GUIDELINES

REPORTING & MANAGEMENT of

URINARY HUMAN CHORIONIC GONADOTROPHIN (hCG) and LUTEINIZING HORMONE (LH) FINDINGS IN MALE ATHLETES

Version 2.0
July 2015
# TABLE OF CONTENTS

1. Objective .................................................................................................................. 3
2. Scope .......................................................................................................................... 3
3. Responsibility .............................................................................................................. 4
4. Introduction .................................................................................................................. 4
5. Pre-analytical Procedure ............................................................................................ 4
6. Assay Requirements .................................................................................................... 5
7. Analytical **Testing** Strategy ....................................................................................... 6
   7.1 Testing for hCG ....................................................................................................... 6
   7.2 Testing for LH .......................................................................................................... 7
8. Interpretation and Reporting of Results ....................................................................... 7
   8.1 hCG results .............................................................................................................. 7
   8.2 LH results ................................................................................................................ 8
9. Results Management .................................................................................................... 9
   9.1 hCG findings ........................................................................................................... 9
   9.2 LH findings .............................................................................................................. 9
10. Definitions .................................................................................................................. 10
    10.1 *Code* Defined Terms ......................................................................................... 10
    10.2 *ISL* Defined Terms ............................................................................................. 11
    10.3 *ISTI* Defined Terms .......................................................................................... 12
    10.4 Other Terms ......................................................................................................... 12
11. References .................................................................................................................. 12
1. **Objective**

These guidelines have been developed to ensure a harmonized approach in the reporting and management of elevated urinary concentrations of human Chorionic Gonadotrophin (hCG) and Luteinizing Hormone (LH).

The finding of the α/β heterodimer of hCG\(^1\) in the urine of male Athletes at concentrations greater than the established Decision Limit (DL) may be an indicator of hCG Use for doping purposes. However, due to the association of elevated urinary hCG with pathologies such as testicular cancer, consideration must be given to possible causes, other than doping, that can produce elevated concentrations of heterodimeric hCG in urine Samples from male Athletes.

Elevated concentrations of total LH\(^2\) in urine of male Athletes may also be an indication of the administration of this banned substance for doping purposes or of the Use of substances that induce the release of endogenous LH, such as gonadotropin-releasing hormone (GnRH) and its synthetic analogues or estrogen blockers (anti-estrogens, aromatase inhibitors). On the other hand, suppressed urinary concentrations of LH in male Athletes may also be an indication, or corroborative finding, of the Use of androgens.

These guidelines aim to assist Laboratories in reporting analytical findings for hCG and LH as well as Anti-Doping Organizations (ADOs) in their results management duties to determine whether an anti-doping rule violation (ADRV) has occurred.

2. **Scope**

These guidelines follow the current rules established in the International Standard for Laboratories (ISL), whose requirements are still fully applicable and shall be respected.

These guidelines outline the Analytical Testing requirements for Laboratories and provide recommendations to ADOs to facilitate the result management of elevated concentrations of hCG and LH in urine Samples of male Athletes.

---

\(^1\) The α/β heterodimer of hCG includes the intact α/β heterodimer as well as the ‘nicked’ α/β heterodimer, in which the β-subunit is (usually) cleaved between residues 47 and 48. Although cleaved, the α and β-subunits in the nicked hCG are held together by non-covalent bonds. Immunoassays developed against ‘intact hCG’ usually measure these two forms of the α/β heterodimeric hCG molecule.

\(^2\) Total LH includes the α/β LH heterodimer as well as the dissociated α- and β-subunits and their degradation products.
3. **Responsibility**

These guidelines are intended for use by WADA-accredited laboratories and ADOs with result management responsibility.

4. **Introduction**

- hCG and LH are prohibited in male *Athletes* only.
- hCG and LH are heterodimeric proteins comprising two polypeptide chains, a common α-subunit and a unique β-subunit (hCGβ, LHβ). Only the α/β heterodimer has biological activity, which is determined by the hormone-specific β-subunit.
- Both hCG and LH occur in urine in different molecular forms, including the intact and nicked α/β heterodimers as well as the dissociated α- and β-subunits and their degradation products (e.g. the β-core fragments, nicked products, etc.).
- In men, hCG and LH stimulate production of testosterone by Leydig cells by binding to and activating CG/LH receptors.
- The heterodimeric hCG is either undetectable or found at very low levels (usually below 2 IU/L) in urine from healthy males. However, elevated levels of heterodimeric hCG, free hCGβ, hCGβ-core fragment are produced by certain malignant tumors, especially testicular cancer. Heterodimeric hCG may also be produced by extra-testicular germ cell tumors. In addition, hCGβ may be produced by various non-trophoblastic cancers.
- Endogenous LH can be usually found at levels <20 IU/L in urine from healthy men. LH has a shorter half-time in circulation than hCG. Circulating LH is subject to negative feedback by the production of endogenous testosterone or the administration of androgens.

