Published in final edited form as:

Electrophoresis. 2008 August ; 29(16): 3477–3483. doi:10.1002/elps.200700785.

A chip-based immunoaffinity capillary electrophoresis assay for assessing hormones in human biological fluids

Edward F. Wellner and Heather Kalish

Nanoscale Immunodiagnostics, Laboratory of Bioengineering and Physical Science, National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD, USA

Abstract

A chip-based capillary electrophoresis system has been designed for assessing the concentrations of four hormones in whole human blood, saliva, and urine. The desired analytes were isolated by immunoextraction using a panel of four analyte-specific antibodies immobilized onto a glass fiber insert within the injection port of the chip. Following extraction, the captured analytes were labeled prior to electro-elution into the chip separation channel, where they were resolved into four individual peaks in circa 2 min. Quantification of each peak was achieved by on-line LIF detection and integration of the area under each peak. Comparison to commercial high-sensitivity immunoassays demonstrated that the chip-based assay provided fast, accurate, and precise measurements for the analytes under investigation. As the availability of commercially available antibodies rapidly expands, the application of this system will greatly increase. Chip-based CE separations of multiple analytes from a single sample also provide a significant advantage in the analysis of small samples.

Keywords

Body fluids; Chip-based immunoaffinity capillary electrophoresis; Hormones; Miniaturization

1 Introduction

The interest in newborn screening for a number of diseases and conditions is increasing. However, the amount of sample obtained from a heel blood draw remains very small. The growing interest in obtaining the greatest amount of information by the least invasive means also dominates the field. Additionally, there is a trend toward the consolidation of many tests on fewer platforms in clinical laboratories [1]. This can be accomplished by either analyzing easily obtainable samples such as urine or saliva from the baby or maximizing the number of analytes that can be screened for in the small amount of blood taken during a heel stick.

The analysis of multiple analytes during single analytical runs has been done by a few methods. Nelson *et al.* [2] were able to isolate and quantitatively measure approximately 50 or more analytes from a single dried blood spot using recycling immunoaffinity chromatography. The results compared favorably with those obtained from standard immunoassay tests, however the amount of time for sample analysis still remained high. In an effort to resolve this issue, Xu and co-workers [3] developed a quadruple-label, time-resolved fluorometric immunoassay for measuring thyroid-stimulating hormone (TSH), immunoreactive trypsin (IRT), 17 α -hydroxyprogesterone (17 α -OHP) and creatine kinase MM (CK-MM) from dried blood spots

[3]. While the preliminary results from a limited number of samples showed good agreement with the individual hormone assays, there were several drawbacks to the method, including sensitivity levels being insufficient to diagnose the diseases under investigation. Some groups have been able to improve upon the time needed for high throughput analysis [1], however, these instruments are not able to detect more than one analyte *per* sample.

CE has yielded significant improvements over traditional HPLC assays including high resolving power, inexpensive materials, small sample volumes, and rapid analysis times [4]. A further improvement on the CE assay is their application to chip-based systems [5]. Phillips and Dickens [6] successfully applied CE coupled with immunological extraction to the measurements of cytokines and other analytes in CSF. Further, using a chip-based CE system, Phillips was able to improve upon CE analysis and perform rapid clinical assays with analysis times almost becoming “real time” or approximately 2 min of analysis time [7].

Using a microchip CE detection system, Wang and Chatrathi [8] rapidly and simultaneously measured renal markers in both synthetic and clinical urine samples, while Wang *et al.* [9] coupled on chip enzymatic assays with microchip CE to rapidly and simultaneously measure lactate and glucose from whole blood and serum. The application of microchip CE to a variety of biological fluids with results achieved in a minimal amount of time offer great promise for microchip CE to be used in a point of care setting.

In this communication, we present a chip-based CE system designed to simultaneously isolate and analyze four hormones from a relatively small amount of blood, saliva or urine. Using this system, we are able to isolate and quantitate within a single run, four hormones present in three different biological fluids, saliva, urine, and whole blood from normal biofluids and from patient samples.

