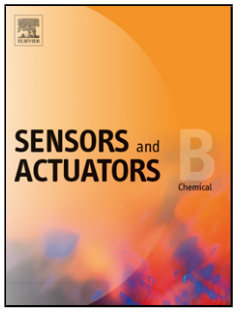
Accepted Manuscript



Title: Smartphone-Based Rapid Quantitative Detection of Luteinizing Hormone Using Gold Immunochromatographic Strip

Authors: Juntao Liu, Yan Fan, Zhuang Kong, Yang Wang, Jinping Luo, Shengwei Xu, Hongyan Jin, Xinxia Cai

PII: S0925-4005(17)32494-2
DOI: https://doi.org/10.1016/j.snb.2017.12.161
Reference: SNB 23849

To appear in: *Sensors and Actuators B*

Received date: 17-7-2017
Revised date: 29-11-2017
Accepted date: 26-12-2017

Please cite this article as: Juntao Liu, Yan Fan, Zhuang Kong, Yang Wang, Jinping Luo, Shengwei Xu, Hongyan Jin, Xinxia Cai, Smartphone-Based Rapid Quantitative Detection of Luteinizing Hormone Using Gold Immunochromatographic Strip, Sensors and Actuators B: Chemical https://doi.org/10.1016/j.snb.2017.12.161

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Smartphone-Based Rapid Quantitative Detection of Luteinizing Hormone Using Gold Immunochromatographic Strip

Juntao Liu[a,b], Yan Fan[a,b],Zhuang Kong[a,b], Yang Wang[a,b], Jinping Luo[a,b], Shengwei Xu[a,b], Hongyan Jin[c,†], Xinxia Cai[a,b,†]

[a] State Key Laboratory of Transducer Technology, Institute of Electronics, Chinese Academy of Sciences, Beijing 100190,China.

[b] University of Chinese Academy of Sciences, Beijing 10090,China.

[c] Obstetrics and Gynecology Department, Peking University First Hospital, Beijing 100034, China.

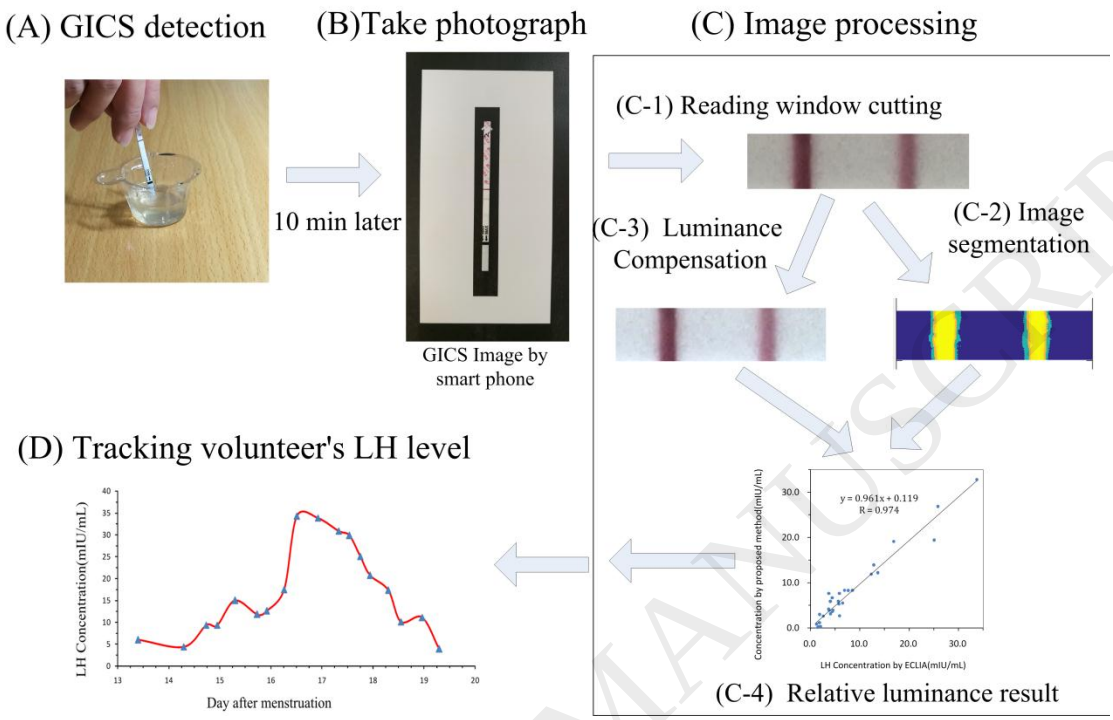
†Corresponding Authors: Xinxia Cai: xxcai@mail.ie.ac.cn, Tel: +86-10-58887193;

Hongyan Jin: maggijhy@163.com, Tel: +86-10-83573895

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Graphical abstract



(A) LH detection with GICS. (B) 10 min later, take photograph with a smart phone. (C) Image process step in the LH determination. (D) Tracking volunteer's LH level of with proposed method

Highlights

- This work provided a novel rapid quantitative determination method for LH with GICS. In summary, the highlights of the work are:
- A luminance-compensation method was applied to reduce environmental interference and increase the detection consistency of GICS image.
- Canny edge detection operator and mathematical morphology were applied to obtain the GICS reading window, and fuzzy c-means (FCM) clustering algorithm were applied to extract the test line and control line.
- The property of the developed quantitative method is demonstrated by the detection of standard LH sample and clinical sample. Results showed that the proposed method could acquire quantitative result in 15 min and also showed high efficiency and high sensitivity, which made it potential to be applied in instantaneous measurement of LH.

ACCEPTED MANUSCRIPT

Abstract

Measurement of the luteinizing hormone (LH) level is very important in assisted reproduction, the diagnosis of ovarian diseases, and evaluation of clinical effects. The objective of the present study was the development of a smartphone-based quantitative method for determining the LH level using a gold immuno-chromatographic strip(GICS). In the developed method, a smartphone is used to obtain the image of the GICS and Canny operator and fuzzy c-means clustering algorithms are used to extract the test and control lines of the GICS. A luminance compensation method is used to reduce environmental interference and increase the detection consistency of the GICS image. Experimental results revealed that the proposed method effectively reduced the relative standard deviation (RSD) of the measurements, afforded high linearity within LH levels of 1.0–83.3 mIU/mL (r = 0.996), and was highly reproducible using different types of smartphones and GICSs. The proposed method was further used to detect clinical serum samples (n = 31), with the achievement of a corresponding correlation coefficient of 0.974 against conventional large biochemical instrument; and to track the urine LH level of a volunteer during ovulation, with quantitative results obtained within 15 min. The method was found to be highly efficient and sensitive and is thus potentially applicable to rapid quantitative LH testing.

