

HUMAN GROWTH HORMONE (HGH) TESTING

1. What is hGH? (/en/questions-answers/human-growth-hormone-hgh-testing#item-638)
2. What effect can hGH have on athletic performance? (/en/questions-answers/human-growth-hormone-hgh-testing#item-639)
3. What are the side effects of hGH abuse? (/en/questions-answers/human-growth-hormone-hgh-testing#item-640)
4. Does a test for hGH exist? (/en/questions-answers/human-growth-hormone-hgh-testing#item-641)
5. Have there been analytical doping cases involving hGH? (/en/questions-answers/human-growth-hormone-hgh-testing#item-642)
6. Which matrix is used for detection of hGH? (/en/questions-answers/human-growth-hormone-hgh-testing#item-643)
7. What is the hGH Isoform Differential Immunoassays (the 'isoforms approach')? (/en/questions-answers/human-growth-hormone-hgh-testing#item-644)
8. What is the hGH Biomarkers Test (the 'markers approach')? (/en/questions-answers/human-growth-hormone-hgh-testing#item-645)
9. Are the hGH tests reliable? (/en/questions-answers/human-growth-hormone-hgh-testing#item-646)
10. Are the Isoform Differential Immunoassays and the hGH Biomarkers Tests complementary? (/en/questions-answers/human-growth-hormone-hgh-testing#item-647)
11. What are GH-releasing factors and are they detectable? (/en/questions-answers/human-growth-hormone-hgh-testing#item-648)
12. When is the best time to test athletes for hGH? (/en/questions-answers/human-growth-hormone-hgh-testing#item-649)
13. Is a urine test for hGH likely to be developed? (/en/questions-answers/human-growth-hormone-hgh-testing#item-650)
14. Can blood samples be stored? (/en/questions-answers/human-growth-hormone-hgh-testing#item-651)

1. WHAT IS HGH?



Human growth hormone (hGH) is a hormone that is naturally produced by the body. It is synthesized and secreted by cells in the anterior pituitary gland located at the base of the brain. Its secretion into the circulation follows a pulsatile pattern resulting in widely fluctuating blood levels that are influenced by multiple factors such as age, gender, sleep, physical activity, diet, stress, fever, steroids and the environment.

In serum, hGH exists as a complex combination of different molecular forms (isoforms), including the major 22-kDa form and minor isoforms such as the 20-kDa form. Furthermore, hGH also exists as aggregates of these isoforms (dimers and oligomers, forming both homo- and heterodimers). Following secretion into the blood circulation, the 22-kDa hGH has a short half-life of 10-20 minutes.

hGH stimulates many metabolic processes in cells. hGH affects protein, fat, carbohydrate and mineral metabolism. The major role of hGH is to stimulate the liver to secrete Insulin-like Growth Factor-I (IGF-I). IGF-I stimulates production of cartilage cells, resulting in bone growth and also plays a key role in muscle protein synthesis and organ growth.

hGH is prohibited both *in-* and *out-of-competition* under section S2 of WADA's List of Prohibited Substances and Methods.

2. WHAT EFFECT CAN HGH HAVE ON ATHLETIC PERFORMANCE?



Some of the effects attributed to hGH, which may explain the attraction for its use as a doping agent, especially in power and endurance sports, include the reduction of body fat (lipolysis), the increase in muscle mass and strength (anabolic effect), as well as its tissue-repairing effects (recovery) on the musculo-skeletal system. The anabolic actions of GH are mostly mediated through IGF-I and include increases in total body protein turnover and muscle synthesis. hGH also appears to be used synergistically with other performance-enhancing drugs, thus having an effect, albeit indirect, on muscle anabolism and athletic performance.

3. WHAT ARE THE SIDE EFFECTS OF HGH ABUSE?



Commonly reported side effects for hGH abuse are: diabetes in prone individuals; worsening of cardiovascular diseases; muscle, joint and bone pain; hypertension and cardiac deficiency; abnormal growth of organs; accelerated osteoarthritis.

In untreated acromegalic individuals (known for pathological over-production of hGH), many of the symptoms described above are observed and life expectancy is known to be significantly reduced.

Because of the role that hGH plays in stimulating IGF-1 secretion, excessive use of hGH may also lead to metabolic dysfunction, including glucose intolerance and other side effects associated with excess levels of IGF-1.

4. DOES A TEST FOR HGH EXIST?



The menace that doping with hGH presents to fair play and the health and well-being of athletes has driven the sport and anti-doping authorities to support the development of analytical methods for its detection. Such tests have been elaborated following two distinct, but complementary scientific approaches: the 'isoforms approach' and the 'markers approach'.

