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Investigation of applicability of ADVIA Centaur Serum Total hCG assay for detection of human chorionic gonadotropin in urine

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Introduction

Human chorionic gonadotropin (hCG) may be misused by male athletes to improve physical performance and therefore, it is a prohibited substance in sports [1]. In doping control, the initial testing and confirmation of hCG is based on its quantification from urine. Concentration greater than 5 mIU/mL may be considered as an indicator of hCG abuse [2]. hCG can be found in urine in various molecular forms (e.g. intact hCG, nicked hCG, free β subunit, β-core fragment) [3]. The measurements are usually carried out with different commercial immunoassays predominately optimized for quantification in serum [4] but validated and demonstrated by the anti-doping laboratories as fit-for-purpose for the determination of hCG in urine [5,6]. Immonassays may differ from their ability to detect different molecular forms of hCG. In general, immunoassays applied for initial testing and confirmation shall use antibodies recognizing different epitopes of the macromolecule analyzed [7]. It is recommended that for initial testing urine samples should be measured with immunoassay capable of detecting total hCG-content and for confirmation immunoassays that detect the intact form of hCG only [2].

We studied the applicability of Siemens ADVIA Centaur Serum Total hCG (ThCG) immunoassay to the detection of hCG in urine. The ADVIA Centaur assay is a two-site sandwich immunoassay. According to the manufacturer, the test recognizes total hCG and free β subunit.

Materials and methods

The ADVIA Centaur assay was validated with hCG positive male urine samples obtained from excretion studies and external quality control (n=18) and hCG negative male urine samples (n=108). Results were used in order to determine the calibration curve stability, linearity, total, within and between run precision and method comparison for Roche Modular

Analytics E170 module for hCG+β assay which recognizes holo-hormone, nicked forms of hCG, the β-core fragment and the free β-subunit. External quality control samples from United Kingdom National External Quality Assessment Service (UK NEQAS) scheme for Pregnancy Testing were analyzed to compare ADVIA Centaur ThCG results with those measured by other quantitative assays and laboratories. Population based distribution of hCG values were determined by measuring 1913 urine samples from male athletes with ThCG assay. Samples were stored refrigerated before analysis.

Results and discussion

All positive urine hCG samples (range 17.9 -137 IU/l) analyzed with Roche E170 hCG assay were positive also when measured with Siemens ADVIA Centaur ThCG assay. Passing-Bablok regression between methods was ADVIA Centaur = 1.14x(Roche E170 hCG) +10.04 (r=0.74). Total precision for ThCG assay was 12.4 %. Within run precision was 10.4 % for the concentration 8 IU/l and between run precision 6.8 % for concentration 25 IU/l. hCG-standard spiked urine samples or diluent were used for linearity testing. Mean recovery-% for samples was 118 % in concentration range 44-381 IU/l. Results for UK NEQAS external quality control samples (Table 1) show that urinary hCG values of Siemens ADVIA Centaur ThCG assay are generally well consistent with the results obtained using other assays and laboratories. The exceptionally low result observed in sample X321 reflects the fact that ThCG method do not recognize hCG β-core fragment, the major endogenous urinary metabolite of hCG, whereas the deviation between the linear ranges of the methods was observed for three samples (X315, X316 and X325) with extremely high hCG concentration which, however, are typically encountered only in the case of pregnancy.

The instrument response for negative male urines (n=108) was clearly elevated (mean 9.9 IU/l) due to non-specific interferences of the urine matrix. The same phenomenon was observed in the distribution of hCG-values collected from 1913 male athletes (Fig 1). Therefore, the incorporation of a statistically determined instrument response threshold should be carefully considered if the method is used as an initial test in routine doping control.

Table 1: UK NEQAS External quality control samples for urinary hCG analyzed with Siemens ADVIA Centaur.

UK NEQAS Distribution No.	Month-Year	Sample	hCG Centaur (IU/l)	GLTM Target (IU/l)
146	Jun-2010	X311	63	59
		X312	8	3
147	Jun-2010	X313	38	39
		X314	40	39
148	Jul-2010	X315	12866	29106
		X316	13256	29986
149	Aug-2010	X317	8	3
		X318	7	4
150	Sep-2010	X319	57	53
		X320	37	33
151	Oct-2010	X321	132	1708
		X322	9	<5
152	Nov-2010	X323	37	42
		X324	10	2
153	Dec-2010	X325	55668	86113

GLTM (Group Laboratory Trimmed Mean) is mean hCG value for all quantitative methods participating this survey. Participants include 3 Abbot Architect, 3 In-house RIA, 3 Roche Elecsys, 2 Siemens ADVIA Centaur and 6 Immulite users.

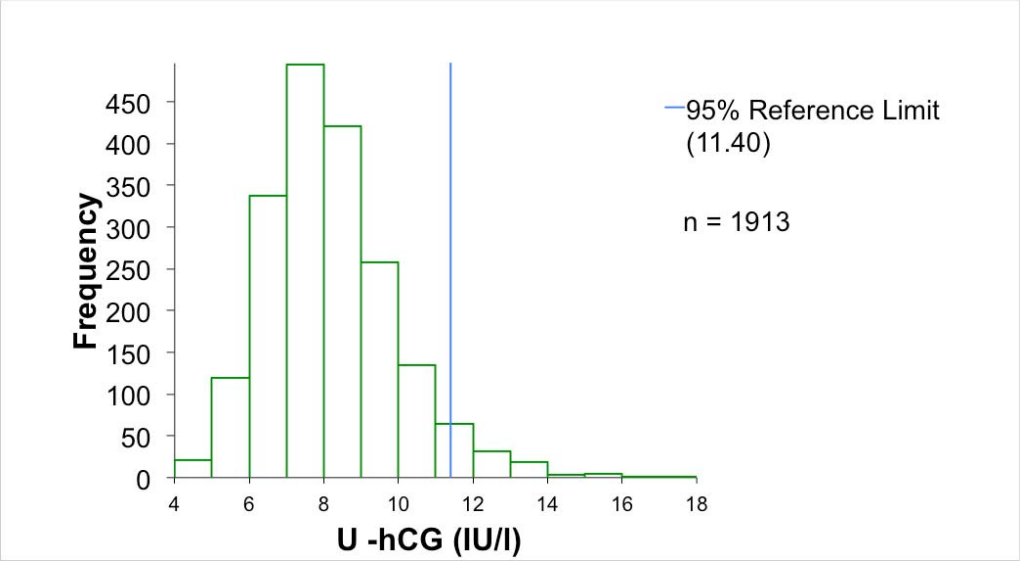

FIG 1: Distribution of urinary hCG concentrations in male athletes measured with Siemens ADVIA Centaur ThCG assay. Statistical analysis suggests hCG value 11.4 IU/l for 95% reference limit.

Conclusions

In this study the applicability of Siemens ADVIA Centaur Serum Total hCG (ThCG) immunoassay to the detection of hCG in urine was evaluated. The method was shown applicable for initial testing purposes, although the use of a well-defined instrument response threshold is mandatory due to high unspecific binding of the method.
For confirmation purposes, however, the method of choice is an assay recognizing the intact form of hCG.

References

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