2 Materials and methods

2.1 Reagents

Recombinant human hormones (follicle-stimulating hormone – FSH, luteinizing hormone – LH, testosterone, and thyroid-stimulating hormone – TSH) and their corresponding mAb were obtained from Accurate Chemical & Scientific (Westbury, NY, USA). 3-Aminopropyltriethoxysilane, carbonyl diimidazole, formamide, and all other chemicals were purchased from Acros Chemicals (Fisher Scientific, Pittsburgh, PA, USA). Streptavidin and hydrazine long-chain biotin were obtained from Pierce Biotechnology (Rockford, IL, USA). Immediately prior to use, all solutions were passed through 0.2 mm NC filters (Millipore, Bedford, MA, USA).

2.2 Sample preparation

Whole heparinized blood (10 mL), saliva (1 mL), and urine (100 mL) samples were obtained from normal volunteers at the George Washington University Medical Center (GWUMC), Washington, DC, USA. Following immunoaffinity depletion of the analytes of interest, the samples were pooled and stored at -80°C in sterile tubes containing a cocktail of protease inhibitors (Calbiochem – EMD Chemicals, San Diego, CA, USA).

These samples were used as normal human model matrices for the study. Standards were prepared by adding a known amount of each recombinant hormone to the appropriate volume of pooled whole blood, saliva, or urine. All standards were made up at 10, 50, 100, and 200 pg/0.5 μL . Additionally, samples (blood, saliva, and urine) were collected from patients seen at the Endocrine Clinic at GWUMC. All samples were collected from consenting subjects and approved by the Institutional Review Board (IRB).

2.3 Instrumentation

The immunoaffinity capillary electrophoresis (ICE) analyses were performed on a Micralyne μ TK microfluidic electrophoresis system (Micralyne, Edmonton, Alberta, Canada). This system consisted of a central control box, which controlled the 4-electrode output, the laser, and the detection input, through a LabView interface run on a PC equipped with Microsoft Windows 2000. The system consisted of four platinum electrodes and an 8 mW 633-nm red diode laser with detection being achieved *via* a confocal epilluminescence microscope focused on the separation channel and adjusted using an *x-y-z* adjustable optical bench. Fluorescent signals were transferred to a Hamamatsu H5773-03 photomultiplier tube (Bridgewater, NJ, USA) and relayed to the computer *via* a 16-bit data acquisition card.

The assays were performed using Micralyne standard T-100 “borofloat” CE chips ($16 \times 95 \times 2.2 \text{ mm}^3$ deep consisting of a 1.1 mm deep etched channel section bonded to a 1.1 mm glass cover). Both the separation and injection channels were 20- μm deep $\times$ 50- μm wide semicircular channels arranged in a cross formation with staggered cross arms (Fig. 1A). At all ends of each channel, a 2.0-mm diameter $\times$ 1.2-mm deep hole or port was present, passing completely through the glass cover into the lower half of the chip. These ports were used for loading samples or buffers into the chip as well as serving as buffer reservoirs on the separation channel. The separation channel was 80.89 mm (75.00 mm to the detector) from the intersection of the cross to port 4 and a sample-loading channel of 9.64 mm between ports 2 and 3. To aid loading and manipulation of surface modification reagents, samples, and buffers, Upchurch NanoPort assemblies (Upchurch Scientific, Oak Harbor, WA, USA) were attached to the upper surface of the chip directly over the opening of each port (Fig. 1B).

2.4 Preparation of the immunoaffinity insert

To easily regenerate the ICE chip and to ensure reusability of the glass chip, antibody-coated immunoaffinity inserts were prepared as previously described [10]. Briefly, AP40 glass fiber filters (Millipore) were silanized in 10% v/v aqueous 3-aminopropyltriethoxy-silane, and derivatized with a 1 mg/mL solution of carbonyl diimidazole dissolved in formamide. The filters were then incubated in a 1 mg/mL solution of streptavidin dissolved in 100 mM phosphate buffer, pH 7.4 before blocking excess side chains with 200 mM Tris-HCl buffer, pH 9.0 and storing the filters in 100 mM phosphate at 4°C.

