therefore, these blood endocrine *Markers* shall be measured in serum *Sample*(s) (see Article 2.0) by Laboratories with appropriate analytical capacity and upon request by the responsible *Testing* Authority (TA) or *WADA*, and results shall be reported in *ADAMS*.

b) The analysis of the blood endocrine *Markers* follows a two (2)-step procedure:

i. An Initial *Testing* Procedure (ITP) based on the quantification of intact IGF-I by top-down Liquid Chromatography-(tandem) Mass Spectrometry (LC-MS^n) or High-Resolution Mass Spectrometry (LC-$HRMS^n$), and on the quantification of P-III-NP using the Siemens ADVIA Centaur P-III-NP chemiluminescence sandwich immunoassay (Siemens Healthcare Laboratory Diagnostics, Camberley, UK), and

ii. A subsequent Confirmation Procedure (CP) may be performed, which consists of the same assay pairing used for the quantification of the concentrations of IGF-I and P-III-NP in the ITP, as well as the identification of IGF-I (in compliance with the ISL *TD* IDCR [3]). A CP shall be performed when at least one of the three blood endocrine *Markers* (IGF-I, P-III-NP, or GH-2000) in the *Sample* constitutes an outlier in the corresponding Passport for elevated values, as determined by the Adaptive Model, triggering an Endocrine – Confirmation Procedure Request ("Endocrine-CPR") in *ADAMS*. A CP may also be performed upon request to the Laboratory (see Article 4.2).

If an outlier for elevated IGF-I from the ITP is confirmed, a subsequent analysis will be performed on the *Sample* applying the hGH Isoform Differential Immunoassay (see ISL *TD* GH [4]).

~~1.0~~2.0 Assay Pre-analytical Procedure

a) The Laboratory should (usually) receive refrigerated (not frozen[i]) "A" and "B" blood *Samples*, which have been collected in ~~blood~~serum tubes containing an inert polymeric serum separator gel and a clotting ~~activation factor~~activator (for example: BD Vacutainer® SST™-II Plus tubes, EU ref 367955; BD Vacutainer® SST™-II Plus Advance tubes, EU ref 367954; BD Vacutainer® SST™ tubes, US ref 367986) in accordance with the *International Standard* for *Testing* ~~and Investigation (ISTI)~~ [16]~~;~~ (*IST*) [5]. The use of alternative collection devices shall be validated by the relevant Laboratory(ies) and approved by *WADA* prior to use for *Sample* collection.

~~*[Comment: Previous studies have demonstrated that IGF-I and P-III-NP concentrations remain stable if the Sample is maintained at a refrigerated temperature for up to 5 days* [17]*.]*~~

b) Alternatively, if the clotting and centrifugation of the blood *Sample* is performed prior to reception at the Laboratory (for example, at the site of *Sample* collection~~),~~) or when a blood *Sample* is shipped from another Laboratory for subcontracted analyses, *Samples* may be received at the Laboratory as frozen/refrigerated ~~blood~~ *Samples* either in the same *Sample* collection tubes or as separated serum in new tubes~~;~~.

c) The Laboratory shall check the status of the *Sample*(s~~) (*e.g.*, evidence of hemolysis~~) and the integrity of the collection tubes (*e.g.,* evidence of breakage of the separating gel). The Laboratory shall note any unusual condition of the *Sample* and record such condition(s) in the ~~Test Report~~Lab Results in *ADAMS*~~;~~.

d) Any *Samples* delivered to the Laboratory in tubes containing an anti-coagulant (for example, ~~*ABP*~~whole blood *Samples* collected in EDTA tubes), or as separated plasma, shall not be analyzed for the blood hGH *Markers* ~~of the Endocrine Module;~~.

e) The Laboratory shall notify and seek advice from the ~~*Testing* Authority~~TA regarding rejection or Analytical *Testing* of *Samples* for which irregularities are noted (see ISL [3]~~).~~[1]).

~~1.1~~2.1 Blood *Samples* ~~received~~Received as ~~non~~Non-separated ~~blood~~ in ~~tubes containing an inert polymeric serum separator gel and a clotting activation factor:~~Serum Tubes

[i] ~~unless~~Unless the blood matrix components have been separated before shipment to the Laboratory.