The Isoform Differential Immunoassays (the Isoforms Test) was first introduced as a research-scale test at the 2004 Summer Olympic Games in Athens, Greece, and later applied at the 2006 Winter Olympic Games in Torino, Italy. Following a process of assay development and validation into a new technical platform (tube-based chemiluminescence technique), which is suitable for the production of commercial kits, the currently applied test kits were firstly used during the 2008 European Football Championship in Austria and Switzerland, as well as during the 2008 Summer Olympic Games in Beijing, China. Currently, all WADA accredited laboratories test for hGH using the Isoforms Test.

The hGH Biomarkers Test (the 'markers approach') was briefly introduced for testing during the 2012 London Olympic and Paralympic Games. However, following the temporary withdrawal from the market of one of its constituent assays, a process of validation of new assays for IGF-1 and P-III-NP has been completed in collaboration with USADA and several research groups, including the method-developer GH-2000 team from the UK and various WADA accredited laboratories. Following the completion of the study on the determination of the Decision Limits (DLs) for the hGH Biomarkers Test, the application of the method will be resumed in a number of WADA accredited laboratories in the near future.

5. HAVE THERE BEEN ANALYTICAL DOPING CASES INVOLVING HGH?



Yes. Since 2010, there have been approximately 15 adverse analytical findings reported following the application of the hGH Isoforms Test. In addition, two athletes were sanctioned following detection of doping with hGH through the application of the hGH Biomarkers Test during the 2012 London Paralympic Games.

6. WHICH MATRIX IS USED FOR DETECTION OF HGH?



Tests for detection of hGH doping are applied in blood serum, which is obtained following the centrifugation in the laboratories of blood collected in specified tubes containing an inert polymeric serum separator gel and a clotting activation factor.

7. WHAT IS THE HGH ISOFORM DIFFERENTIAL IMMUNOASSAYS (THE 'ISOFORMS APPROACH')?



Since the total levels of hGH secreted into circulation vary naturally, are widely fluctuating over time and may be influenced by several factors not associated with doping, it is practically impossible to develop an anti-doping test based simply on the measurement of increased total hGH concentrations. However, doping with recombinant hGH (recGH) alters the naturally constant proportions between the different isoforms of hGH present in blood of an individual. The hGH Isoform Differential Immunoassays were developed to detect these changes in the proportions of different hGH isoforms after recombinant recGH injection.

8. WHAT IS THE HGH BIOMARKERS TEST (THE 'MARKERS APPROACH')?



hGH affects the expression of many different proteins which may serve as biological markers of hGH activity. These include markers of hGH action in the liver such as IGF-I, as well as markers of hGH action on soft tissue collagen turnover, such as the N-terminal peptide of procollagen type III (P-III-NP). The measurement of these two hGH markers in serum may serve to uncover the manipulation of the hGH/IGF-I axis independently of the doping substance used, be it recGH or other agents used to increase circulating hGH [for example analogs of GH-releasing hormone (GHRH), hGH secretagogues such as GH-releasing peptides (GHRP) or even hGH gene doping]. The detection and quantification of such biomarkers of hGH activity constitute the rationale of the indirect method for detection of doping with hGH, referred to as the 'markers approach'.

9. ARE THE hGH TESTS RELIABLE?



The concepts and development of both hGH tests have been systematically reviewed by international independent experts in such fields as hGH and IGF-I, endocrinology, immunoassay, analytical chemistry, pharmacology, laboratory work, anti-doping, etc., and published in international scientific journals.

The 2013 Court of Arbitration for Sport (CAS) decision on the FIS vs. Veerpalu case confirmed that the current Isoforms Test is robust and scientifically reliable. As a result of criticism expressed by the CAS Panel on the statistical procedure originally applied to the determination of the test's DLs, new statistical analyses were performed on an increased number of hGH doping control data (samples from athletes treated under real doping control conditions of sample collection, transportation, storage and analysis, including standardized analytical protocols and instrumentation) by two independent teams of statisticians. The results of the study have been published in an independent peer-reviewed scientific journal specialized in GH and IGF-I research.

The revised DLs have been incorporated into a new version of the Guidelines on hGH Isoform Differential Immunoassays for anti-doping analyses, available on WADA's website. These Guidelines continue to provide direction on the sample pre-analytical preparation procedure, the performance of the test and the interpretation and reporting of the test results.