All of the capture antibodies were examined for cross-reactivity by 2-D Western blotting against all of the analytes of interest. Additionally, their binding characteristics were checked by precipitin curve binding assays and antibodies with matched association/dissociation constants selected for immobilization. This was performed to reduce nonspecific binding and ensure that all isolated analytes would elute from their capture antibodies within a predicted timeframe. Each antibody was biotinylated *via* its carbohydrate moieties in the Fc or tail of the molecule using hydrazine biotin (Pierce Biotechnology) according to the manufacturer's instructions.

The biotinylated antibodies were incubated with the streptavidin-coated filter for 30 min at 37°C, followed by 30 min incubation with a solution of 1 mg/mL biotin to block free biotin-binding sites on the streptavidin filters. One hundred 2-mm diameter disks were punched from the filter and each batch tested for uniformity and specificity by running five randomly selected disks against a standard solution containing a stock solution of each analyte. To ensure adequate quality control and to check for batch-to-batch variability, ten disks were randomly selected from each batch and tested by incubating them with a solution containing 100 pg of each analyte. Additionally, during sample analysis, a panel of standards was run every ten samples to check for antibody degradation. A 10% loss in antibody efficiency was considered a cut-off point and the disk or batch of disks were discarded.

2.5 ICE analysis

ICE analysis was run in an identical manner to that previously described [7] and illustrated in Fig. 2. Briefly, before performing the assay, the chip channels were flushed with 100 mM phosphate buffer by attaching 360- μm od, 50- μm id poly-ether-ether-ketone (PEEK) tubing (Upchurch Scientific) to the chip ports 1 and 3. The buffer was pumped at a flow rate of 1 $\mu\text{L}/\text{min}$ using a Harvard syringe pump (Pump 11; Harvard Scientific Apparatus, Holliston, MA, USA). The PEEK lines were removed prior to filling ports 1, 3, and 4 with 100 mM phosphate buffer and plugging them to prevent leakage from port 2. The assay was performed in three steps. Step 1 involved introducing an 0.5 μL (500 nL) sample into port 2 using a 0.5 mL syringe and incubating with the immunoaffinity disk for 1 min. During this time the immobilized antibodies bound their respective analytes. Following the incubation, the sample was removed from port 2 using a 0.5 mL syringe thus allowing the noncaptured materials to be removed for further analysis. Step 2 involved the introduction of 500 nL of a 1 mg/mL solution of AlexaFluor 633 laser dye dissolved in 100 mM phosphate buffer, pH 7.5 into the immunoaffinity port and incubating for a further 5 min. During this time, the succinimide groups on the dye reacted with amine groups on the bound analytes to specifically label the bound analytes. The unreacted dye was removed and the port washed five times by introducing 500 nL of phosphate buffer. Finally, in the third step, the port was filled with 100 mM phosphate buffer, pH 1.5. The low pH dissociated the antibody-analyte complex thus releasing the captured analytes for electrophoretic analysis. The CE electrodes were placed into all of the four ports and using the LabView interface, the CE run was programmed to electro-kinetically migrate a 500 pL plug of sample into the intersection where the sample channel meet the main separation channel. This was achieved by applying a 1 kV potential between ports 2 and 3 for 8 s while keeping ports 1 and 4 at ground to prevent leakage of the sample into the separation channel. Separation of the injected sample was achieved by applying a 6 kV potential between ports 1 and 4, while maintaining ports 2 and 3 at ground to stop sample flow back into the injection channel. The separated peaks were detected on-line by the LIF detector.

2.6 Validation

In order to validate the accuracy and precision of the chip-based assay, split samples were analyzed by commercial high-sensitivity enzyme-linked immunoassay kits (ELISA; Accurate Chemical) run according to the manufacturer's instructions. Additionally, patient samples were compared to results obtained by conventional hormone assays performed in the GWUMC clinical laboratories.

3 Results

3.1 Chip characteristics

The immunoaffinity disk contained circa 488 ng of the immobilized antibody mixture, which was shown to contain equal amounts of each antihormone antibody. Saturation and dilution experiments using known hormone standards demonstrated the saturation and LOD characteristics of the immunoaffinity disk in all three biological fluids (Table 1). The ICE system was operated at room temperature, which was less than ideal for maintaining antibody activity, but even under this condition it was found that the immunoaffinity disk could be regenerated up to ten times before losing significant binding efficiency (a loss of 10% efficiency).