Keywords: Luteinizing hormone, Gold immunochromatographic strip, Quantitative detection, Assisted reproduction

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1. Introduction

The luteinizing hormone (LH) is one of the hormones produced by gonadotropic cells in the anterior pituitary gland[1]. It enables the theca cells in the ovaries to generate androgens and hormonal precursors for estrogen production[2]. Normally, functional ovarian activity involves the synergic action of the two pituitary gonadotropins, follicle-stimulating hormone (FSH), and LH, which together determine follicular growth, maturation, ovulation, and luteinization[3,4]. The LH concentration significantly impacts the morphological and functional changes that occur in the oocyte, as well as determines the meiotic status of the oocyte and its ability to be fertilized[6]. An acute rise in the LH level, referred to as "LH surge," triggers ovulation and the development of the corpus luteum[7,8]. LH detection is thus often employed in assisted reproduction[9,10]. The measurement of the LH level is also very important in the diagnosis of ovarian diseases and evaluation of clinical effects [11,12,13]. While the LH is secreted as pulses, it is necessary to monitor its concentration over a sufficient period of time to obtain reliable information about its level in the blood. The LH contains fewer sialic acid residues than the FSH, and the former is thus more rapidly cleared from the circulation, resulting in more pronounced LH pulses. This is reflected by the 30-min half-life of the LH compared with the 149minof FSH[14].It is therefore necessary to minimize the waiting time for an LH detection test, with point of care (POC) measurement being the best alternative for this purpose.

Traditional detection methods such as enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), and electro-chemiluminescence immunoassay (ECLIA) require specialized equipment and professional personnel, and this makes them unsuitable for POC measurements. Gold immunochromatographic strip (GICS) is a fast immuno-labeled analysis technology that boomed in the early 1990s[5].GICS has many advantages such as simplicity, low cost, high speed, and non-requirement for skilled technicians. The technology is thus practicable for POC LH measurement. However, GICS is colorimetric and involves naked-eye analysis. This causes significant variability of its results among different users, for which reason its utilization has been substantially limited [8].However, different methods for fast quantitative GICS detection have been proposed. Jiang proposed a method for the rapid quantitative detection of alpha fetoprotein (AFP)using GICS and a reflective optical fiber module [17]. During the operation of the employed stepper motor, the signals from the test and control lines of the GICS are sequentially acquired by the reflective optical detection module. In this system, a mechanical scanning device is used to obtain an image of the GICS reading window. An attractive alternative method is quantitative detection based on the GICS image. Along this line, Sumonphan[18] used a commercial optical scanner (Canon N 640Pex)to obtain the GICS image, while Wu [19] introduced a chromogenic rapid test reader for measuring the concentration of prostate-specific antigen (PSA). The systems used in these works afforded more accurate measurements and shorter test times. However, auxiliary optical apparatus were required to obtain the GICS image, for which reason the methods are unsuitable for POC LH measurement.

ACCEPTED MANUSCRIPT

A more reasonable approach to POC LH measurement is the use of a readily available digital photo detection system such as a smartphone with a built-in camera [20,21]. However, the image of a GICS obtained by a smartphone would be significantly affected by environmental illumination[22,23]. The elimination of this environmental interference to enhance the measurement consistency has been a major and difficult research issue regarding quantitative GICS detection. In the present study, a fast quantitative GICS detection method that utilizes a smartphone was developed. In the proposed method, a smartphone is used to obtain the GICS image. A canny edge detection operator is then used to extract the GICS reading window, while a fuzzy c-means (FCM) clustering algorithm is used to extract the test and control lines from the reading window. To reduce environmental interference, luminance compensation using color constancy algorithms is employed. The relative luminance based on the color intensities of the test and control lines is calculated and the linear fitting curve of the relative luminance versus the LH concentration is obtained through the detection of a standard LH solution. The effectiveness of the proposed method was demonstrated by applying it to the detection of a standard LH sample and a clinical serum sample.

2. Experimental

2.1 Apparatus and Reagents

The smartphones that were used in the present experiments were a Huawei Honor v8(Huawei Technologies Co. Ltd., Shenzhen, China), Samsung Galaxy Note 2 (Samsung Group, Korea), and Xiaomi Mi 5X (Xiaomi Inc., Beijing, China). An HP Color LaserJet Pro M277DW (HP Development Company, L.P.,USA) was employed. The clinical serum samples were detected by an electro-chemiluminescence immuno-assay (ECLIA) system (Beckman Acess2, Beckman Coulter, Inc., USA), while the urine samples were detected by an ELISA system(BioTek ELx808IU, BioTek Instruments, Inc., USA).Reagent-grade water with a specific resistance of 18.2M was supplied through a Michem ultrapure water apparatus (Michem Instruments Co., Chengdu, China).

The other experimental equipment included a human LH ELISA Kit (HengYuan Bio-technology Co., Ltd., Shanghai, China), two types of GICSs (Runbio Biotech Co., Ltd., ShanTou, China), and a standard LH sample (Beckman Coulter Inc., USA). All the buffer reagents and other inorganic chemicals were purchased from Sigma–Aldrich Chemical Co. Ltd (St. Louis, MO, USA). All the chemicals were of analytical reagent grade and used without further purification. All the experiments were performed at ambient temperature. The utilized clinical serum samples were obtained from the Peking University First Hospital.

2.2 Clinical samples and volunteers

Thirty-one clinical serum samples were obtained from the Peking University First Hospital for comparative analyses. The samples had been previously detected at the hospital by an ECLIA system to determine their LH concentrations. The employed urine samples were provided by volunteers. The study protocol was approved by the Human Research Ethics Committee of Peking University First Hospital.