~~Reception~~	~~Both *Samples* "A" and "B" shall be centrifuged for 10-15 min at 1300-1500 g as soon as possible after reception at the Laboratory.~~ ~~The "A" *Sample* shall be used for the initial and confirmatory (if needed) quantifications (see below).~~ ~~The "B" *Sample* shall be step-frozen and stored until use, if needed (see below).~~
~~Aliquoting and analysis~~*Sample* Processing upon Reception	– Both "A" and "B" *Samples* shall be centrifuged for 10-15 min at 1300-1500 g as soon as possible after reception at the Laboratory. "A" *Sample* ~~One Aliquot of the "A" *Sample* serum shall be taken for initial quantification.~~ – ~~The remaining "A" serum fraction~~ If the "A" *Sample* is not opened to be analyzed within five (5) days from *Sample* collection, then the Laboratory may ~~be kept~~ : • Keep the centrifuged "A" *Sample* in the *Sample* collection tube ~~or aliquoted~~ and step-freeze it (at approx. -15°C or less and according to the tube manufacturer's instructions) until thawing and aliquoting for analysis, or • Aliquot the separated serum fraction into new vials ~~with label(s)~~ (ensuring that appropriate Laboratory ~~Internal~~ Chain of Custody (see ISL *TD* LCOC) [6] is maintained~~.~~), which shall be stored frozen (at approx. -15°C or less) until thawing for analysis. ~~For initial quantification:~~ ~~– the Aliquot may be analyzed immediately after aliquoting; or~~ ~~– the Aliquot shall be stored at approximately 4 °C if analyzed within 24h (within a maximum of five (5) days from *Sample* collection); or~~ ~~– the Aliquot shall be frozen (-20°C) if the analysis will be conducted more than 24h after aliquoting.~~ – ~~For the confirmatory quantification, one new Aliquot of the~~ "B" *Sample* The centrifuged "B" *Sample* shall be step-frozen and stored (at approx. -15°C or less and according to the tube manufacturer's instructions) until use, if needed (see below). *[Comment: If the Laboratory transfers the Aliquot into new vials for frozen storage, the vials should ensure proper sealing for optimal storage (cryovials with an "O-ring"). Thawing of Sample(s) for analysis should be done stepwise; Samples shall not be thawed under hot water or any other similar process that risks raising the temperature of the Sample above room temperature. Thawing overnight under refrigeration (2-8 °C) is recommended.]*~~"A" *Sample* shall be analyzed immediately after aliquoting.~~ ~~*[Comment: When analyses specific to the ABP are requested for blood (serum) Samples (i.e., Markers of the Endocrine Module or blood steroid Markers as part of the Steroidal Module), only the "A" Sample should be considered for the initial and the confirmatory quantifications of the Markers. In cases where the "A" Sample is not suitable for the performance of ABP Markers quantification (e.g., there is insufficient Sample volume; the Sample container has not been properly sealed or has been broken; the Sample's integrity has been compromised in any way; the "A" Sample is missing), a splitting procedure of the "B" Sample could be performed, as detailed in the ISL[3].]*~~

~~Storage~~ ~~[The same storage conditions apply for *Samples* received in conditions described in section 4.2]~~*Sample* Processing for Analysis	~~Storage for up to three (3) months → at approximately -20 °C.~~ ~~Storage for more than three (3) months → freeze at approximately -20 °C and transfer to approximately -70 to -80 °C.~~ a) ~~*[Comment: If*~~ITP An Aliquot of the ~~*separated*~~"A" *Sample* serum fraction ~~*is*~~shall be taken for the ITP of the blood endocrine *Markers*, and shall be processed as follows: • It may be analyzed immediately after aliquoting; or • It may be stored refrigerated (2-8 °C) if analyzed within a maximum of five (5) days from *Sample* collection; or • It shall be stored frozen (at approx. -15 °C or less) if the analysis will be conducted more than five (5) days from *Sample* collection. The remaining "A" serum fraction may be kept in the *Sample* collection tube~~*, it shall be step-frozen for storage according to the tube manufacturer's instructions until analysis.*~~ ~~*If the Laboratory transfers the Aliquot*~~ or aliquoted into new ~~*vials for*~~vial(s) and shall be stored frozen ~~*storage,*~~(at approx. -15 °C or less) if the ~~*vials should ensure proper sealing for optimal storage (cryovials with an "O-ring").*~~ ~~*Thawing of Sample(s) for*~~ analysis ~~*should also be done stepwise. Samples shall not be thawed under hot water or any other similar process that risks raising the temperature of the Sample above room temperature. Thawing overnight at 4°C is recommended.]*~~will be conducted more than five (5) days from *Sample* collection[ii]. b) CP The CP shall be performed on a new Aliquot of the remaining "A" *Sample* serum fraction and shall be conducted immediately after aliquoting.

