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Validation of urinary hCG analysis using automatic immunoassay analyzer PATHFAST® based on the chemiluminescence enzyme immunoassay (CLEIA) with Magtration® technology

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Introduction

In accordance with the WADA ISL 5.2.4.3.1.3, human Chorionic Gonadotropin (hCG) should be determined by two immunoassays which use antibodies recognizing different binding sites (epitopes) [1–3]. For the analysis of hCG in our laboratory, the Immulite® 1000 is used for the initial screening procedure. The epitopes of both kits are different and the former is an anti-common β2: anti-common β1 (specificity for nicked hCG, non-nicked hCG and free β subunit), the latter is an anti-hCG dimmer: anti-common β1 (specificity for non-nicked hCG only) [4]. Manufacturers often remove the ELISA kits from the market without any notice (e.g. IMx™ from Abbott Laboratories, IL, USA, in 2005) [5]. We need to be prepared for such situations. PATHFAST® is a fully automatic immunoassay analyzer, which combines progressive chemiluminescence technology with patented Magtration® technology. The hCG analysis for fertility diagnostics using PATHFAST® is validated for whole blood, serum and plasma by the manufacturer; however the clinical use of urine samples is not covered by Japan's Pharmaceutical Affairs Law. For doping control purposes, we conducted the validation of urinary hCG analysis using PATHFAST®.

Materials and Methods

The PATHFAST® immunoanalyser was evaluated and compared with the Immulite® 1000 (DPC/Siemens, IL, USA) used in the routine screening test. The PATHFAST® system was from Mitsubishi Chemical Medience Corporation (Tokyo, Japan). The reagent kits were the PATHFAST® HCG preg (1110-3000) 60 tests. Two-point calibration was performed by every 4 weeks (WHO 4th International Standard 75/589). The specificities of the

PATHFAST® were against nicked hCG, non-nicked hCG and free β subunit. The cross reactivities are shown in Table 1. The hCG analysis with PATHFAST® was conducted using chemiluminescence enzyme immunoassay (CLEIA) and Magtration® technology. Dilution buffer (25 μL), alkaline phosphatase conjugated anti HCG MoAb (50 μL) and anti HCG MoAb coated magnetic particles (50 μL) were added to the urine samples (25 μL). After the immunoreaction (37°C, 5 min), bound/free separation (Magtration®) was conducted. Chemiluminescent substrate (100 μL) was added for enzyme reaction, and chemiluminescence was measured (461 nm). The assay principle is shown in Figure-1.

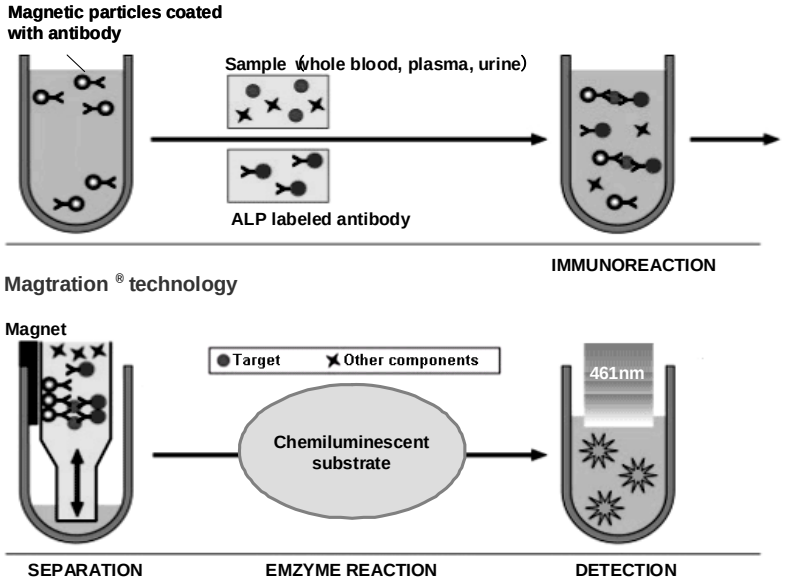

Figure-1. Assay principle of PATHFAST®

Method validation

Specificity: Five different male urine specimens were analyzed by PATHFAST® and Immulite®1000. Sensitivity: Five different urine specimens were fortified with 5mIU/mL of total β-hCG (WHO 4th IS 75/589) and analyzed to investigate whether the kit could detect the MRPL samples. Linearity: Dilution linearity was evaluated using serial dilution of five urine samples of known hCG concentrations. Five different urine specimens were spiked with 0.5, 1, 2, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 mIU/mL of total β-hCG (WHO 4th IS 75/589) and analyzed. Limit of detection: Analytical sensitivity was defined as the concentration at two times standard deviation from the mean of the urine without HCG in 20 consecutive replicates and represents the lowest concentration that could be distinguished from zero. Intra-assay and inter-assay precision: Three urine samples of low (5 mIU/mL), middle (20 mIU/mL) and high (100 mIU/mL) concentrations of total β-hCG were prepared

and analyzed once a day on three different days. Robustness: The influence of the urinary pH value on analytical data was investigated. A total of 18 samples with pH 4.5 (n=6), 6.5 (n=6) and 8 (n=6) were analyzed to investigate the influence of the urinary pH value on analytical results of hCG quantification. Applicability: The WADA external quality assurance sample (WADA 2011 EQAS-01) was analyzed to check the applicability. This sample was a pooled urine collected from an excretion study with recombinant hCG.