A characteristic electropherogram is illustrated in Fig. 3 showing the resolution of all four hormones. This pattern demonstrated less than a 5% variation in migration times over ten separations. The hormones were resolved in the following order: TSH, testosterone, LH, and FSH. Table 2 summarizes the precision and accuracy of ICE for measuring known amounts of the four hormones in all three biological fluids. Intra- and interassay coefficients of variation

(CVs) were calculated by running a standard mixture of all four hormones five times, on the same chip, on the same day or on five consecutive days. Intraassay CVs for TSH, testosterone, LH and FSH were found to be 4.45, 7.62, 3.95, and 5.22%, respectively. The interassay CVs were 5.55, 8.23, 4.69, and 4.16% for the same order.

Originally, the average run-time for an ICE assay was approximately 14 min, with 12 min being used to introduce the sample, capture it, wash, and label the captured analytes, however, the actual electrophoretic separation was performed in only 2 min. This meant that four samples could be run *per* hour using a single chip. Improvements in antibody binding reduced the time required for analyte capture to 1 min, which together with a more efficient washing procedure greatly improved the overall time required for performing the assay. The improved system was shown to now be capable running six samples *per* hour using a single chip. However, when multiple chips were prepared and the prerun preparations were performed in a staggered sequence, up to 18 samples could be run in an hour. Although this is still reasonably slow compared to more conventional hormone assays, the increased sensitivity and ability to measure multiple analytes within the same sample give ICE a definite advantage. Not only can four analytes being measured simultaneously within the same sample but the extremely small sample requirements (0.5 μ L) offered by the chip system greatly out-weighs the disadvantage of assay speed.

3.2 Correlation with standard immunoassays

Split samples were used to compare the immunoaffinity chip analyses to results obtained by commercial ELISA kits. The r^2 values for whole blood, when the data sets were analyzed by linear regression using a commercially available program (GraphPad 4; GraphPad Software, San Diego, CA, USA), demonstrated values of 0.983 for FSH, 0.969 for LH, 0.941 for testosterone, and 0.974 for TSH. The r^2 values for urine were slightly lower but still acceptable, being 0.969 for FSH, 0.957 for LH, 0.932 for testosterone, and 0.961 for TSH, respectively. Correlations between the two assay systems when saliva samples were analyzed were even less correlated with r^2 values of 0.877 for FSH, 0.902 for LH, 0.875 for testosterone, and 0.938 for TSH.

3.3 Measurement of hormones in normal biological fluids

Analysis of the three different biological fluids demonstrated that the immunoaffinity disk was reasonably efficient at recovery of the analytes of interest. However, the most reliable and consistent results were obtained with whole blood samples. In whole blood the immunoaffinity extraction could be easily performed without prior sample preparation (Fig. 4). In both saliva and urine samples, centrifugation at 10 000 $\times$ g and pH adjustment had to be performed before the immunoaffinity extraction. In both cases, cellular debris or acidic pH greatly affected the outcome of the assay. In the former situation, the cellular debris often resulted in higher concentrations possibly caused by nonspecific binding to the capture antibodies or release of cell-bound hormones. The pH was found to seriously hinder the accuracy of the assay system, the acidic conditions found in urine causing hindrance to the initial antibody–analyte binding, thus causing false negative or reduced measurements. In both cases, centrifugation and dialysis were required before accurate measurements could be obtained. Figure 5 illustrates the findings in both saliva and urine pre and postcleanup.