~~1.2~~2.2 Blood *Samples* ~~received~~Received as ~~frozen/refrigerated centrifuged blood/serum *Samples*:~~Centrifuged and Frozen/Refrigerated

Sample Processing upon Reception	a) If *Samples* are received frozen, they ~~should~~shall remain frozen until thawing and aliquoting for analysis~~ as described in this Article 4.2~~. b) If *Samples* are received refrigerated, ~~they~~the "A" *Sample* should be processed ~~as~~ to obtain an Aliquot for analysis as soon as possible (as per Article 4~~2~~.1~~.~~.), while the "B" *Sample* shall be stored frozen (at approx. -15 °C or less) until aliquoting for analysis.
~~Aliquoting~~Aliquot, Storage and ~~analysis~~Analysis	a) ITP i. Once a serum Aliquot of the "A" *Sample* ~~"A"~~ is ~~thawed, one Aliquot shall be~~ taken for ~~initial quantification. This Aliquot~~ the ITP of the blood endocrine *Markers*, it should: • Be analyzed immediately or may be stored ~~at approximately 4~~refrigerated (2-8 °C ~~for~~) if analyzed within a maximum of ~~24h before~~ five (5) days from *Sample* collection; or • Stored frozen (at approx. -15 °C or less) if the analysis~~.~~ is to be conducted after five (5) days from *Sample* collection [3].

[ii] It is recommended that the Laboratory stores the serum *Samples* frozen (at approx. -70 °C or less) if the TA (or *WADA*) has requested the Laboratory to place them into long-term storage (> 3 months) for Further Analysis purposes (see also ISL Article 5.3.7.2).

	i.ii. The remaining "A" serum fraction ~~may be kept in the *Sample* collection tube or aliquoted into new vial(s) with label(s) ensuring Laboratory Internal Chain of Custody is maintained.~~ shall be stored as per Article 2.1 above. b) ~~For the confirmatory quantification, one~~CP The CP shall be performed on a new Aliquot of the remaining "A" *Sample* serum fraction and shall be ~~analyzed~~conducted immediately after aliquoting.

~~Analytical *Testing* Procedure Requirements~~

[Comment to Articles 2.1 and 2.2: When analyses specific to the ABP are requested, only the "A" Sample shall be considered for the ITP and CP. In cases where the "A" Sample is not suitable for the performance of the ABP Markers analysis (e.g., there is insufficient Sample volume; the Sample container has not been properly sealed or has been broken; the Sample's integrity has been compromised in any way; the "A" Sample is missing), a splitting procedure of the "B" Sample could be performed, as detailed in the ISL [1].]

~~2.0~~3.0 Analytical *Testing* Procedure Validation and Analysis Requirements

~~Prior to~~For the implementation of the ~~Analytical *Testing* Procedures~~ATPs for the ~~quantification~~analysis of ~~IGF-I and P-III-NP~~the blood endocrine *Markers* of the *ABP* in routine *Doping Control* analysis, the Laboratory shall fulfil the following requisites:

a) Validate the ~~Analytical *Testing*~~Quantitative Procedures~~, including~~ (for ITP and CP) for measuring the ~~determination of *Marker* concentrations, as well as~~ the ~~assays' Limit of Quantification (LOQ), Repeatability (s_r), Intermediate Precision (s_w), Bias and Measurement Uncertainty (u_c);~~Qualitative Procedure (for CP) for IGF-I identification, as per the ISL *TD* VAL [7] requirements.

b) The ~~Analytical *Testing* Procedures~~validated ATPs shall meet the acceptance values for the parameters of assay performance applicable to the separate quantification of IGF-I and P-III-NP ~~assay performance,~~concentrations as specified in ~~Table~~ Article 3.1 ~~and Table~~ (as applicable).