ACCEPTED MANUSCRIPT

2.3 GICS detection procedure

2.3.1 Detection principle and structure of GICS

In a typical GICS, a colloidal gold-labeled antibody is immobilized on a conjugate pad. This is a temporary adsorption and can be flushed away by any buffer solution. A primary antibody(referred to as the capture antibody)against the target analyte is immobilized on the test line, while a secondary antibody against the labeled conjugate antibody is immobilized on the control line. When the conjugate-pad side of the GICS is dipped into the specimen solution, the specific colloidal gold-labeled antibody(detector reagent)on the conjugate pad would be solubilized by redissolving in the specimen solution and react with the target analyte (if present in the solution) to form a gold/antibody/antigen compound. The liquid then migrates to the absorption pad by capillary action. When the liquid flows through the test line, the gold/antibody/antigen compound reacts with the capture antibody and accumulates in the test line, forming a red band on the membrane. The excess labeled antibody conjugate is captured in the control line by the secondary antibody, while the buffer or excess solution goes to the absorption pad. The color intensity in the test line corresponds to the amount of the target analyte, the concentration of which can be determined from the strength of the test line[15,16]. Figure 1 shows a schematic of the general sandwich format of a GICS.

Figure 1

2.3.2 GICS detection

The GICS detection procedure is presented in the flowchart in Figure 2.In this study, the LH sample was first placed in a test tube and the conjugate-pad side of the GICS was dipped into the sample solution for 20 s. The GICS was then taken out and laid flat on a paper template. After 10 min, a smartphone was used to photograph the GICS and the obtained image was subsequently processed as described in Section 2.4. Finally, the concentration of the LH was calculated based on the relative luminance using a linear fitting curve. The detection requires no more than 15 min, which makes it especially applicable to point-of-care tests(POCTs).

Figure 2

2.3.3 Condition of image collection

In this work, we designed a paper template with special patterns, as shown in Figure 2 (B). The pattern comprised two concentric black rectangular frames with a white arrow at the center. The inner and outer dimensions of the outer frame were 7.0 × 13.5 and 10.0 × 16.5 cm, respectively. The outer dimensions of the inner frame were 1.5 × 10.0 cm. Before taking the photographs, the GICS was placed on the inner frame, covering the white arrow at the center, with the flow direction the same as that of the arrow. The automatic exposure mode was used to obtain the photographs and the images were saved in jpg format (3264 × 1840 pixels). During the photographing, care was taken to ensure that the border of the image was within the

ACCEPTED MANUSCRIPT

relevant black rectangular frame. This was to ensure that the captured images always had similar structures, to significantly shorten the reading window cutting time.

2.4 Processing of GICS Image

The step of the smartphone-based LH level determination involving the processing of the obtained GICS image is described below.

1) Canny operator for cutting of GICS reading window

Canny edge detection is a technique for extracting useful structural information from different visual objects and can be used to substantially reduce the amount of data to be processed[24]. In the present study, the GICS images were uniformly obtained. Hence, the structures of the different images were very similar and the approximate location of the GICS could be calculated. The unwanted part of the image could thus be cut based on the structure of the paper template. This means that the processed image was smaller than the original one, enabling significant reduction of the computational cost. The processed GICS images were subsequently converted into grayscale and the canny operator was used to extract their edges. However, the edges of an image obtained by this method do not produce a continuous curve. Thus, mathematical morphology was used to obtain the boundaries of the GICS and extract the GICS reading window from the original image.

2) Fuzzy c-means clustering algorithm segmentation

To determine the locations of the test and control lines, the signal data was segmented by using a fuzzy c-means (FCM) algorithm to assign pixels of the image to three classes in terms of test line, control line and background part. An FCM is an unsupervised clustering algorithm that has been successfully applied to a number of problems such as feature analysis, clustering, and classifier designing [25,26]. It is an iterative optimization algorithm that minimizes the object function

$$J_m(U, v_1, \dots, v_c) = \sum_{j=0}^{n} \sum_{i=1}^{c} (u_{ij})^m (d_{ij})^2 \quad (1)$$

where $u_{ij} \in [0,1]$, $\sum_{i=1}^{c} u_{ij} = 1$, $\forall j = 1, \dots, n$, $X=\{x_1, x_2, \dots x_n\}$ is the data set, $U = [u_{ij}]_{c\times n}$ is the fuzzy c-partition matrix, $V = [v_i]_c$ is the cluster center matrix, n is the number of data points, c is the number of clusters, $m \in [1,\infty)$ is a weighting constant, and $d_{ij} = \|v_i - x_i\|$ is the distance(usually Euclidian distance) between the i th cluster center and the j th data point.

In this work, to reduce the computation cost, clustering was applied to the GICS reading window extracted from the image. Considering the image characteristic of the GICS, the parameters of the segmentation algorithm in the present study were as follows: number of clusters, c = 3, weighting constant m = 2. An example of the segmentation is shown in Figure 2(C-2).

3) Luminance compensation using color constancy algorithms

Actually, the surface of a given object would produce different reflectance specters under different lighting conditions. Hence, the image of a GICS obtained by a smartphone would be significantly affected by the environmental illumination conditions. To reduce the environmental interference, a luminance compensation method that utilizes a Max RGB color constancy algorithm was employed. The use of a Max RGB algorithm is

ACCEPTED MANUSCRIPT

an extremely simple method for estimating the chromaticity of a scene illumination for evaluation of the color constancy[27,28]. It assumes that there is a white patch in the scene of every image, and that the maximum value of each color channel of an RGB-format image appears in this white patch. Thus, with the assumption of the presence of the white patch, the maximum value of each of the R, G, and B channels of an image is the illumination color. In this work, we considered the background part of the GICS reading window as the white patch. Hence, before the GICS detection, it was necessary to determine the standard parameters of the assumed white patch. For this purpose, we prepared several GICSs, which were used to detect LH solutions of different concentrations, and used a scanner to obtain standard images of the GICSs. Through the above-described image processing method, the background part of the reading window of each GICS was extracted, and the average values (R_a, G_a, and B_a) of the R, G, and B channels were calculated and stored as the standard parameters in the smartphone. These standard parameters could be used to detect GICSs of a similar type. To conduct the LH detection of the present samples, the GICS images obtained by a smartphone were processed by the above algorithm to extract the background part of the reading window. The R, G, and B components of the background part were then determined and the respective average values for the three channels (R_o, G_o, and B_o) were calculated.