For more information on the process followed to determine the test's DLs, please consult Hanley JA *et al.* "hGH isoforms differential immunoassays applied to blood samples from athletes: decision limits for anti-doping testing". *Growth Hormone & IGF Research*, 2014.

The scientific validity and efficacy of the hGH biomarkers approach has been documented in multiple scientific publications for over a decade. A series of placebo-controlled recombinant recGH administration studies performed in Europe and Australia has shown that both IGF-I and P-III-NP rise substantially following recGH administration in a dose-dependent manner. These markers have been evaluated for several confounding factors that might influence the scores of the discriminant functions, including age, gender, ethnicity, exercise, diurnal and day-to-day variation, intra-individual variation, bony and soft tissue injury, sporting discipline, and body habitus (physique). Nevertheless, before resuming its implementation in WADA-accredited laboratories, the study on the determination of the DLs for the new assays will be also subjected to independent peer review and publication in an international scientific journal.

10. ARE THE ISOFORM DIFFERENTIAL IMMUNOASSAYS AND THE hGH BIOMARKERS TESTS COMPLEMENTARY?



These two tests are complementary in nature and in time window of hGH detection. The hGH Isoforms Test detects the alteration of proportions (ratios) between hGH isoforms up to 24-48 hours after recGH administration. The Biomarkers Test is based on measuring the increased synthesis of two biological markers of hGH bioactivity, namely IGF-I and P-III-NP, and it may not detect the initial phase of recGH use, but does so at later times and for a longer period than the Isoforms Test.

11. WHAT ARE GH-RELEASING FACTORS AND ARE THEY DETECTABLE?



GH-releasing factors include the GH Releasing Hormone (GHRH) and its analogues, GH secretagogues (GHS) and GH-releasing peptides (GHRPs). These compounds induce the secretion of endogenous hGH by the pituitary gland. Therefore, they are used in place of recGH to attain the effects of hGH doping, and possibly to mask detection of recGH use with the Isoforms Test. However, anti-doping laboratories have developed mass spectrometry-based methods for their detection, and adverse analytical findings have been reported following their detection in doping control samples. In addition, their use may be detected with the indirect hGH Biomarkers Test.

12. WHEN IS THE BEST TIME TO TEST ATHLETES FOR hGH?



As for any other doping controls, test distribution plans for hGH testing should be based on the implementation of 'Intelligent Testing' strategies aimed at making an effective and efficient use of available testing resources. Athletes use hGH as a doping agent for extended periods of time in order to benefit from its purported performance-enhancing effects (in contrast, for example, to other doping agents such as stimulants, which may have an immediate effect). In addition, doping athletes suspect that they may be tested for hGH during competition periods.

Therefore, testing for hGH should be prioritized to out-of-competition periods, and be based on intelligent, non-notice and unpredictable testing. Target testing of suspected athletes based on intelligence information (e.g. information about doping routines), prior suspicious test results or dubious and unexpected sporting achievements is also recommended. Due to their complementary nature in detecting hGH doping, it is advised that samples are tested with both the Isoforms and the Biomarkers Tests.

13. IS A URINE TEST FOR HGH LIKELY TO BE DEVELOPED?



According to the majority of international experts, the blood matrix is the most suitable matrix for the detection of hGH.

hGH in urine is found in extremely small quantities (less than 1% than that found in blood), and not much information is available regarding the excretion of different hGH isoforms in urine .

Research efforts undertaken to develop urine-based detection methods for hGH have been unsuccessful so far. However, WADA continues collaborating with research teams to explore this possibility using novel scientific ideas and technical strategies.

14. CAN BLOOD SAMPLES BE STORED?



Freezing blood serum is a scientifically acceptable procedure that allows for the preservation of substances in samples for future testing and detection.

Research has shown that hGH is stable in serum if frozen under the right conditions (please refer to the **hGH Guidelines for Assay Pre-analytical Procedure** ([//www.wada-ama.org/sites/default/files/resources/files/WADA-Guidelines-for-hGH-Differential-Immunoassays-v2.1-2014-EN.pdf](http://www.wada-ama.org/sites/default/files/resources/files/WADA-Guidelines-for-hGH-Differential-Immunoassays-v2.1-2014-EN.pdf)), section 6.2).

WADA encourages Anti-Doping Organizations under the World Anti-Doping Code to store blood samples when relevant (e.g. in cases of unexpected results, intelligence available to the anti-doping organization, etc.). Storing serum for future testing has a significant deterrent effect.

The 2015 World Anti-Doping Code makes it possible to open a disciplinary proceeding within ten years from the date an anti-doping rule violation occurred.