3.4 Analysis of patient samples

The ICE system was applied to the analysis of 30 patient samples with hormonal disorders in order to test the efficacy of the system. In general, it was found that the patient biofluids performed in a similar manner to the normal, spiked samples and preanalysis cleanup had to be performed on the saliva and urine prior to analysis. Additionally, in the patient samples it was found that whole blood was the biofluids of choice giving good correlations with the

clinical laboratory findings. Both saliva and urine yielded lower results although in the same pattern, providing a potential source for noninvasive sampling. This pattern for each biofluids is illustrated in Fig. 6, where a female patient with high TSH was easily analyzed by ICE with a 97.6% correlation with the clinical laboratory results when whole blood was used; a 94.5% correlation when saliva was examined and a 93.3% correlation when urine was examined. Table 3 details the findings in whole blood samples taken from patients with other disorders.

4 Discussion

The applications and advantages of microchip CE over CE and traditional analytical techniques, such as HPLC or ELISA, have been covered in some excellent review articles [11–13]. Microchip CE is currently being used in basic, clinical, and forensic science [14–18] and shows great potential for use in a point-of-care setting. Advantages of using microchip CE over traditional CE include the fabrication of chips in inexpensive materials to make them disposable, reduced reagent and solvent consumption, thus preserving precious samples and decreasing operational costs, and gaining valuable analysis time because of less sample preparation time and faster analysis rates. These advantages become increasingly important when dealing with pediatric or neonatal samples or when diagnostic delays can be costly to the patient. In this communication, the isolation and quantitation of four different hormones from three different biological fluids, whole blood, saliva and urine, using microchip-CE has been demonstrated.

The use of urine or saliva, instead of whole blood, is advantageous due to noninvasive collection methods, yet their analysis can be complicated and often requires sample pretreatment to obtain reliable and reproducible results. Urine analysis is often hampered by pH, high inorganic ion concentrations, and low sensitivity [19–21]. Microchip CE analysis of saliva can be performed without sample pretreatment [22,23] as long as cellular debris, turbidity, and the composition of the saliva as a result of bacterial metabolism do not interfere with the analytes or the reproducibility[24]. The analysis of whole blood yielded the expected concentration of added hormones without sample pretreatment, while the analysis of saliva and urine yielded lower and higher concentrations respectively, than what was expected. After pH adjustment and clarification the saliva and urine concentrations correlated with the known amounts added.

Immunoaffinity CE or ICE has been used to selectively concentrate analytes and increase sensitivity, however using ICE can take a long time to perform in the traditional capillary format. Adding immunological extraction to the microchip format further decreases the sample preparation time and can enhance the sensitivity of microchip CE [7,25]. Yet, as the results presented in this paper show, microchip-ICE may lead to artificially inflated or deflated values for analyte concentrations if care is not taken in the sample preparation or if immunological capture is enhanced by nonspecific binding and labeling of cellular debris. The hormone levels present in saliva decreased after sample treatment. Thus, inflated concentrations, possibly due to cellular debris present in untreated saliva samples which had been captured and labeled pretreatment, were effectively removed.

The ICE microchip system was able to effectively isolate TSH, testosterone, LH, and FSH from whole blood, urine, and saliva samples with low intra- and interassay CVs. Additionally the chip system showed greater sensitivity than the traditional ELISA assay, however, it was at a cost to analysis time. Overall the ability to measure multiple hormones from a single sample, which in itself was a smaller amount than needed for nonmicrochip analytical methods, holds the greatest potential in the development of new analytical techniques, applicable to neonatal and precious samples. Further, the compact size of the apparatus lends itself to point-of-care monitoring, especially when disposable or reusable immunoaffinity inserts are used.

Such disks are capable of immobilizing several different antibodies [10] thus expanding the analytical panels that can be used with the ICE system.

Acknowledgments

The authors would like to thank Dr. Terry M. Phillips for his valuable contributions to this work.