The following diagonal transformation matrix based on a Von Kries model was obtained for the proposed luminance compensation method:

$$D = \begin{bmatrix} R_a/R_o & 0 & 0 \\ 0 & G_a/G_o & 0 \\ 0 & 0 & B_a/B_o \end{bmatrix} \tag{2}$$

The original image can thus be converted into the luminance compensation image by the following diagonal matrix production:

$$I_c = D * I_0 \tag{3}$$

where I_o is the original image, and I_c is the luminance compensation image.

4) Concentration

As mentioned earlier, the luminance of a signal from a test line or control line affords a basis for the quantitative evaluation of the LH level. After the luminance compensation step, segmentation of the luminance compensation image was used to extract the corrected test line, control line, and background part, and calculate the relative luminance R_L of the GICS. The relative luminance was introduced to determine the concentration of the measured substance and can be calculated as follows:

$$R_L = \frac{L_T}{L_C} = \frac{\frac{\sum_{i=1}^{T} I_T}{N} - \frac{\sum_{i=1}^{B} I_B}{K}}{\frac{\sum_{i=1}^{C} I_C}{M} - \frac{\sum_{i=1}^{B} I_B}{K}} \tag{4}$$

where L_T and L_C are the luminance values of the test line and control line, respectively; *T*, *C*, and *B*are the pixels in the test line, control line, and background part, respectively; and I_T, I_C, and I_B are the gray levels of the

ACCEPTED MANUSCRIPT

pixels in the test line, control line, and background part, respectively. The following equation applies:

$$I = 0.299R + 0.587G + 0.114B \quad (5)$$

Through the detection of standard LH samples of different concentrations, the R_L values of the samples were determined and a linear fitting curve of R_L versus LH concentration was obtained by least squares. The linear fitting curve was further used to determine the LH concentrations of other samples using the images of the GICSs obtained by a smartphone.

3. Results and discussion

3.1 Performance of proposed method

3.1.1 Time response curve of GICS

It is obvious that the strengths of the test and control lines vary with time. It is therefore important to determine when to image the GICS. The present experiments were performed as follows. A standard LH sample (40 mIU/mL) was placed in a test tube and a GICS strip was dipped into it to react with the LH. Twenty seconds later, the GICS was placed in a Laser-scan and scanned for a minute to obtain varying GICS images. The relative luminance values of the test and control lines were then calculated from the images. As shown in Figure 3, the strengths of the test and control lines initially increase with time and then begin to steady after about 10 min. The photographs of the GICSs after 10 min of the reaction were thus adopted to decrease the detection error due to variation of the strengths of the test and control lines.

Figure 3

3.1.2 Performance of luminance compensation method

To evaluate the performance of the proposed luminance compensation method, two measurement processes were considered, namely, with the luminance compensation and without it. The repeatabilities of the two processes were also compared. First, a GICS was used to detect the standard LH solution of concentration 40 mIU/mL. A smartphone was then used to obtain photographs of the reacted GICS under three different conditions, namely, in a bright environment, a dim environment, and under the flash lamp of the smartphone. The GICS images were then subjected to the two measurement processes to determine the relative luminance values. The relative standard deviations(RSDs) of the results for the respective processes were calculated and analyzed.

Table 1 presents the results for the two processes, where "A" indicates the process with luminance compensation, and "B" the process without luminance compensation. As can be observed, the RSD for the former is 4.26%, while that for the latter is 8.04%, which confirms that the proposed luminance compensation method significantly improves the detection performance.

Table 1

ACCEPTED MANUSCRIPT

3.1.3 Analytical performance of proposed method

The performance of the proposed method was evaluated using standard LH solutions of different concentrations. A standard LH sample (250 mIU/mL) was diluted to 83.3, 50.0, 20.0, 10.0, 5.0, 2.0 and 1.0 mIU/mL, respectively, with an assay buffer. The other steps of the process, such as the immune reaction, photographing, and image processing, were performed as described above. The assay buffer was also detected as a negative signal. As can be seen from Figure 4(A), the relative luminance increases with increasing LH concentration, with the calibration plots revealing a good linear relationship (with a linear correlation coefficient of 0.996) between the relative luminance and the LH concentration for values of 1.0–83.3 mIU/mL of the latter. Generally, during the reproductive years, the basal LH levels of a woman is within 2–15 mIU/mL. LH, rapidly increasing (LH surge) to 20–100 mIU/mL and then decreasing to 4–10 mIU/mL at the beginning and in the late stage of ovulation, respectively. The proposed method may thus be feasible for determining the LH level of serum samples.

To further evaluate the improvement of the measurement process afforded by luminance compensation, A given GICS image was processed with and without luminance compensation. As can be observed from the results in Figure 4(B), the calibration plot for the measurement without luminance compensation has a lower linear correlation coefficient(r = 0.986) and a poorer error bar compared to the process with luminance compensation, indicating a higher linear correlation and repeatability of the former.

Figure 4

3.1.4 Evaluation of selectivity of proposed method

Good selectivity is very important for bio-analysis detection methods. To characterize the specificity of the proposed method, the cross-reactivity between the analyte and the interfering substance was investigated. For this purpose, estradiol (E2), follicle-stimulating hormone (FSH), dopamine(DA), uric acid (UA), neuron-specific enolase (NSE), and carcinoembryonic antigen (CEA) were used as the interfering substances, with concentrations of 580 pg/mL, 25 mIU/mL, 1ng/mL, 1ng/mL, 1ng/mL and 1ng/mL, respectively. Standard LH solutions with concentrations of 2 and 10 mIU/mL, respectively, were also tested for comparison. Expectedly, only the GICS reacted with the LH, which yielded an obvious test line, whereas the test line for the interring substances were negligible. The relative luminance values are shown in Figure 5. Compared with the relative luminance for only LH (10 mIU/mL),the values for the reaction in the presence of the interfering substances are negligible. This indicates that the interfering substances do not noticeably interfere with the LH detection. Furthermore, it is obvious from Figure 5 that an LH concentration as low as 2.0 mIU/mL can be detected with a signal-to-noise ratio of 3.