~~b)~~c) The Laboratory shall apply the validated ATPs in accordance with the ATP Analysis Requirements specified in Article 3.2 ~~(as applicable).~~.

~~**Table 1**: Acceptance Criteria for Parameters of Assay Performance for the Endocrine Module~~

3.1 ATP Validation Requirements (see also ISL *TD* VAL [7])

~~**Validation Parameters**~~**Endocrine *Marker***	**IGF-I**	**P-III-NP**
Identification of *Markers*	The Laboratory shall validate the Qualitative Procedure for confirmation of the identity of IGF-I in accordance with the requirements of the ISL *TD* IDCR [3] and ISL *TD* VAL [7]	N/A
~~Maximum LOQ~~ **Limit of Quantification (LOQ)**	~~≤~~≤ 50 ng/mL	~~≤~~≤ 1 ng/mL
Working Range	50 -1000 ng/mL	1 – 20 ng/mL

~~Maximum~~ **Relative Standard Combined** ~~Standard~~ **Measurement Uncertainty** ~~(~~, u_{c_Max}, ~~%)~~ **(%),**	≤(≤) 30% at LOQ (≤) 20% at > 150 ng/mL	≤≤ 15%

3.2 ATP Analysis Requirements

Endocrine *Marker*	IGF-I	P-III-NP
Test Method and Instrumentation	Quantitative Procedure (ITP and CP): Top-down (intact) Liquid Chromatography combined with tandem Mass Spectrometry based on triple quadrupole (LC-MS^n) or High-Resolution Mass Spectrometry analyzer (LC-HRMS). Qualitative Procedure (CP): LC-MS^n (or LC-HRMS) (in compliance with the ISL *TD* IDCR [3])	Quantitative Procedure (ITP and CP): Siemens ADVIA Centaur P-III-NP chemiluminescence immunoassay
Aliquot	Shall be measured (ITP and CP) in singlicate (1x) on one (1) serum Aliquot not greater than (≤) 50 µL	Shall be measured (ITP and CP) in singlicate (1x) on one (1) serum Aliquot according to manufacturer's instructions.
Internal Standards	Stable isotope-labeled IGF-I (*e.g.,* NIST or ProSpec ^{15}N-IGF-I).	N/A
Calibration	A freshly prepared Single Point Calibrator (SPC) shall be included in each analytical batch. The recombinant human IGF-I calibrator from NIST (SRM 2926) should be used to prepare the SPC. Any other calibration material shall be validated against the NIST SRM 2926 calibrator.	As per kit instructions
Quality Controls	The QCs shall be prepared either from authentic serum or by spiking a standard solution(s), independent from that used for the calibrator(s), into serum. Following preparation, all QC material shall be aliquoted and stored frozen (preferably at approx. -70 °C or less for long-term storage) until use. At least one (1) serum QC sample representative of the low part of the working range (*e.g.*, at a concentration within the first quartile of the working range) and one (1) serum QC sample representative of the high part of the working range (*e.g.*, at a concentration within the fourth quartile of the working range) shall be used. The QCs may be prepared independently for each *Marker* or containing both *Markers* at the appropriate concentrations in a single QC sample. For the CP, at least one QC sample, depending on the initial quantification results for the *Markers*, shall be included in each confirmatory analytical batch.	

~~2.0~~4.0 ~~Analytical *Testing*~~Confirmation Procedure ~~Accreditation Requirements~~for the Endocrine Module

4.1 ~~Demonstrate readiness~~Confirmation Procedure Requests for ~~assay implementation~~Blood Endocrine *Markers* Triggered through ~~method validation~~*ADAMS*

a) Once the ITP data of the blood endocrine *Markers* is entered and ~~successful participation~~matched with the corresponding *Doping Control* Form (DCF) *in* ~~at least one *WADA*-approved educational External Quality Assessment Scheme (EQAS) round~~ *ADAMS*, the Adaptive Model automatically updates the endocrine Passport. If an outlier is identified based on an abnormally high GH-2000 score, IGF-I, and/or ~~inter-~~P-III-NP value, an Endocrine-CPR is triggered and sent automatically to the Laboratory ~~collaborative study. In cases of identified deficiencies, proper corrective action~~through *ADAMS*. The Laboratory shall ensure the reception and management of CPR notifications using a dedicated *ADAMS* account(s~~)~~).

b) The TA[iii] shall inform the Laboratory whether to proceed or not with the CP of the blood endocrine *Markers*, within fourteen (14) days from the receipt of the Endocrine-CPR notification.

i. Upon receipt of confirmation to proceed with the CP, the Laboratory shall proceed with the CP of the blood endocrine *Markers* as soon as possible.