5. **Pre-analytical Procedure**

- Following reception, “A” *Samples* should be analyzed for hCG and/or LH as quickly as possible or refrigerated.
- Before aliquoting for analysis, the urine *Sample* should be homogenized in the *Sample* bottle.
- *Aliquots* taken for analysis should be analyzed immediately or stored at 4°C for up to 96 hours until analysis. *Aliquots* should not be frozen.
- If stored at 4°C, *Aliquots* should be re-suspended after removal from refrigerated storage (e.g. by pipetting, vortexing or shaking).
• **Aliquots** should be allowed to stand at room temperature for at least 30 minutes to allow for any particulate matter to re-dissolve before being loaded into the instrument for analysis. Failure to dissolve the precipitate may cause false low hCG/LH values.

• If stored at -20°C, **Samples** should be analyzed or transferred to -70°C as soon as possible (this applies, in particular, to the Confirmation Procedures when performed on “A” Samples following the determination of a Presumptive Adverse Analytical Finding as well as on “B” Samples, if applicable).

• For long-term storage when Further Analysis is required, it is recommended that Samples be stored frozen at -70°C to avoid the dissociation and degradation of the α/β heterodimers into free α- and β-subunits and their fragments.

### 6. Assay Requirements

• For the measurement of heterodimeric hCG and total LH concentrations in urine **Samples**, **Laboratories** shall apply assays that have been validated and demonstrated as fit-for-purpose.

• For the determination of hCG in urine, **Laboratories** shall apply assays which are specific for the α/β heterodimer of hCG\(^1,3\). Assays that measure other molecular forms (e.g. free subunits or degradation fragments) in addition to the α/β heterodimer of hCG should not be used.

• In contrast, for the estimation of LH in urine, **Laboratories** shall apply assays capable of measuring the total content of LH\(^2\) immunoreactivity, *i.e.* targeting as many molecular forms as possible, *e.g.* the α/β heterodimer, the free β-chain and the β-core fragment.

• Parameters of heterodimeric hCG\(^1\) assay performance should be validated on-site, including, for example, the determination of the assay’s Limit of Quantification *(LOQ)*, within-Laboratory Repeatability (*s\(_r\)*), Intermediate Precision (*s\(_w\)*), bias and relative standard combined Measurement Uncertainty (*u\(_c\) %)*.

---

\(^3\) Men with “familial hCG”, an apparently physiological and non-pathological anomaly of hCG secretion, have consistently elevated concentrations of hCGβ in serum and urine. This may cause a positive finding if an assay for “total” hCG is used. Therefore, such assays should not be used for Doping Control purposes.
The acceptance values for these parameters of heterodimeric hCG\(^1\) assay performance are specified in the table below:

<table>
<thead>
<tr>
<th>Validation parameter</th>
<th>Acceptance Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( s_r ) (intra-assay Relative Standard Deviation, RSD %)</td>
</tr>
<tr>
<td></td>
<td>( s_w ) (inter-assay RSD %)</td>
</tr>
<tr>
<td>( \text{LOQ}^4 ) (IU/L)</td>
<td></td>
</tr>
<tr>
<td>( u_c ) (%)</td>
<td></td>
</tr>
</tbody>
</table>

7. Analytical Testing Strategy

7.1 Testing for hCG

- For the Initial Testing Procedure, Laboratories should apply an immunoassay that specifically detects the \( \alpha/\beta \) heterodimer of hCG\(^1\) (e.g. Roche hCG-STAT, Perkin-Elmer AutoDelfia, Delfia Xpress or any other assay validated to be fit-for-purpose).