Figure 5

ACCEPTED MANUSCRIPT

3.1.5 Evaluation of repeatability of proposed method

The repeatability of the proposed method was also investigated by an assay of a standard LH solution of concentration 40 mIU/mL. Five different GICSs were respectively used to detect the standard LH solution and a smartphone was used to take the photographs (n>3) of the reacted GICSs under different conditions. The images were then processed to obtain the relative luminance. Based on the experimental results, the coefficients of variation (CVs) of the GICS images were calculated (see Table 2).As can be seen, the CVs for the different GICSs are within 0.69%–6.06%, with the overall value being 7.45%, indicating that the proposed method is acceptably repeatable.

Table 2

3.1.6 Reproducibility by different smartphones

The images obtained by different types of smartphones vary. To evaluate the reproducibility of the proposed measurement method with respect to the employed smartphone, the GICS-based detection process was performed using three different types of smartphones, namely, a Samsung Note 2, Mi 5X, and Huawei Hornor V8, respectively. Standard LH samples of concentrations 50.0, 25.0, 10.0, and 2.5 mIU/mL were detected. After 10 min of detection using the GICS, the three smartphones were used to photograph the GICS and the obtained images were processed by the proposed method. Finally, the relative standard deviation (RSD) of the relative luminance values of the images were calculated and analyzed. Table 3 gives the results for the three smartphones (A. Samsung Note 2, B. Mi 5X, C. Huawei Hornor V8). As can be observed from Table 3, the RSDs are within 1.71%–7.78%, which indicates that the proposed method is well reproducible by different types of smartphones.

Table 3

3.1.7 Reproducibility by different GICSs

There are several types of GICSs available for LH detection and a robust quantitative detection method should be reproducible by different types of GICSs. The GICSs produced by different manufacturers have the same detection principle and similar structures, but differ with respect to the color intensities of the test and control lines. It is thus necessary to obtain the linear fitting curves for the different GICSs before their application to LH detection. In this study, two different types of GICS (GICS A and GICS B) produced by Runbio Biotech Co., Ltd., ShanTou, China were considered using the present algorithm. GICSs A and B were used to detect standard LH samples and the corresponding linear fitting curves were obtained, with equations C_{LH}= I*52.65 - 10.00 and C_{LH} = I*33.33 - 7.74, respectively, where I is the relative luminance and C_{LH} is in mIU/mL. As can be observed, the two equations have quite different slopes and intercepts. The two GICSs were respectively used to detect several other LH samples and the corresponding concentrations were

ACCEPTED MANUSCRIPT

calculated using the above linear fitting curves. The determined concentrations were compared with the standard concentrations and the relative errors were calculated, as presented in Table 4. The relative errors for GICSs A and B can be observed to range within -2.35%–7.93%and -5.95%–8.80%. This shows that the proposed LH detection method is sufficiently accurate and reproducible by different GICSs.

Table 4

3.2 Analytical performance of clinical serum samples

The proposed method was used to assay clinical serum samples obtained from the Peking University First Hospital. The serum samples had been previously detected by an ECLIA instrument in the hospital and the determined concentrations, which were within1.23 – 33.75 mIU/mL, were considered as the standard values. First, the linear fitting curve of the relative luminance versus the LH concentration was established through the detection of standard LH samples. Thirty-one clinical serum samples were then detected using the above-described procedure and the corresponding LH concentrations were calculated using the linear fitting curve. Figure 6 shows the correlation between the results obtained by the ECLIA instrument and those of the proposed method. Good agreement, represented by r = 0.974, was determined. This confirms the potential of the proposed method for LH detection in clinical diagnostics.

Figure 6

3.3 Application in monitoring LH levels in urine

The LH level in urine is often used in assisted reproduction and is also important to the diagnosis of ovarian diseases and the evaluation of clinical effects. To evaluate the effectiveness of the proposed method for urine sample analysis, several urine samples obtained from volunteers were assayed by both the proposed method and the classical enzyme linked immune-sorbent assay (ELISA) method. As indicated in Table 5, the relative errors between the two methods are within -5.72% – 6.63%, indicating that the detection results of the proposed method acceptably agree with the reference ELISA data.

Table 5

To monitor the feasibility and applicability in monitoring LH level in urine of the proposed method, we considered a 35 years old female volunteer with a stable menstrual cycle (30±1 days) and tracked her urine LH level during ovulation. The detection began on the 13th day after menstruation. Every day, she provided her urine sample and its LH level was detected by a GICS. A smartphone (Huawei Honor v8) was used to take the photograph of the GICS and the obtained image was processed through the proposed method to quantify the LH level. The results of the daily detection, which lasted for 7 days, are shown in Figure 7. It can be seen from the figure that the basal LH level is about 5 mIU/mL, and the peak level higher than 35 mIU/mL, which

ACCEPTED MANUSCRIPT

is about six times the basal level. The peak level appeared approximately on the 17th day after menstruation.

Figure 7

From all the above results, it can be seen that the proposed method could be used to quantitatively assess the LH level within 15 min and with high efficiency and sensitivity. The fast quantitative assessment and clinical chemistry of the proposed method are mutually complementary. If time is not critical and the appropriate equipment is available, laboratory-based methods would be suitable. However, when the LH level is to be determined at home or work, rapid quantitative assessment is preferred.

