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Detection of total β-hCG in doping control – A case study

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Introduction

In the Cologne laboratory the initial testing procedure for human choriogonadotropin (hCG) in urine of male athletes is performed by chemiluminescene enzyme immunoassay with the total β-hCG Kit, analysis system Access2 from Beckman Coulter. In case of a suspicious screening result the test has to be repeated with a second antibody recognising different epitopes of the macromolecule than that one applied for the initial testing procedure [1]. Here the hCG STAT kit from Roche (Roche Diagnostics GmbH, Mannheim, Germany) is chosen [3]. In 2007 and 2008 both German WADA accredited laboratories, Cologne and Kreischa, received several samples from the same male athlete showing constantly suspicious screening results for total β-hCG, but no indication for the presence of hCG (complete molecule, consisting of α- and β-subunit). As a follow-up-procedure one suspicious sample was analysed with three different assays for total β-hCG (two in Cologne, one in Kreischa) and two different assays for hCG and LH (Cologne and Kreischa respectively). The results of this follow-up as well as recommendations to the result management authorities are presented.

Material and Methods

In **Cologne** laboratory the analyses have been performed with the following kits according to the manufacturers instruction:

Access2: total β-hCG (Beckman Coulter, Krefeld, Germany) ***E 2,3***
ROCHE Elecsys 2010: hCG STAT (Roche Diagnostics GmbH, Mannheim, Germany) ***E 2,5***
ROCHE Elecsys 2010: hCG+β (Roche Diagnostics GmbH, Mannheim, Germany) ***E 2,3***
Access2: LH (Beckman Coulter, Krefeld, Germany)

In **Kreischa** laboratory the analyses have been performed with the following kits according to the manufacturers instruction:

Immulite one hCG und β-hCG (Siemens Healthcare Diagnost., Eschborn, Germany) ***E 2,3***

Delfia intact hCG (PerkinElmer, Rodgau-Juegesheim, Germany) ***E 2,5***

Immulite one LH (Siemens Healthcare Diagnostics, Eschborn, Germany)

E: Epitope, see table 2

Table 1: Results of three urine samples from one male athlete analysed by four different kits.

Date of sample collection	Köln Beckman Coulter	Köln Roche	Köln Roche	Kreischa Delfia	Kreischa Immulite
	β-hCG	β-hCG	intact hCG	intact hCG	β-hCG
	[IU/L]	[IU/L]	[IU/L]	[IU/L]	[IU/L]
Sep 08	24	20	< 1	n.a	n.a.
Jun 08	26	25	< 1	0,4	38
Apr 08	22	16	< 1	n.a.	n.a.

n.a. = not analysed

Discussion

The unusual and constant occurrence of low β-hCG concentrations in several urine samples collected from the same male athlete needed a follow-up. In a first step different kits have been applied by 2 laboratories in order to exclude any cross-reactivity to unknown substances. Also LH was analysed again without any abnormal result. The analytical result of the doping control sample was reported as 'Atypical Finding' with the following comment:

This sample was found to contain an abnormal concentration of β-hCG (subunit of hCG), but hCG (complete molecule consisting of α- and β -subunit) was absent. It is recommended that the following information should be considered before any further action is taken: An abnormal concentration of β-hCG may be caused by hCG administration, or an β-hCG secreting tumour. It is also recommended that this information is treated as any other confidential medical information. Further tests and detailed medical investigations are necessary to come to a final conclusion.

In April 2008 the result management authority was asked to collect 3 more urine samples and in addition a blood sample. The production of subunits of hCG is under separate genetic control. Differential production of the subunits has been observed in cancer patients. The number of patients who produce only the free β subunit is very small. The concentration of β-hCG in blood may help to compare those data with patients suffering from tumours. Free

β-hCG has been indicated as a superior tumor marker for testicular cancer and possibly other malignancies [3]. Further ongoing medical investigations of the athlete did not show any hint for a disease. This case showed that the application of a hCG-kit detecting the complete molecule for confirmation (as done in Cologne until this case occurred) may lead to a false negative result. It seems that there is a possibility that low ß-hCG concentrations are excreted in urine without presence of the complete molecule as it was observed in former cases (reported as 'Adverse Analytical Finding'). Concerning this deviation the case was not considered as an 'Adverse Analytical Finding'.

Table 2: Commonly identified epitopes on hCG, its free subunits and degradation products (from 2,4).

E	Epitope	Description	Nonnicked hCG	Nicked hCG	hCG terminal	Nonnicked free β	Nicked free β	β-core fragment
1	Anti-hCG dimer	Site at subunit interface on nonnicked hCG	X					
2	Anti-common β1	Mutual site on hCG, free β and β-core	X	X	X	X	X	X
3	Anti-common β2	Separate mutual site on hCG and free β (β-core?)	X	X	X	X	X	
4	Anti- β C-terminal	Mutual site on hCG and free β only	X	X		X	X	
5	Anti-common α	Mutual site on hCG and free α	X	X	X	X	X	
6	Anti-free β	Free subunit-specific site, hidden on hCG				X		
7	Anti-nonnicked free β	Free subunit-specific site, close to nicking site				X	X	
8	Anti-free β + β-core	Mutual site on free b and β-core fragment						X

References:

[1] World Anti-Doping Agency. ISL 6.0, Montreal (2009) http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-IS-Laboratories/WADA_Int.Standard_Laboratories_2009_EN.pdf (access date: 1.2.2009)

[2] Cole, LA. (1997) Immunoassay of human chorionic gonadotropin, its free subunits, and metabolites. *Clin Chem.* **43**, 2233-2243

[3] Roche Diagnostics (2003) Produktinformation, HCG STAT Elecsys®: Intaktes humanes Choriongonadotropin 03300811 190

[4] Gronowski, AM. (ed.) Handbook of clinical laboratory testing during pregnancy, Humana Press, Totowa, NJ, 2004