- For the Confirmation Procedure(s), in accordance with ISL provision 5.2.4.3.1.3 on the application of affinity binding assays, Laboratories should apply another immunoassay that specifically detects the \( \alpha/\beta \) heterodimer of hCG\(^1\) (different from the assay applied for the Initial Testing Procedure)\(^5\).

- For Samples producing a Presumptive Adverse Analytical Finding for the \( \alpha/\beta \) heterodimer of hCG, the “A” Sample Confirmation Procedure should be performed as soon as possible. Alternatively, the remainder of the “A” Sample and the “B” Sample should be frozen immediately at -70°C until thawing for analysis.

---

\(^4\) LOQ is defined as the lowest concentration meeting the specified criteria for assay \( s_r \) and \( s_w \).

\(^5\) Laboratories that do not have the analytical capacity to perform the Confirmation Procedure with a second assay specific for the \( \alpha/\beta \) heterodimer of hCG shall have, upon consultation with the responsible Testing Authority, the Sample shipped to and analyzed by another Laboratory that has such analytical capacity.
• For both “A” and “B” **Confirmation Procedures**, three **Sample Aliquots** shall be measured, except in cases of limited **Sample** volume, in which case a lower maximum number of replicates may be used.

7.2 **Testing for LH**

• **Laboratories** should estimate the concentrations of total LH\(^2\) in urine during the **Initial Testing Procedure** by applying an immunoassay capable of detecting as many molecular forms of LH as possible (e.g. Immulite LH, Delfia LH or any other assay validated to be fit-for-purpose).

• If the **Initial Testing Procedure** produces a **Presumptive Adverse Analytical Finding** for LH, the **Laboratory** should test the **Sample** for the presence of GnRH or its synthetic analogues (e.g. Leuprolide)\(^6\). **Testing** for anti-estrogenic substances and aromatase inhibitors should be part of the **Laboratory**’s standard **Testing** menu.

8. **Interpretation and Reporting of Results**

8.1 **hCG results**

• For urine **Samples** with values of specific gravity (SG) **greater than 1.020**, hCG concentrations shall be adjusted to SG = 1.020\(^7\).

• The **Laboratory** shall report an **Adverse Analytical Finding** for hCG if, following a **Presumptive Adverse Analytical Finding** from the **Initial Testing Procedure**, the **Confirmation Procedure** confirms the presence of the hCG-\(\alpha/\beta\) heterodimer at concentrations (after adjustment if urine SG > 1.020) greater than the **DL** of 5 IU/L.

• In case of an **Adverse Analytical Finding** for hCG, a comment shall be added to the Test Report describing the hCG finding and recommending the **ADO** to advise the **Athlete** to undergo clinical investigations to exclude any pathological cause of the elevated urinary hCG.

---

\(^6\) **Laboratories** that do not have the analytical capacity to perform analyses for GnRH or its synthetic analogues shall have, upon consultation with the responsible **Testing Authority**, the **Sample** shipped to and analyzed by another **Laboratory** that has such analytical capacity.

\(^7\) For urine **Samples** with values of SG higher than 1.020, the hCG and LH concentrations in the **Sample** shall be adjusted according to the formula:

\[
\text{Conc. }_{1.020} \text{ (IU/L)} = [(1.020-1) / (\text{SG}_{\text{Sample}} - 1)] \cdot \text{Conc. }_{\text{measured}} \text{ (IU/L)}
\]
8.2 LH results

- For urine Samples with values of SG greater than 1.020, LH concentrations shall be adjusted to SG = 1.0207.

- The Laboratory shall consider a Presumptive Adverse Analytical Finding for LH if results from the Initial Testing Procedure for total LH (after adjustment if urine SG > 1.020) are higher than 20 IU/L.

- When Testing for GnRH and/or its synthetic analogues, anti-estrogenic substances and aromatase inhibitors, the Laboratory shall report an Adverse Analytical Finding if the Prohibited Substance is confirmed in the Sample at any concentration level (in accordance with the TD IDCR (1)).

- When there is a Presumptive Adverse Analytical Finding for LH, and tests performed to detect the presence of GnRH and/or its synthetic analogues, anti-estrogenic substances and aromatase inhibitors produce negative results, the Laboratory shall report the finding as an Atypical Finding for LH.

