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Abbott IMx® total β-hCG-kit versus Roche Elecsys® hCG STAT-kit: Validation and performance comparison

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Introduction

In 2005 Abbott removed its hCG-kit (in vitro test 3A63-20) from the German market without any further notice. Later on the worldwide distribution was stopped. Up to this time this microparticle enzyme immunoassay (MEIA) was used by our laboratory for the screening of human Chorionic Gonadotropin [hCG] misuse. For any necessary confirmation of suspicious samples after screening the total β-hCG-kit (in vitro test 1A06-22) from Abbott was applied.

Both kits were easy to handle and fulfilled the criteria of sensitivity, minimum required performance limit of 5 mIU/ml urine, according to the anti-doping regulation of the World Anti-doping Agency [WADA]. The two Abbott MEIA kits were performed with the same IMx® benchtop machine, whereas the antibody binding site (epitope) of both kits were different. The epitope of the IMx® hCG-kit was an anti-common a: anti-β C-terminal epitope showing specifity for nicked and non-nicked hCG. The IMx® total β-hCG kit is an anti-β C-terminal: anti-common b1 epitope, specific for nicked and nonnicked hCG as well as for the free β subunit [1].

The Abbott IMx® machine is followed by the AxSYM® system Plus, but only offering a total β-hCG kit. The AxSYM® system offers a wide range of different analytical parameters for clinical diagnostics. This system is very space consuming in comparison to the well established IMx® machine. Due to these disadvantages we looked for a cost-effective alternative method.

The screening procedure for about 7000 samples per year was changed. We decided to use the Abbott IMx® total β-hCG kit for routine analysis and chose the Elecsys® hCG STAT kit (Roche Diagnostics) for confirmation, due to the availability of this system at our university. The applied electro chemiluminescence immuno assay [ELICA] is able to detect the intact holo-hormone (with epitope anti-hCG dimer: anti-common β2). The free β-subunit as well as

nicked hCG forms will not be recognized. According to the manufacturer the test was only validated for plasma. It shows low cross reactivity with LH and FSH and no cross reactivity with TSH according to the manufacturer's description. A manufacturers comparison with the Abbott IMx® hCG kit showed a good correlation (r = 0,96). For application in doping analysis a validation of the Elecsys® system 2010, hCG STAT kit was performed for hCG in urinary matrix and is described in detail. The results of both systems have been compared [2].

Material and Methods

All analyses have been performed with the IMx® system, total β-hCG-kit (in vitro test 1A06-22), total β-hCG calibrators (9C21-01), total β-hCG controls (9C21-12, all Abbott, Wiesbaden, Germany) and the Elecsys® system 2010 utilizing the hCG STAT kit (1971289) and the hCG STAT CalSet (1731670, all Roche Diagnostics, Mannheim, Germany). Calibration was performed according to the manufacturer's instructions.

For all investigations urine samples from 10 healthy male volunteers, aged between 25 and 30 without any medication and moderate sport activity (up to 5 h per week) were collected and stored at 4°C prior preparation and immediate analysis.

Specificity of both kits was tested by analyzing 10 different native urine samples. To determine the identification capacity 10 different native urines were spiked with 5 mIU total β-hCG/ml (total β-hCG control, manufactured volumetrically and referenced to the World Health Organization 4th International Standard 75/589 for β-hCG, Abbott, Wiesbaden, Germany). For robustness 18 native urines were spiked with 15 mIU total β-hCG/ml and the pH-value per 6 samples was adjusted to 4,5, 6,5 and 8,0 respectively. In a second step 3 times 3 urines were spiked with LH and FSH at a concentration of 20, 50 and 100 mIU/ml each (Menogon, 1 ampoule containing 75 I.E. FSH and 75 I.E. LH, Ferring, Kiel, Germany). For the estimation of linearity 6 native urine samples were spiked with 5, 10, 25, 50, 75 and 150 mIU total β-hCG /ml.

Results

Specificity: all 10 analyzed native urine samples showed a total β-hCG concentration below 1 mIU/ml urine. The mean value for the Abbott total β-hCG kit was (0,2 ±0,3) mIU/ml, for the Roche hCG STAT kit (0,6 ±0,2) mIU/ml was estimated.

Identification capacity: total β-hCG could be identified in each urine sample spiked with 5 mIU total β-hCG/ml. The mean value for the Abbott kit was calculated with (4,6 ±0,6) mIU/ml and (4,4 ±0,2) mIU/ml for the Roche kit.

Robustness: the different pH values of the urine samples did not have any influence on the identification of the spiked hCG (Figure 1). Results for cross reactivity against LH and FSH are shown in Figure 2.

Linearity: linearity was verified with the Mandel test and confirmed for the IMx® and Elecsys® system [Figure 3, 4].

Discussion

The Roche hCG STAT kit fulfilled the validation criteria for doping analysis regarding specificity, identification capacity and linearity. The molecule binding site (epitope) is different from that used in screening (Abbott IMx total β-hCG). The results concerning cross reactivity against LH and FSH differed to the manufactures investigations, where 500 mIU/ml LH and 1000 mIU/ml FSH did show a cross reactivity of 0,29% for LH and 0,09% for FSH [2]. One reason may be that those tests have been performed in plasma while urine may show a wider range of cross reactivity. It is also possible that the applied Menogon is not 100% free of hCG. Here these investigations should be repeated with pure standards of LH and FSH. Nevertheless the data are acceptable as an earlier investigation showed that in 232 urine doping control samples a LH concentration of 20 mIU/ml was not exceeded. The LH intra-day variation in one healthy male volunteer ranged between 2 and 8 mIU/ml [3].

