



World Anti-Doping Program

**GUIDELINES**

**REPORTING & MANAGEMENT**

**of**

**HUMAN CHORIONIC  
GONADOTROPHIN (hCG)  
FINDINGS**

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## 1. **Objective**

These guidelines have been developed to ensure a harmonized approach in the reporting and management of elevated concentrations of human Chorionic Gonadotrophin (hCG). The finding of hCG in the urine of male *Athletes* at concentrations greater than 5 mIU/mL may be an indicator of hCG use for doping purposes. However, due to the complexity of hCG isoform composition in urine and the reported association of some hCG molecular forms with pathophysiological conditions such as cancer, consideration must be given to possible causes, other than doping, that can produce elevated hCG concentrations in urine *Samples* from male *Athletes*.

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

These guidelines incorporate instructions on the reporting of hCG values greater than 5 mIU/mL.

## 2. **Scope**

These guidelines follow the current rules established in the World Anti-Doping Program's *International Standard for Laboratories (ISL)*, whose requirements are still fully applicable and shall be respected. These guidelines also contain recommendations to facilitate the result management of elevated concentrations of hCG in the urine *Samples* of male *Athletes*.

Unlike the ISL, these guidelines are not mandatory, and *ADOs* are free to decide how to incorporate them into their current rules and procedures. These guidelines can be incorporated in whole or in part and can be amended, reworded, or an alternative approach adopted to best fit *ADO's* needs.

*ADOs* should refer to the latest version of the *Prohibited List* (a Level 2, mandatory document of the World Anti-Doping Program), Section S2. Peptide Hormones, Growth Factors and Related Substances.

## 3. **Responsibility**

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

## 4. Definitions

### 4.1 Code Defined Terms

'*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*.

'*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 participates in sport at the international level (as defined by each International Federation), the national level (as defined by each *National Anti-Doping Organization*, including but not limited to those *Persons* in its *Registered Testing Pool*), and any other competitor in sport who is otherwise subject to the jurisdiction of any *Signatory* or other sports organization accepting the *Code*. All provisions of the *Code*, including, for example, *Testing* and therapeutic use exemptions, must be applied to international- and national-level competitors. Some *National Anti-Doping Organizations* may elect to test and apply anti-doping rules to recreational-level or masters competitors who are not current or potential national caliber competitors. *National Anti-Doping Organizations* are not required, however, to apply all aspects of the *Code* to such *Persons*. Specific national rules may be established for *Doping Control* for non-international-level or non-national-level competitors without being in conflict with the *Code*. Thus, a country could elect to test recreational-level competitors but not require therapeutic use exemptions or whereabouts information. In the same manner, a *Major Event Organization* holding an *Event* only for masters-level competitors could elect to test the competitors but not require advance therapeutic use exemptions or whereabouts information. For purposes of Article 2.8 (Administration or *Attempted Administration*) 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*.

'*International Standard*': 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*.

'*Prohibited List*': the List identifying the *Prohibited Substances* and *Prohibited Methods*.

'*Prohibited Method*': Any method so described in the *Prohibited List*.

'*Prohibited Substance*': Any substance so described in the *Prohibited List*.

'*Sample/Specimen*': Any biological material collected for the purposes of *Doping Control*.

## 4.2 ISL Defined Terms

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

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

'Initial Testing Procedure (Screen 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* in excess of a defined threshold.

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

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

'Presumptive 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.

## 4.3 Other Terms

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

## 5. Laboratory determination of hCG in urine

- Following reception, "A" *Samples* should be refrigerated and analyzed for hCG as quickly as possible;
- For the measurement of hCG 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 follow ISL provision 5.2.4.3.1.3 on the application of affinity binding assays (e.g. immunoassays) for detection of macromolecules in urine *Samples*;
- It is strongly recommended that, for the Initial Testing Procedure, Laboratories apply immunoassays capable of detecting the total hCG content in urine, which should include many of the molecular forms of hCG found in urine (e.g. intact hCG, nicked hCG, free  $\beta$ -subunit,  $\beta$ -core fragment, etc). In contrast, for Confirmation Procedures, Laboratories should apply immunoassays that specifically detect the intact form of hCG only;
- For *Samples* producing a concentration of total hCG above 5 mIU/mL on the Initial Testing Procedure for the "A" *Sample*, perform the Confirmation Procedure on an additional Aliquot of the "A" *Sample* as soon as possible<sup>1,2</sup>.

## 6. Laboratory reporting guidelines

- The Laboratory shall report an *Adverse Analytical Finding (AAF)* for hCG if, following a Presumptive Analytical Finding from the Initial Testing Procedure, the Confirmation Procedure confirms the presence of intact hCG at concentrations greater than 5 mIU/mL (after adjusting to a urine specific gravity (SG) of 1.020<sup>3</sup>);

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<sup>1</sup> Alternatively, the remainder of the "A" *Sample* and the "B" *Sample* should be frozen immediately (preferably at -80°C) until thawing and taking of an Aliquot for confirmation analysis.

<sup>2</sup> To avoid the presence of particulate matter, test Aliquots should be allowed to thaw by standing at room temperature for at least 30 min. or be incubated at 37°C with mixing (vortexing) for 5-10 min. Test Aliquots shall be homogenized and centrifuged before analysis.