- Detection of total LH at reduced concentration levels below 1 IU/L may serve as corroborative evidence of LH suppression due to Use of exogenous androgens or to indirect androgen doping [2]. If available, serial urine LH measurements may be more sensitive to identifying such suppression. Such results for LH should be reported as an Atypical Finding for LH (with a comment in the Test Report indicating that LH values are suppressed below 1 IU/L) and interpreted in parallel with the values obtained for the Markers of the “steroid profile” and any previous urine LH measurements. The Laboratory may make a recommendation to the Testing Authority to store the Sample for Further Analysis.
9. Results Management

9.1 hCG findings

- When a Sample is reported as an Adverse Analytical Finding for hCG, the ADO should alert the Athlete and advise that clinical investigations be performed within a reasonable time frame to exclude pathological causes of the elevated urinary hCG concentrations. No provisional suspension shall be imposed on the Athlete during the course of the clinical investigations. The ADO should advise WADA when clinical investigations are conducted on an Athlete.

- The ADO should also conduct at least one (1) follow-up no-notice test within a reasonable time frame (e.g. within 2 weeks) following the initial finding. If possible, the follow-up Sample should be analyzed at the same Laboratory and using the same immunoassay that produced the Adverse Analytical Finding.

- If no clinical evidence is provided or the clinical investigations determine that there is no pathological condition associated with the elevated hCG concentrations, the results management process is followed as in the case for Use of other Prohibited Substance(s) or Prohibited Method(s). The results of the follow-up Sample should also be considered when evaluating the initial Adverse Analytical Finding and the clinical information.

- If medical information is provided by the Athlete to support the claim that the result is due to a physiological or pathological condition, such information shall be taken into account and should lead the ADO to stop the result management process of the case as an ADRV.

9.2 LH findings

- When a Sample is reported as an Atypical Finding for LH, the ADO should conduct at least one (1) follow-up no-notice test on the Athlete. The follow-up Sample should be analyzed at the same Laboratory that produced the Atypical Finding.

- The ADO should consider the results of the follow-up test for LH in parallel with the evaluation of the longitudinal “steroid profile” of the Athlete and any

---

8 Refer to the WADA Medical Evaluation Document for hCG findings (in preparation).

9 An Adverse Analytical Finding for the heterodimeric hCG does not exclude the possibility of a pathological cause. Most cases of testicular cancer are associated with elevated serum and urine concentrations of heterodimeric hCG, as well as with the presence of free hCGβ and hCGβ-core fragment in urine. In such cases, it is a responsibility of the Athlete to provide medical information or clinical evidence demonstrating that the heterodimeric hCG finding is the result of a pathological condition.
available previous urine LH measurements. This evaluation should be done in consultancy with an Expert Panel.

- If GnRH and/or its synthetic analogues, anti-estrogenic substances or aromatase inhibitors are confirmed in the urine Sample at any level and reported as an Adverse Analytical Finding, the results management process is followed, as in the case for Use of any other Prohibited Substance(s) or Prohibited Method(s).

10. Definitions

10.1 Code Defined Terms

Adverse Analytical Finding (AAF): A report from a Laboratory or other WADA-approved entity that, consistent with the International Standard for Laboratories and related Technical Documents, identifies in a Sample the presence of a Prohibited Substance or its Metabolites or Markers (including elevated quantities of endogenous substances) or evidence of the Use of a Prohibited Method.

Anti-Doping Organization (ADO): A Signatory that is responsible for adopting rules for initiating, implementing or enforcing any part of the Doping Control process. This includes, for example, the International Olympic Committee, the International Paralympic Committee, other Major Event Organizations that conduct Testing at their Events, WADA, International Federations, and National Anti-Doping Organizations.

Athlete: Any Person who competes in sport at the international level (as defined by each International Federation) or the national level (as defined by each National Anti-Doping Organization). An Anti-Doping Organization has discretion to apply anti-doping rules to an Athlete who is neither an International-Level Athlete nor a National-Level Athlete, and thus to bring them within the definition of "Athlete." In relation to Athletes who are neither International-Level nor National-Level Athletes, an Anti-Doping Organization may elect to: conduct limited Testing or no Testing at all; analyze Samples for less than the full menu of Prohibited Substances; require limited or no whereabouts information; or not require advance TUEs. However, if an Article 2.1, 2.3 or 2.5 anti-doping rule violation is committed by any Athlete over whom an Anti-Doping Organization has authority who competes below the international or national level, then the Consequences set forth in the Code (except Article 14.3.2) must be applied. For purposes of Article 2.8 and Article 2.9 and for purposes of anti-doping information and education, any Person who participates in sport under the authority of any Signatory, government, or other sports organization accepting the Code is an Athlete.