References

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

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

[3] Voss, S., Gotzmann, A., Geyer, H., Mareck, U., Schänzer,W. (2005) Evaluation of LH concentration in male and female urines. Effects of a single LH application on the steroid profile. In: Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U. (eds.) *Recent Advances in Doping Analysis (13)*, Köln, pp 423-426

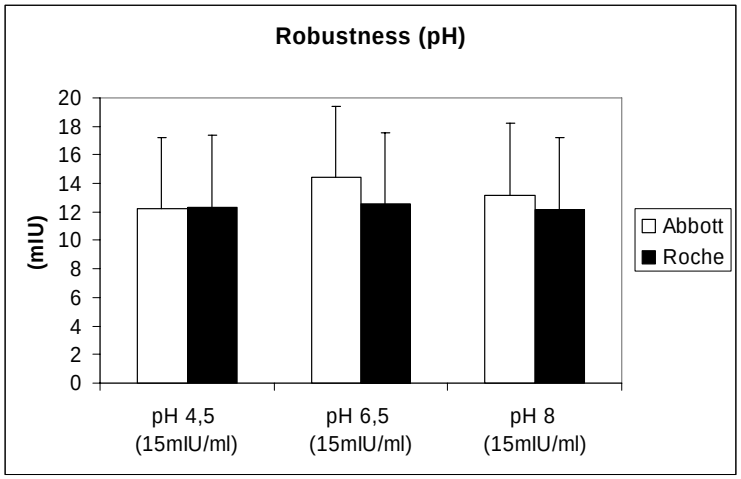

FIG. 1: Total β-hCG concentration in spiked urine samples at different pH-values: mean values (n=6) and standard deviation.

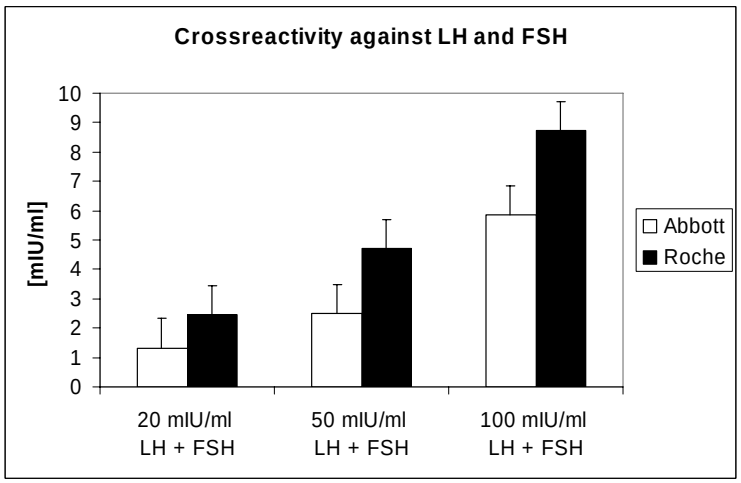

FIG. 2: Crossreactivity against LH and FSH: mean values (n=3) and standard deviation.

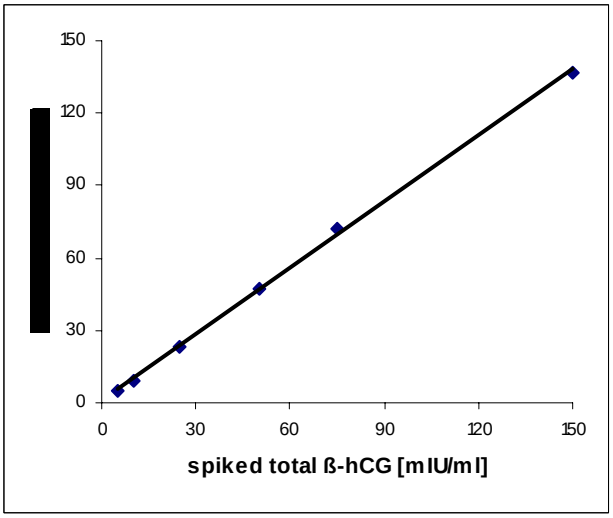

FIG. 3 Linearity-test Abott IMx (r=0,9995)

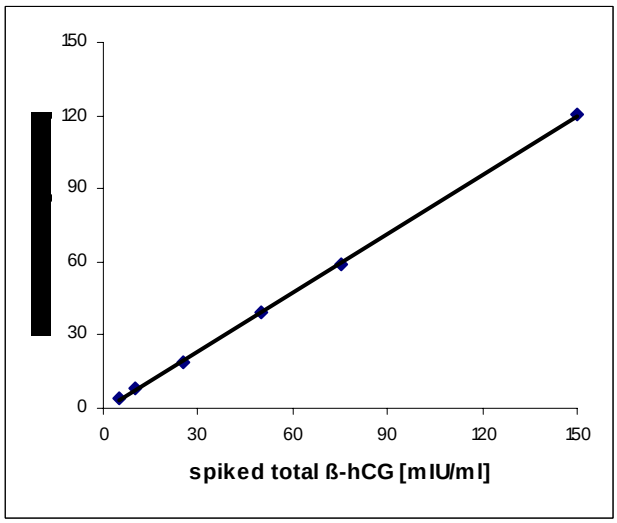

FIG. 4 Linearity-test Roche Elecsy, (r=0,9999)