~~i.~~ii. Any justification from the TA or the PC[iii] to not proceed with the CP shall be ~~documented and implemented;~~provided in writing according to Article 8.6 of the ISL *TD* APMU [8]. In such cases, the Laboratory shall update the Lab Results in *ADAMS* for the *Sample* with a comment stating that the TA or the PC[iii], as applicable, requested to not perform the CP, and the reasons given.

iii. ~~Obtain ISO/IEC 17025 accreditation~~In the absence of communication from the TA or the PC[iii] within fourteen (14) days from the Endocrine-CPR notification, the Laboratory shall proceed with the CP of the blood endocrine *Markers*.

c) When the Laboratory receives an Endocrine-CPR for ~~the~~ a *Sample* for which *Adverse Analytical Finding*(s) (*AAF*) have been reported for other *Prohibited Substance*(s) or *Prohibited Method*(s), the Laboratory shall consult the TA about the need to conduct the CP for the blood endocrine *Markers*.

4.2 Confirmation Procedure Requests from the ***Testing*** ~~Procedures~~Authority, the Passport Custodian, the *Athlete* Passport Management Unit or *WADA*.

The Adaptive Model will also flag abnormally low or variable endocrine *Markers*. However, in such cases the Laboratory will not receive an automatic notification through *ADAMS*. Instead, the *Athlete* Passport Management Unit (APMU) will advise the PC (who will advise the TA, if different) on whether the *Sample*, or other *Samples* from the corresponding Passport, shall be subjected to a CP for the blood endocrine *Markers* and the application of the hGH Isoform Differential Immunoassay. Therefore, in these cases the Laboratory shall receive a written request from the TA[iii], or *WADA*, before proceeding with the CP.

4.3 Confirmation of Blood Endocrine *Marker* Values

The CP for the blood endocrine *Markers* following receipt of an Endocrine-CPR, or upon request, consists of the application of the same assay pairing used for the quantification of ~~hGH *Markers* in blood~~ the concentrations of IGF-I and P-III-NP in the ITP as ~~part of the Endocrine Module from an Accreditation Body that is a full member of the International Laboratory Accreditation Cooperation~~

[iii] The APMU or PC, where the PC is not the TA, may contact, in writing, the Laboratory regarding performance of a CP of the blood endocrine *Markers* on behalf of the TA. In such cases, the APMU (which may have been bestowed such authority by the PC) or the PC shall copy the relevant TA.

(ILAC) and a signatory to the ILAC Mutual Recognition Agreement (ILAC MRA).well as the identification of IGF-I (in compliance with the ISL *TD* IDCR [3]).

Quality Controls (QCs) and Reagents

- QC samples: Laboratories shall implement well-characterized and stable internal QC sample(s), which are not subject to assay lot variations, for the performance of the tests under different assay conditions (different assay lots, different analysts, *etc.*). Following preparation/reception by the Laboratory, all QC material should be aliquoted and stored frozen (preferably at – 80°C for long-term storage) until use. These QC samples should include:
  - QC_{low}: Serum obtained from healthy individual(s), which is demonstrated to contain concentrations of IGF-I not greater than (≤) 200 ng/mL and P-III-NP not greater than (≤) 5 ng/mL;
  - QC_{high}: Serum obtained from hGH administration studies or another appropriate source that has been demonstrated to contain concentrations of IGF-I greater than (≥) 500 ng/mL and P-III-NP greater than (≥) 10 ng/mL.