Atypical Finding (ATF): a report from a Laboratory or other WADA-approved entity which requires further investigation as provided by the International Standard for Laboratories or related Technical Documents prior to the determination of an Adverse Analytical Finding.

International Standard (IS): A standard adopted by WADA in support of the Code. Compliance with an International Standard (as opposed to another alternative standard, practice or procedure) shall be sufficient to conclude that the procedures addressed by the International Standard were performed properly. International Standards shall include any Technical Documents issued pursuant to the International Standard.

Sample or Specimen: Any biological material collected for the purposes of Doping Control.

Testing: The parts of the Doping Control process involving test distribution planning, Sample collection, Sample handling, and Sample transport to the laboratory.
Use: The utilization, application, ingestion, injection or consumption by any means whatsoever of any Prohibited Substance or Prohibited Method.


10.2 ISL Defined Terms

Aliquot: A portion of the Sample or biological fluid or tissue (e.g., urine, blood) obtained from the Athlete used in the analytical process.

Analytical Testing: The parts of the Doping Control process involving Sample handling, analysis and reporting following receipt in the Laboratory.

Confirmation Procedure: An analytical test procedure whose purpose is to identify the presence or to measure the concentration/ratio of one or more specific Prohibited Substances, Metabolite(s) of a Prohibited Substance, or Marker(s) of the Use of a Prohibited Substance or Method in a Sample.

[Comment: A Confirmation Procedure for a threshold substance shall also indicate a concentration/ratio of the Prohibited Substance greater than the applicable Decision Limit (as noted in the TD DL).]

Decision Limit (DL): a concentration, accounting for the maximum permitted combined uncertainty, above which an Adverse Analytical Finding shall be reported.

Further Analysis: Any analysis for any substance or method except where an Athlete has previously been notified of an asserted anti-doping rule violation based on an Adverse Analytical Finding for that substance or method.

Initial Testing Procedure: An analytical test procedure whose purpose is to identify those Samples which may contain a Prohibited Substance, Metabolite(s) of a Prohibited Substance, or Marker(s) of the Use of a Prohibited Substance or Prohibited Method or the quantity of a Prohibited Substance, Metabolite(s) of a Prohibited Substance, or Marker(s) of the Use of a Prohibited Substance or Prohibited Method.

International Standard for Laboratories (ISL): The International Standard applicable to Laboratories.

Laboratory(ies): (A) WADA-accredited laboratory(ies) applying test methods and processes to provide evidentiary data for the detection of Prohibited Substances, Methods or Markers on the Prohibited List and, if applicable, quantification of a Threshold Substance in Samples of urine and other biological matrices in the context of anti-doping activities.

Measurement Uncertainty (MU): Parameter associated with a measurement result that characterizes the dispersion of quantity values attributed to a measurand.

[Comment: Knowledge of the MU increases the confidence in the validity of a measurement result.]

Presumptive Adverse Analytical Finding: The status of a Sample test result for which there is a suspicious result in the Initial Testing Procedure, but for which a confirmation test has not yet been performed.
10.3 ISTI Defined Terms

Testing Authority: The organization that has authorized a particular Sample collection, whether (1) an Anti-Doping Organization (for example, the International Olympic Committee or other Major Event Organization, WADA, an International Federation, or a National Anti-Doping Organization); or (2) another organization conducting Testing pursuant to the authority of and in accordance with the rules of the Anti-Doping Organization (for example, a National Federation that is a member of an International Federation).

10.4 Other Terms

Exogenous: refers to a substance which is not ordinarily capable of being produced by the body naturally.

Expert Panel: The Experts, with knowledge in the concerned field, chosen by the Anti-Doping Organization and/or Athlete Passport Management Unit, who are responsible for providing an evaluation of the Passport. For the Haematological Module, Experts should have knowledge in one or more of the fields of clinical haematology (diagnosis of blood pathological conditions), sports medicine or exercise physiology. For the Steroidal Module, the Experts should have knowledge in Laboratory analysis, steroid doping and/or endocrinology.

11. References