Abbreviations

CV	coefficients of variation
FSH	follicle-stimulating hormone
ICE	immunoaffinity capillary electrophoresis
LH	luteinizing hormone
TSH	thyroid-stimulating hormone

References

1. LePage J, Peters S, Lehotay DC. Clin Biochem 2004;37:415–417. [PubMed: 15087259]
2. Nelson KB, Dambrosia JM, Grether JK, Phillips TM. Ann Neurol 1998;44:665–675. [PubMed: 9778266]
3. Venkatramani CJ, Xu JZ, Phillips JB. Anal Chem 1996;68:1486–1492.
4. Stover FS. Electrophoresis 1990;11:750–756. [PubMed: 2257845]
5. Harrison DJ, Fluri K, Seiler K, Fan Z. Science 1993;261:895–897. [PubMed: 17783736]
6. Phillips TM, Dickens BF. Electrophoresis 1998;19:2991–2996. [PubMed: 9870401]
7. Phillips TM. Electrophoresis 2004;25:1652–1659. [PubMed: 15188254]
8. Wang J, Chatrathi MP. Anal Chem 2003;75:525–529. [PubMed: 12585479]
9. Wang J, Chatrathi MP, Collins GE. Anal Chim Acta 2007;585:11–16. [PubMed: 17386641]
10. Phillips TM, Wellner EF. Electrophoresis 2007;28:3041–3048. [PubMed: 17724696]
11. Verpoorte E. Electrophoresis 2002;23:677–712. [PubMed: 11891702]
12. Colyer CL, Tang T, Chiem N, Harrison DJ. Electrophoresis 1997;18:1733–1741. [PubMed: 9372264]
13. Kutter JP. Trends Anal Chem 2000;19:352–363.
14. Beard NP, de Mello AJ. Electrophoresis 2002;23:1722–1730. [PubMed: 12179994]
15. Alonso A, Albarran C, Martin P, Garcia P. Electrophoresis 2006;27:5101–5109. [PubMed: 17120261]
16. Huang H, Bu Y, Zhou GH. World J Gastroenterol 2006;12:3814–3820. [PubMed: 16804964]
17. Jin LJ, Ferrance J, Landers JP. BioTechniques 2001;31:1332–1335. 1338–1340, 1342. [PubMed: 11768663]passim
18. Baena B, Cifuentes A, Barbas C. Electrophoresis 2005;26:2622–2636. [PubMed: 15934051]
19. Garcia CD, Henry CS. Analyst 2004;129:579–584. [PubMed: 15213822]
20. Tuma P, Samcova E, Opekar F, Jurka V, Stulik K. Electrophoresis 2007;28:2174–2180. [PubMed: 17557358]
21. Veraart JR, Lingeman H, Brinkman UA. J Chromatogr A 1999;856:483–514. [PubMed: 10526801]
22. Annovazzi L, Cattaneo V, Viglio S, Perani E. Electrophoresis 2004;25:1255–1263. [PubMed: 15174046]

23. Tanaka Y, Naruishi N, Nakayama Y, Higashi T, Wakida S. J Chromatogr A 2006;1109:132–137. [PubMed: 16364340]
24. Schipper RG, Silletti E, Vingerhoeds MH. Arch Oral Biol 2007;52:1114–1135. [PubMed: 17692813]
25. Guzman NA, Phillips TM. Anal Chem 2005;77:61A–67A.

Figure 1.

Diagram and photograph of the immunoaffinity chip. (A) Diagram indicating the location of the immunoaffinity port and the port numbers used in the assay description. (B) Photograph of the immunoaffinity chip complete with attached nano-ports, showing the larger reservoir ports on-chip ports 1 and 4 and the smaller regular ports on-chip ports 2 and 3.

Figure 2.

Diagrammatic illustration of the principle of the assay. Step 1: the sample containing the analyte of interest is introduced into the immunoaffinity port and is captured by the immobilized antibody. The nonreactive materials in the sample are then removed. Step 2: the AlexaFluor dye is introduced and binds to the captured analyte *via* free amino groups present on the analyte. Nonreactive dye is removed. Step 3: an acidic elution buffer is introduced thus releasing the labeled analyte, which is separated on the chip and detected as an electropherogram.

Figure 3.

A typical electropherogram illustrating resolution of the five analyte peaks in spiked whole blood in under 2 min. The peaks are numbered 1, TSH; 2, testosterone; 3, LH; 4, FSH. The baseline has been averaged for clarity and the running conditions are outlined in Section 2.5.

Figure 4.

Recovery of the four analytes in whole blood indicating the lack of interference in this complex biological matrix. Open bars, TSH; left-angled bars, testosterone; right-angled bars, LH; gray bars, FSH.