4.4 *[Comment: Four (4) separate QC samples may also be used, as long as they contain* hGH Isoform Differential Immunoassay Requests ("Isoform-Requests") triggered through *ADAMS*

a) Once the CP data of the blood endocrine *Markers* is entered and matched with the corresponding Passport in *ADAMS*, the Adaptive Model automatically updates the endocrine Passport with the confirmed *Marker* values.

b) If the ITP triggers an *Atypical Passport Finding* (*ATPF*) for high IGF-I, which is confirmed in the subsequent CP, an Isoform-Request for the performance of the hGH Isoform Differential Immunoassay (see ISL *TD* GH [4]) is triggered and sent automatically to the Laboratory through *ADAMS* (see Figure 1).

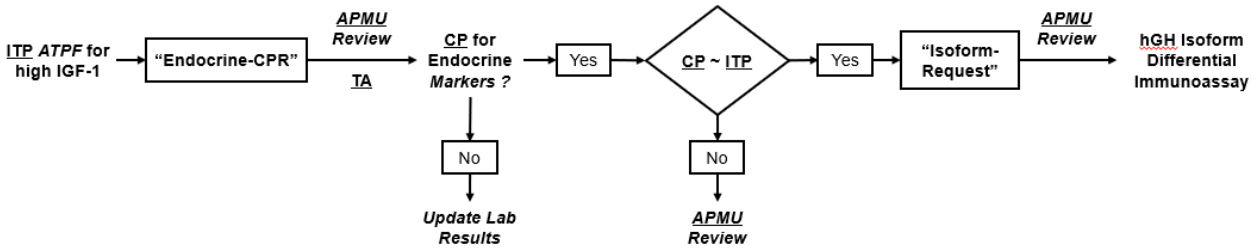

Figure 1: Steps leading to the application of the hGH Isoform Differential Immunoassay to *Samples* with elevated IGF-1 identified in the ITP.

5.0 Reporting Results for the Blood Endocrine *Markers*

The IGF-I and P-III-NP *at the necessary* concentrations *(e.g., QC_{IGF-I_low}, QC_{IGF-I_high}, QC_{PIIINP_low} and QC_{PIIINP_high}).]*

- Reagents: With every new batch of reagents (new lot number), the following evaluation steps should be implemented before including the new batch into routine operations for P-III-NP quantification:

- Each of the QC samples shall be determined at least three (3) times whenever a new batch of reagents is obtained. The number of replicates per determination shall be conducted as stipulated by the assay manufacturers. The QCs may be measured in a single assay or over a range of assays. If, for any QC, the difference between the mean concentration for the new batch and that for the preceding batch is more than 20%, the new batch shall not be implemented into routine operations and an investigation of the new batch shall be conducted.
- In order to detect small but systematic changes over time, it is recommended that the performance of a new batch of reagents is controlled, for example, through a cumulative sum (CUSUM) chart/table, which is established for each QC based on the difference between the mean(s) of the new batch and the initial value(s). When using the CUSUM, results should be assessed using customary procedures as detailed at *http://itl.nist.gov/div898/handbook/pmc/section3/pmc323.htm*

Analytical *Testing* Procedure and Reporting of Test Results

Initial Quantification of the *Markers*

- One Aliquot taken from the original "A" *Sample* shall be analyzed once (x1) to quantify intact IGF-I and P-III-NP;
- QC Sample(s), at low- and high-levels of the *Markers* (see Article 5.3), shall be included in each initial quantification analytical batch;

a) The concentrations of IGF-1 and P-III-NP shall be reported in *ADAMS* in nanograms per milliliter (ng/mL);).

[Comment: for the purposes of the Endocrine Module of the ABP, the GH-2000 score does not need to be calculated or reported by the Laboratory since it will be automatically calculated in ADAMS [15].]

[Comment to Article 5.0 a): For the purposes of the Endocrine Module of the ABP, the GH-2000 score does not need to be calculated or reported by the Laboratory; it will be automatically calculated in ADAMS].

b) If the measured *Marker* concentration(s) of IGF-I and/or P-III-NP is below the LOQ of the assay, the Laboratory shall report a value of "-1" for itsthe affected concentration in *ADAMS*.

b)c) If the concentration(s) of IGF-I and/or P-III-NP cannot be determined or the *Marker*(s) cannot be identified, the affected *Marker(s)* shall be reported as "-1" and the Laboratory shall make a corresponding comment in the Test Report on why the *Marker* could not be quantifiedLab Results in *ADAMS* (*e.g.,* the measurement of the *Marker* is not possible due to unusual matrix interferences);).