3.4 Comparison of different quantitative GICS determination methods

The quantitative GICS-based detection offers a practical approach to point-of-care testing and many studies have been devoted to establishing techniques for such. Table 6 compares the presently proposed method with other similar quantitative determination methods. Jiang[17] proposed a rapid GICS method for the quantitative detection of AFP using a reflective optical fiber. Under the propulsion of a stepper motor, the signals of the test and control lines of the GICS are sequentially acquired by a reflective optical detection module. Because of the limited irradiated area of the optical fiber, a mechanical scanning device is required to obtain the image of the reading window of the GICS. This makes the detection module bulky. The use of a GICS image for the quantitative detection is a good alternative approach. Sumonphan[18] proposed a GICS-based method for estimating Nevarapine (NVP) drug concentration. The GICS images are acquired by a commercial optical scanner (Canon N 640Pex) and processed by support vector regression (SVR)to estimate the drug concentration. Wu[19] proposed a system for the rapid quantitative estimation of prostate-specific antigen (PSA) using a homemade chromogenic rapid test reader. The reader captures the reflectance of the signal line of the GICS via a charge-coupled device camera and the image is processed to determine the PSA concentration. The methods presented in references [18] and [19] require special optical inspection equipment, which makes them unsuitable for POCT. David[29] developed a cell-phone-based reflective optical detection apparatus for the quantitative detection of the thyroid stimulating hormone (TSH). The flash of the cell phone camera is used as the light source. An accessory module with an optical fiber and collimating lens are used to channel the light of the flash to illuminate the GICS. The system was evaluated by using it to detect human serum and was found to exhibit good sensitivity and reproducibility. Owing to its high megapixel imaging sensor and ability for real-time image processing, a smartphone can be explicitly used to sense different chemical and biological samples through the use of a GICS or paper-based chip. Preechaburana[30] used a mobile phone as the measurement platform for acquiring a high dynamic range (HDR) image of a GICS for the detection of N-terminal proBNP (NT-proBNP). Cold light sources were used to produce three illuminations and three images were acquired in the automatic exposure mode. This method was used to detect an NT-proBNP solution with good performance within the diagnostics range. The systems presented in references [29] and [30] both require an auxiliary module, which limits their application. In

ACCEPTED MANUSCRIPT

contrast, in the presently proposed method, a smartphone is used to acquire the GICS image and no other accessory apparatus is required. Environmental interference is reduced by a luminance compensation constancy algorithm. Experimental implementation of the method revealed high efficiency and sensitivity, which makes it a powerful potential tool for medical POC diagnostics.

Table 6

4. Conclusion

A novel rapid quantitative LH detection method using a GICS was developed. In the proposed method, a smartphone is used to obtain an image of the GICS, with a luminance compensation method used to reduce environmental interference and increase the detection consistency. Canny edge detection and mathematical morphology are used to obtain the GICS reading window, and a fuzzy c-means clustering algorithm is used to extract the test and control lines of the GICS. The effectiveness of the method was demonstrated by using it to detect standard LH samples and clinical samples. The experimental results revealed a correlation coefficient of 0.996for standard LH concentrations of 1.0–83.3 mIU/mL, and a limit detection of 2.0 mIU/mL (S/N = 3). The proposed method was also shown to retain its effectiveness for different types of smartphones and GICSs. Thirty-one clinical serum samples were further used to compare the results of the proposed method with those obtained by a large ECLIA instrument, with a correlation coefficient of 0.974 observed. The utilization of the proposed method to track the LH level in the urine of a 35 year-old female volunteer during ovulation showed that the method could be used to obtain quantitative results within 15 min, with high efficiency and sensitivity. This indicates the applicability of the method for LH field testing.

Acknowledgements

Research sponsored by the NSFC (No. 61527815, 61673024, 61771452 and 61471342), the National Key Research and Development Program (No. 2017YFA0205902）and the key research program of frontier sciences, Chinese Academy of Sciences (QYZDJ-SSW-SYS015).

ACCEPTED MANUSCRIPT

References

[1] Jiang X. L., Dias J.A., He X.L., 2014. Structural biology of glycoprotein hormones and their receptors: Insights to signaling, Mol Cell Endocrinol, 382 (1): 424–451.

[2] Baiasch J, bregues F., 2003. Pregnancy after administration of high dose recombinant human LH alone to support final stages of follicular maturation in a woman with long-standing hypogonadotrophic hypogonadism. Reprod Biomed Online. 6(4):427-431

[3] Ruvoio G, Bosco L, Pane A, et al. 2007. Lower apoptosis rate in human cumulus cells after administration of recombinant luteinizing hormone to women undergoing ovarian stimulation for in vitro fertilization procedures. Fertil Steri , 87(3): 542 -546.

[4] Ferraretti A P, Gianaroii L, Magii M C, et al., 2004. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques [J].Feritl Steril, 82(6):1521 -1526.

[5] Muhammad S, Abdel-Nasser K, Muhammad D. 2015. Designs, formats and applications of lateral flow assay: A literature review. J Saudi Chem Soc, 19: 689 – 705

[6] Zeev Shoham, M.D. 2002. The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. Fertil Steril, 77(6):1170 -1177.

[7] Makoto U., Kazuo Y., Kaoru N., Satoshi T., Hiroshi D., Yasunori N., Kazutaka O., and Toshiaki O., 1992. Subunit-specific sulphation of oligosaccharides relating to chargeheterogeneity in porcine lutrophin isoforms, Glycobiology, 2 (3): 225–231

[8] Endo N., Tanaka T.. 2015. Effect of intermittent administration of neurokinin 3 receptor agonist on luteinizing hormone secretion, estrus, and ovulation in feed-restricted goats. Small Ruminant Res, 127: 50-57

[9] Shoham z. 2002. The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. Fertil Steril, 77(6):1170-1177

[10]Balasch J, Fábregues F. 2002. Is luteinizing hormone needed for optimal ovulation induction. Curr Opin Obstet Gynecol. 14(3): 265-74.

[11] Orvieto R, Meltcer S, Liberty G, et al. 2012. Does day-3 LH/FSH ratio influence in vitro fertilization outcome in PCOS patients undergoing controlled ovarian hyperstimulation with different GnRHanalogue. Gynecol Endocrinol, 28(6): 422-424.

[12] Pezzuto A, Ferrari B, Coppola F, et al. 2010. LH supplementation in down-regulated women undergoing assisted reproduction with baseline low serum LH levels. Gynecol Endocrinol. 26(2): 118-24.

[13] O'dea L, O'brien F, Currie K, et al. 2008. Follicular development induced by recombinant luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in anovulatory women with LH and FSH deficiency: evidence of a threshold effect. Curr Med Res Opin, 24(10): 2785-2793.

[14] Bogdanove EM, Gay VL. 1969. Studies on the disappearance of LH and FSH in the rat: a quantitative approach to adenohypophysial secretory kinetics. Endocrinol, 84:1118–31.