Table-1. Cross reactivity in PATHFAST®

Cross-reactant	Conc.	Cross reactivity
LH	1,000mIU/mL	0.759%
TSH	1,000μIU/mL	0.111%
FSH	1,000mIU/mL	0.0004%
hGH	1,000ng/mL	not detactable
Prolactin	1,000ng/mL	not detactable

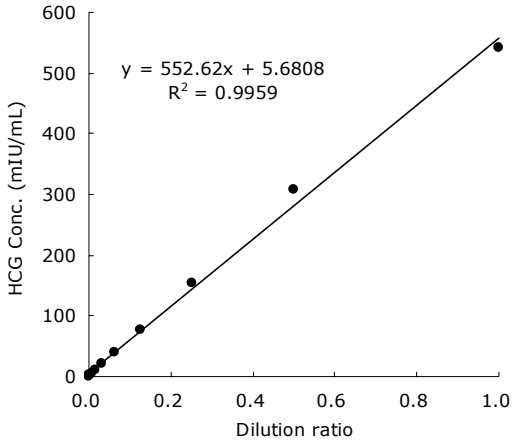

Figure-2. Linearity of PATHFAST

Results and Discussion

Specificity: The mean values estimated by in PATHFAST® and Immulite®1000 were 0.9±0.3 mIU/mL and 1.3±0.2 mIU/mL, respectively. Sensitivity: The concentration estimated by PATHFAST® was 5.4±0.7 mIU/mL. Total β-hCG could be detected in WADA MRPL samples (5 mIU/mL) using PATHFAST®. Linearity: Dilution linear curve ranging from 0.5 to 500 mIU/mL was obtained with satisfactory correlation coefficients (Figure-2). Limit of detection: Analytical sensitivity was 1.73 mIU/mL. Intra-assay and inter-assay precision: The inter-assay precision ranged from 6.6 to 7.2% for Immulite® 1000 and 4.5 to 8.7% for PATHFAST® (Table-2). Robustness: No influence of urinary pH value on detection of hCG was observed (Table-3). Applicability: The concentration measured by PATHFAST® was 26.3 mIU/mL (n=3), while a nominal concentration was 28.2 mIU/mL (Immulite®), and excellent accuracy (6.7%) was obtained.

In conclusion, total β-hCG could be analyzed in human urine using the PATHFAST® assay system. The PATHFAST® system combines the accuracy of a full-scale laboratory with the flexibility of a mobile solution. However, its maximum throughput is 6 samples for 26 min. Hence, with its space-saving design and large degree of flexibility PATHFAST® is suited as a back-up system in the laboratory or for confirmation analysis.

The PATHFAST® system is able to analyze LH and FSH concentrations in whole blood, serum and plasma samples. Therefore, further validation study of urinary LH for doping control purposes will be conducted.

Table-2. Inter-assay precision Immulite®1000 and PATHFAST®

Inter-day precision (%) 3 days, n=9					
Low(5 mIU/mL)		Middle(20 mIU/mL)		High(100 mIU/mL)	
Immulite®1000	PATHFAST®	Immulite®1000	PATHFAST®	Immulite®1000	PATHFAST®
7.2	7.0	6.6	8.7	6.7	4.5

Table-3. β-hCG concentration in spiked samples (15mIU/mL) at different pH values

	pH4.5		pH6.5		pH8.0	
	Immulite® 1000	PATHFAST®	Immulite® 1000	PATHFAST®	Immulite® 1000	PATHFAST®
mean (n=6)	16.7	13.6	16.5	15.6	15.8	13.9
SDn-1	2.4	2.7	1.5	1.6	2.6	2.7
CV(%)	0.1	0.2	0.1	0.1	0.2	0.2

(Unit: mIU/mL)

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References

[1] World Anti-Doping Agency. The International Standard for Laboratories ver. 6.0. Montreal (2009) http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-IS-Laboratories/ISL/WADA_Int_Standard_Laboratories_2009_EN.pdf (access date 10.01. 2012)

[2] Shahzad K, Kim DH, Kang MJ. (2007) Analytic evaluation of the beta-human chorionic gonadotropin assay on the Abbott IMx and Elecsys2010 for its use in doping control. *Clin Biochem* 40, 1259-1265.

[3] Robinson N, Sottas PE, Saugy M. (2010) Evaluation of two immunoassays for the measurement of human chorionic gonadotropin in urine for anti-doping purposes. Clin Lab 56, 197-206.

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

[5] Gotzmann A, Voß SC, Schänzer W. (2007) Abbott IMx® total β-hCG-kit versus Roche Elecsys hCG STAT-kit: Validation and performance comparison. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis (15)*, Köln, pp 351-353.