<sup>3</sup> For urine *Samples* with values of SG different from 1.020, the hCG concentration in the *Sample* shall be adjusted according to the formula:

$$\text{Conc. hCG}_{1.020} \text{ (mIU/mL)} = [(1.020-1) / (\text{SG}_{\text{Sample}} - 1)] \bullet \text{Conc. hCG}_{\text{measured}} \text{ (mIU/mL)}$$

- In cases when the Confirmation Procedure fails to confirm the findings of the Initial Testing Procedure and does not demonstrate the presence of intact hCG at concentrations greater than 5 mIU/mL (after adjusting to a urine SG of 1.020<sup>3</sup>), the Laboratory shall report the *Sample* as an *Atypical Finding (ATF)*<sup>4</sup>. 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 for the causes of the hCG finding and, if necessary, to conduct further investigations in the form of follow-up no-notice tests (at least 2);
- In all cases, and at any concentration, the Laboratory will report an *AAF* for hCG if, based on any reliable analytical method, the Laboratory can demonstrate that the *Prohibited Substance* is of exogenous origin (e.g. detection of chemical structures only found in recombinant hCG). In such case, no further investigation is necessary.

## 7. Results Management

- When a *Sample* is reported as an *AAF* for hCG, the results management process is followed, as in the case for use of other *Prohibited Substances* or *Methods*<sup>5</sup>;
- When a *Sample* is reported as an *ATF* for hCG, the *ADO* should alert the *Athlete* and advise that clinical investigations be performed to address the possibility of a pathophysiological condition as the cause of the elevated total urinary hCG concentrations. The *ADO* should also advise *WADA* when clinical investigations are conducted on an *Athlete*;
- In addition, when a *Sample* is reported as an *ATF* and the results of the clinical investigations performed on the *Athlete* do not show a pathophysiological cause for the elevated total hCG finding, the *ADO* should conduct at least two (2) follow-up no-notice tests on the *Athlete*. The follow-up *Samples* should be analyzed at the same Laboratory that produced the *ATF* report;
- If the follow up tests reflect similar suspicious results with increased total hCG concentrations determined in the Initial Testing Procedures at the levels reported for the initial test (after adjusting to a urine SG of 1.020<sup>3</sup>) together with negative (< 5 mIU/mL) intact hCG from the Confirmation Procedures, the *ADO*

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<sup>4</sup> In order to confirm the atypical result, it is strongly recommended that in cases when the Confirmation Procedure fails to confirm the findings of the Initial Testing Procedure, the Laboratory repeats the analysis on the *Sample* with the total hCG assay employed for the Initial Testing Procedure before reporting the *ATF*.

<sup>5</sup> An *AAF* for intact hCG at levels higher than 5 mIU/mL does not totally exclude a possible pathological cause. Some cases of testicular cancer, in particular, may be associated with elevated levels of intact hCG, in addition to the presence of hCG free  $\beta$ -subunit and/or  $\beta$ -core fragment. In such cases, it is a responsibility of the *Athlete* to provide medical information or clinical evidence demonstrating that the hCG finding is the result of a pathological condition.

should conclude that no ADRV has occurred and no further investigations are necessary. This information shall be documented in the dossier of the *Athlete* concerned and shared with *WADA* (and other *ADOs* as relevant);

- If the Initial Testing Procedure(s) for a follow-up test produces elevated values for total hCG which differ from the initial test (after adjusting to a urine SG of 1.020<sup>3</sup>) and with a negative (<5 mIU/mL) intact hCG Confirmation Procedure, the *ADO* should treat the results as suspicious <sup>6</sup> and contact *WADA* for further instructions on the results management process of the case;
- If a follow-up test produces a Presumptive Analytical Finding from the repeat of the Initial Testing Procedure, plus the Confirmation Procedure confirms the presence of intact hCG at concentrations greater than 5 mIU/mL (after adjusting to a urine SG of 1.020<sup>3</sup>) and is reported as an *AAF*, the results management process is followed, as in the case for use of other *Prohibited Substances or Methods*<sup>4</sup>;
- 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 in the result management of the case;
- A copy of the report confirming an elevated concentration of hCG, including the results of the initial and subsequent follow-up tests, and any related analytical or clinical information, shall be forwarded to *WADA* .

#### Confirmation of exogenous origin

- Once it is determined through a reliable analytical method (e.g. detection of chemical structures characteristic of recombinant hCG) that the detected hCG is of exogenous origin and reported as an *AAF*, the results management process is followed, as in the case for use of any other *Prohibited Substances or Methods*.

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<sup>6</sup> In these cases, the *Sample* is considered suspicious since urinary total hCG concentrations associated with physiological or pathological conditions (e.g. 'familial' hCG, cancer) are usually maintained at a constant level or increased over time with disease progression. Therefore, decreased concentrations of total hCG in follow-up test(s) may be indicative of previous use of the substance for doping purposes, while increased concentrations may warrant further clinical investigations. These suspicious cases should be further elucidated by performing a battery of hCG tests, specific for different hCG isoforms, at specialized reference laboratories.