[15] Li J. F., Zou M. Q., Chen Y., Xue Q., Zhang F., Li B. B., Wang Y. F., Qi X. H., Yang Y., 2013. Gold immunochromatographic strips for enhanced detection of Avian influenza and Newcastle disease virusesOriginal, Analytica Chimica Acta, 782: 54-58

ACCEPTED MANUSCRIPT

[16] Liu B., Wang L. L., Tong B., Zhang Y., Wei S., Pan M. F., 2016. Wang S., Development and comparison of immunochromatographic strips with three nanomaterial labels: Colloidal gold, nanogold-polyaniline-nanogold microspheres (GPGs) and colloidal carbon for visual detection of salbutamol, Biosens. Bioelectron., 85(15): 337-342

[17] Jiang H.Y., Du M., Ke D.Z., 2012. A Rapid Quantitative Determination Method of AFP Concentration with Gold Immunochromatographic Strip, Journal of Computers, 7(12): 2868-2875

[18] Sumonphan E., Auephanwiriyakul S., Theera-Umpon N., 2008. Interpretation of nevirapine concentration from immunochromatographic strip test using support vector regression. Proceedings of 2008 IEEE International Conference on Mechatronics and Automation. 8:633-637

[19] Wu C. C., Lin H. Y., Wang C. P., Lu L. F., Yu T. H., Hung W. C., Houng J. Y., Chung F. M., Lee Y. J., Hu J. J., 2015. Evaluation of a rapid quantitative determination method of PSA concentration with gold immunochromatographic strips. BMC Urology, 15: 109-116

[20] Iftak Hussaina, Munmun Dasa, Kamal Uddin Ahamadb, Pabitra Nath, 2017. Water salinity detection using a smartphone, Sens Actuators B Chem., 239 : 1042–1050

[21] Yang X., Y. Wang, W. Liu, Y. Zhang, F. Zheng, S. Wang, et al., 2016. A portable system for on-site quantification of formaldehyde in air based on G-quadruplex halves coupled with a smart phone reader. Biosens. Bioelectron. 75:48–54.

[22] Jia M.Y., Wu Q. S., Li H., Zhang Y., Guan Y. F., Feng L., 2015. The calibration of cellphone camera-based colorimetric sensor array and its application in the determination of glucose in urine. Biosens. Bioelectron., 74: 1029-1037.

[23] Liu J.T., Kong Zh., Wang Y., Fan Y., Luo J.P., Xu S.W., Jin H.Y., Cai X.X., 2017. A Rapid Quantitative Determination Method of Luteinizing Hormone with Gold Immunochromatographic Strip, IEEE Engineering on medicine and biology society, 17: 17-20.

[24] Canny J., 1986. A computational approach to edge detection. IEEE Transactions on Pattern Analysis and Machine Intelli, 8: 679-698.

[25] Zhou X., Shen Q., Liu L., 2008. An improved FCM method based on particle swarm optimization for color image segmentation. Journal of Computational Information Systems, 4(5): 2097-2102.

[26] Han J., Ma K. K., 2002. Fuzzy Color Histogram and Its Use in Color Image Retrieval. IEEE Transactions on Image Processing, 11: 944-952.

[27] Gijsenij A., Gevers T., 2011. Color Constancy using Natural Image Statistics and Scene Semantics. IEEE Transactions on Pattern Analysis and Machine Intelligence, 33 (4): 687-698.

[28] Weijer V. D., Joost, Gevers T., Gijsenij A., 2007. Edge-based color constancy. IEEE Transactions on Image Processing, 16: 2207-2214.

[29] David J.Y., Park T.S., Yoon J.Y. 2013. Cell-phone-based measurement of TSH using Mie scatter optimized lateral flow assays. Biosens. Bioelectron., 40: 180–185.

[30] P. Preechaburana, S. Macken, A. Suska, D. Filippini. 2011. HDR imaging evaluation of a NT-proBNP test with a mobile phone. Biosens. Bioelectron., 26(5): 2107-2113

ACCEPTED MANUSCRIPT

Biography

Xinxia Cai received her PhD degree in Bioelectronics,Electronics &Electrical Engineering from Glasgow University in UK. Xinxia Cai is a professor of biosensors at Institute of Electronics, Chinese Academy of Sciences, while she is a chief scientist of the national major scientific research project on nano-science, a winner of national science fund for distinguished young scholars. In 1995, she held research position on biosensors at Tokyo Institute of Technology in Japan. She has published in excess of 150 journal and conference papers and has received 25 invention patents.

Hongyan Jin received her PhD degree in obstetrics and gynecology from Fudan University in 2005. Dr. Hongyan Jin is an associate research fellow at Obstetrics and Gynecology Department, Peking University First Hospital. She has published in excess of 30 journal and conference papers and has received the Young Scholar Award of American Association for cancer research (AACR) three times. Her current research interests include reproductive endocrinology and diagnosis of ovarian diseases.

Juntao Liu received M.E. degree in Instrument Science and Technology and B.E. degree in Precision Instrument and Machinery from Beihang University. In 2009, he received PhD degree in Physical Electronics from Institute of Electronics, Chinese Academy of Sciences. His current research interests include biomedical detection technology and mobile medical technology. He has published in excess of 50 journal and conference papers and has received 10 invention patents.

Jinping Luo completed her PhD in Institute of Electronics, Chinese Academy of Sciences. And work in this institute now. she is good at biochemical detection and biosensor fabrication.

Shengwei Xu completed his B.E. in Beihang University, now is a PhD student in Institute of Electronics, Chinese Academy of Sciences in this institute now. He is good at biomedical Systems.

Yang Wang is a PhD student in Institute of Electronics, Chinese Academy of Sciences.

Fan Yan is a PhD student in Institute of Electronics, Chinese Academy of Sciences.

Zhuang Kong is a MS student in Institute of Electronics, Chinese Academy of Sciences.

