

World Anti-Doping Program

GUIDELINES

REPORTING & MANAGEMENT of URINARY HUMAN CHORIONIC GONADOTROPHIN (hCG) and LUTEINIZING HORMONE (LH) FINDINGS IN MALE ATHLETES

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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 hCG¹ 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 LH² 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 (hCG β , LH β). 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 hCG β , hCG β -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, hCG β 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 LH² 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 hCG¹ 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 hCG β 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 hCG¹ assay performance are specified in the table below:

Validation parameter	Acceptance Criterion
s_r (intra-assay Relative Standard Deviation, <i>RSD</i> %)	≤ 10% (at 5 IU/L)
s_w (inter-assay <i>RSD</i> %)	≤ 20% (at 5 IU/L)
LOQ⁴ (IU/L)	≤ 2.0 IU/L
u_c (%)	≤ 20% (at 5 IU/L)

7. Analytical Testing Strategy

7.1 Testing for hCG

- For the Initial Testing Procedure, Laboratories should apply an immunoassay that specifically detects the α/β heterodimer of hCG¹ (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 α/β heterodimer of hCG¹ (different from the assay applied for the Initial Testing Procedure)⁵.
- For *Samples* producing a Presumptive Adverse Analytical Finding for the α/β 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 α/β 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 LH² 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 = 1.020⁷.
- 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- α/β 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 $SG = 1.020^7$.
- 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 concentrations⁸. **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*⁹.
- 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 in to 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 hCG β and hCG β -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*.

WADA: The World Anti-Doping Agency.

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

1. WADA Technical Document TDIDCR (current version). Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for Doping Control Purposes.
[https://www.wada-ama.org/en/resources/search?ff0\]=field_resource_collections%3A30](https://www.wada-ama.org/en/resources/search?ff0]=field_resource_collections%3A30)
2. Goebel C, Howe CJ, Ho KK, Nelson A, Kazlauskas R, Trout GJ. Screening for testosterone abuse in male athletes using the measurement of urinary LH, a revision of the paradigm. *Drug Test Anal.* **1**:511-517, 2009.