

PROJECT REVIEW

“Development of valid immunoassay methods for LH in urine”

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Luteinizing hormone (LH) is one of the prohibited gonadotrophins named in the WADA 2008 Prohibited List. LH is a naturally occurring hormone which is secreted by the pituitary gland. In males LH stimulates testosterone production by the testes. Whilst LH has been prohibited in sport for some years, it is only relatively recently that human LH has become available as a commercial pharmaceutical product due to recombinant biotechnology. Thus there is now a pressing need to establish valid reference range(s) for endogenous LH levels in urine to assist in the detection of doping with recombinant LH as well as to improve the standardisation of urine LH measurement as an adjunct to detection of suppressed LH levels that accompany doping by use of exogenous androgens and hCG. Whilst there are several commercially available immunoassays optimised for serum but which can be used to measure LH in urine, the numerical values obtained from the different kits are not in good agreement. There is also the question of whether kits calibrated using natural pituitary-purified LH give accurate results when measuring degradation metabolites of recombinant LH excreted in urine. Thus there is a need to establish comparability of LH measurement amongst the over 30 WADA laboratories who use a variety of techniques for the measurement of LH. This project will measure LH in a range of urines using a variety of commercial assays in order to establish comparability of measurement and establish upper and lower reference ranges for normal subjects. An attempt will also be made to determine if any assays or combination of assays can be used to validly detect recombinant LH as well as distinguishing reliably from endogenous LH.

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Results and Conclusions

The primary objective of this project was to determine which method or methods that can be successfully used to measure luteinizing hormone (LH) in urine. The measurement of LH is important for two reasons. The first is that the administration of testosterone and other androgenic hormones leads to a suppression of the secretion of endogenous LH and the second is the recent availability of recombinant LH which may elevate LH values. There are several different methods used by WADA laboratories to measure LH in urine all of which are based on antibody reactions. However it was clear that the different methods can give very different numerical values. Controlled studies using a variety of samples and a number of methods have shown that the Siemens Immulite is the preferred method for measuring LH in urine particularly when the suppression of LH is being used as an aid to detect doping with testosterone. Most other methods are not useful for this purpose because the LH or at least that portion of the LH molecule to which the methods respond is not stable in urine and hence low and apparently suppressed values can and do result from degradation on storage. The values of LH in urine as measured by the Immulite are relatively stable on storage for at least two weeks at room temperature and for months at -200C.

Studies involving the administration of recombinant LH have shown that the injection of the recommended dose of LH does not lead to any elevation of measured LH in urine. This was not unexpected given the dose and the rate of natural LH production. A short term elevation of urinary LH was observed when thirty two times the recommended dose was injected. A method using SDS-PAGE with Western blotting has been developed which can distinguish between pituitary and recombinant LH in both standards and in samples extracted from urine. It is clear from this work that the LH excreted in urine has a different apparent molecular weight to the parent LH.

As a result of this project it is now feasible to use LH suppression as an adjunct procedure with T/E measurement to detect testosterone doping and to detect and confirm the use of large doses of recombinant LH.

Publications/Presentations

Goebel, C, Agon, VV, Handelsman, DJ, Trout, GJ, Kazlauskas, R : Measurement of endogenous and recombinant LH in urine. Manfred Donike Workshop, 28th Cologne Workshop on Dope Analysis, 7.-12.3. 2010.