Figure 5.

Immunoaffinity chip measurement of the four hormones in saliva and urine pre- and postsample cleanup. (A) The open bars represent saliva samples spiked with 100 pg of the four different hormones without any premeasurement cleanup. The closed bars represent saliva sample post centrifugation, to remove cellular debris, and adjustment of the pH to 7.0. (B) Identical measurements made on spiked urine pre (open bars) and post (filled bars) centrifugation and pH adjustment to 7.0.

Figure 6.

Electropherograms of whole blood, saliva, and urine from a patient with clinically diagnosed thyroid disease. (A) Electropherogram of whole blood; (B) electropherogram of saliva from the same patient; (C) electropherogram of a urine sample from the same patient. Running conditions as outlined in Section 2.5. The peaks are eluted as described in Fig. 3. In this female patient, testosterone was minimally detected as seen in the small peak 2 but TSH was seen as an abnormally high peak (peak 1).

Table 1
LOD and saturation limits for the immunoaffinity disk

Analyte	Whole blood		Saliva		Urine	
	Saturation (ng)	LOD (pg)	Saturation (ng)	LOD (pg)	Saturation (ng)	LOD (pg)
TSH	2.1	0.6	1.8	1.2	1.7	1.3
Testosterone	2.3	0.5	2.0	0.8	1.9	1.1
LH	2.2	0.6	2.1	1.1	2.0	1.7
FSH	2.1	0.9	1.9	1.1	1.8	1.4

Table 2

Hormone recovery in the different biological fluids

Analyte	Amount added ^{a)}	Whole blood	Saliva ^{b)}	Urine ^{b)}
TSH	10	9.5 ± 0.3	8.8 ± 3.4	8.3 ± 3.6
	50	47.8 ± 2.2	46.9 ± 4.8	42.7 ± 5.4
	100	98.4 ± 2.7	96.4 ± 5.1	91.8 ± 6.4
	200	197.4 ± 3.1	194.4 ± 6.2	193.2 ± 5.8
Testosterone	10	8.9 ± 4.6	8.4 ± 5.2	8.1 ± 2.2
	50	44.8 ± 4.3	45.2 ± 4.1	43.6 ± 3.2
	100	94.6 ± 2.8	93.7 ± 3.5	94.2 ± 3.3
	200	192.8 ± 4.5	191.5 ± 5.6	190.2 ± 6.7
LH	10	9.4 ± 2.8	9.1 ± 3.1	8.8 ± 5.3
	50	47.3 ± 1.9	45.5 ± 3.6	46.2 ± 3.3
	100	97.1 ± 3.1	95.8 ± 4.4	43.6 ± 3.9
	200	198.1 ± 2.5	195.4 ± 3.3	196.2 ± 3.1
FSH	10	8.9 ± 5.7	8.6 ± 6.2	8.8 ± 2.7
	50	47.2 ± 2.8	45.5 ± 3.9	44.8 ± 6.2
	100	97.2 ± 3.3	95.8 ± 4.1	96.9 ± 4.4
	200	197.2 ± 3.6	192.4 ± 6.1	194.8 ± 2.7

^{a)} All values expressed in pg. *n* = 10 runs ± SEM.^{b)} Post sample cleanup.

Table 3

Comparison between ICE and clinical laboratory measurements of whole blood hormone concentrations in the patient study groups

Patient diagnosis	Hormone	ICE	Clinical laboratories ^{a)}
Hypothyroidism	TSH	122.8 ± 6.6	143.9 ± 10.5
Male infertility	Testosterone	35.7 ± 4.5	41.2 ± 8.4
	LH	6.2 ± 1.9	7.8 ± 1.6
	FSH	9.9 ± 1.5	12.8 ± 2.7
Polycystic ovary	Testosterone	216.4 ± 21.5	245.1 ± 32.7
	LH	5.2 ± 1.3	7.5 ± 1.6
	FSH	139.9 ± 10.5	125.8 ± 12.7

n = 10 subject in each diagnostic group. All values expressed as pg/mL.

^{a)}Values converted to pg/mL.