- An observation of hemolysis of the *Sample* should be recorded in the comments section of the Laboratory Test Report in *ADAMS*.

Confirmatory Quantification of the *Markers*

If requested by the *Testing* Authority (TA), *Results Management* Authority (RMA) or *WADA*, the Laboratory shall proceed with the confirmatory quantification of the *Markers* of the Endocrine Module.

[Comment: An APMU or Passport Custodian (PC), where the PC is not the TA, may request a confirmatory quantification on behalf of the TA or RMA. In such cases, the APMU or PC shall copy the relevant TA or RMA, as applicable, on all written requests to the Laboratory for confirmatory quantifications.]

When a confirmatory quantification analysis is requested:

- One new Aliquot taken from the original "A" *Sample* shall be analyzed once (x1) to:
  - quantify intact IGF-I and P-III-NP; and
  - identify IGF-I (as per the TD IDCR [14]);
- At least one QC Sample (see Article 5.3), depending on initial quantification results, shall be included in each confirmatory quantification analytical batch;
- The concentrations of IGF-I and P-III-NP shall be reported in *ADAMS* in nanograms per milliliters (ng/mL);
- If the measured *Marker* concentration is below the LOQ of the assay, the Laboratory shall report a value of "-1" for its concentration in *ADAMS* and the Laboratory shall make a comment in the Test Report on why the *Marker* could not be quantified (e.g., the measurement of the *Marker* is not possible due to unusual matrix interferences);
- An observation of hemolysis of the *Sample* should be recorded in the comments section of the Laboratory Test Report in *ADAMS*.

Table 2. Analytical *Testing* Procedure Validation and Performance Requirements for the initial and confirmatory quantification of IGF-I in blood (serum) *Samples* by top-down LC-MS^n for the Endocrine Module of the *ABP*.

Method and Instrumentation	Top-down (intact IGF-I) Liquid Chromatography combined with (Tandem) Mass Spectrometry based on triple quadrupole or HRMS (LC-MS^n; n ≥ 1).
Range of the Method	Shall cover the ranges of IGF-I concentrations normally found in males and females and demonstrate linearity between **50–1000 ng/mL**, at least.
Limit of Quantification (LOQ)	The LOQ shall not be greater than (≤) **50 ng/mL**.
Maximum Relative Combined Standard Measurement Uncertainty u_c (%)	The estimated u_c (%) shall not be greater than (≤) **20%**.
Sample	IGF-I quantification shall be conducted using a volume not greater than (≤) **50 µL** of serum per analysis.
Internal Standard	Stable isotope-labeled IGF-I (*e.g.*, NIST[iv] or ProSpec[v] -^{15}N-IGF-I).
Calibration	A freshly prepared single point calibrator (SPC) shall be included in each analytical batch. The Recombinant Human IGF-I calibrator from NIST (SRM 2926[vi]) should be used to prepare the SPC. Any other calibration material shall be validated against the NIST SRM 2926 calibrator.

[A]pplicable links
[iv] https://shop.nist.gov/ccrz__ProductDetails?sku=2927&cclcl=en_US
[v] https://www.prospecbio.com/igf1_n15_human
[vi] https://shop.nist.gov/ccrz__ProductDetails?sku=2926&cclcl=en_US

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[2] *WADA Technical Document* ISL *TD* ATP: Analytical *Testing* Procedures.

[3] *WADA Technical Document* ISL *TD* IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the *Identity* of Analytes for *Doping Control* Purposes.

[4] *WADA Technical Document* ISL *TD* GH: Human Growth Hormone (hGH) Isoform Differential Immunoassays for Doing Control Analyses.

[5] The World Anti-Doping *Code International Standard* for *Testing* (IST).

[6] *WADA Technical Document* ISL *TD* LCOC: Laboratory Chain of Custody.

[7] *WADA Technical Document* ISL *TD* VAL: Minimum Requirements for Validation of Analytical *Testing* Procedures for *Doping Control.*

[8] *WADA Technical Document* ISL *TD* APMU: Athlete Passport Management Unit Requirements and Procedures

[Comment to Article 6.0: Current versions of WADA International Standards and Technical Documents may be found at https://www.wada-ama.org/en/what-we-do/international-standards]