ACCEPTED MANUSCRIPT

FigureCaptions

Figure 1. Schematic of the general sandwich form of a GICS

Figure 2. Procedure of the proposed method: (A) Sample detection using a GICS, (B) photographing of the GICS 10 min later using a smartphone, (C) image processing to determine the LH level, and (D) tracking the LH level

Figure 3. Time variation of the GCIS luminance

Figure 4. Relationship between the concentration of the LH and the relative luminance: (A)Linear curve of the proposed method with luminance compensation, and (B)linear curve of the proposed method without luminance compensation

Figure 5. Selectivity of the proposed method

Figure 6. Correlation between the ECLIA method and the proposed method

Figure 7. Variation of the LH level in the urine of a female volunteer during ovulation

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure

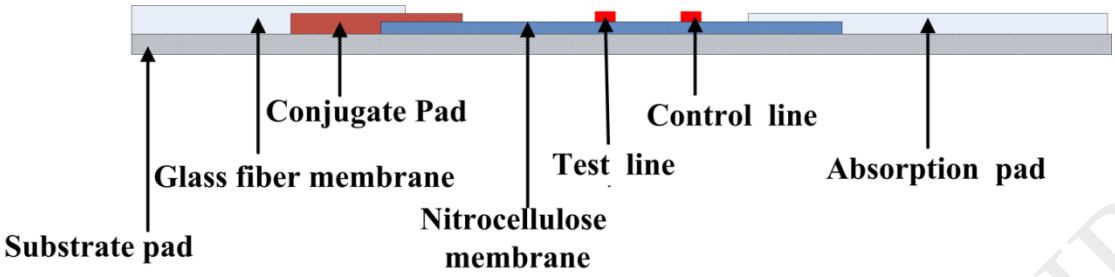


Figure 1. Schematic of the general sandwich form of a GICS

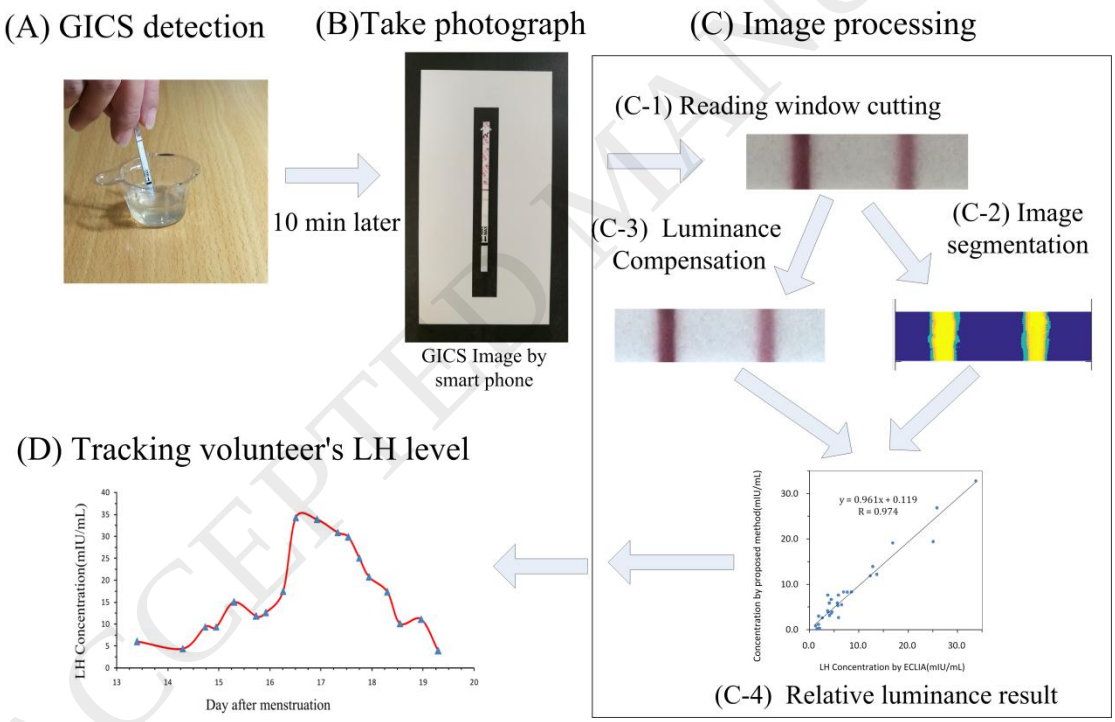


Figure 2. Procedure of the proposed method: (A) Sample detection using a GICS, (B) photographing of the GICS 10 min later using a smart phone, (C) image processing to determine the LH level, and (D) tracking the LH level

ACCEPTED MANUSCRIPT

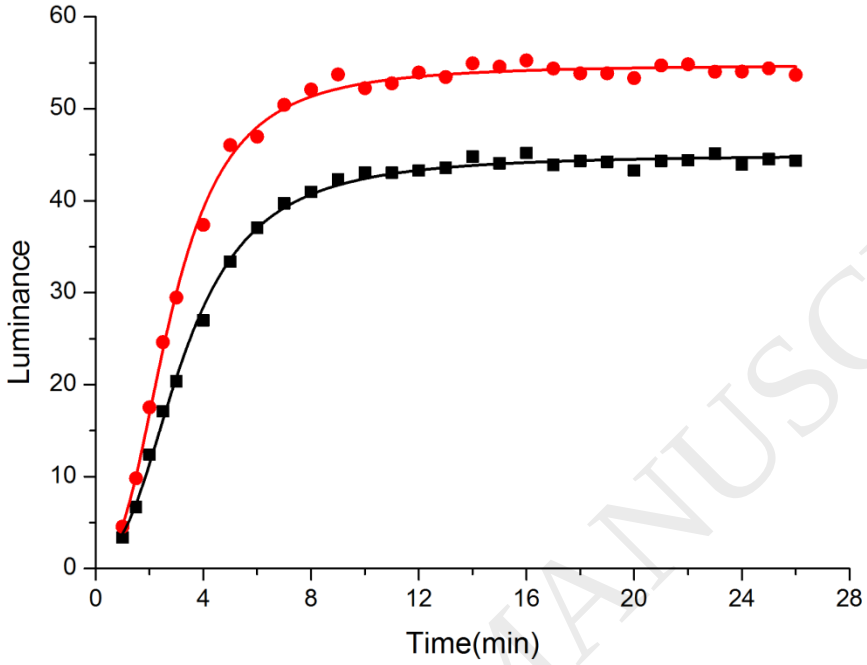


Figure 3. Time variation of the GCIS luminance

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

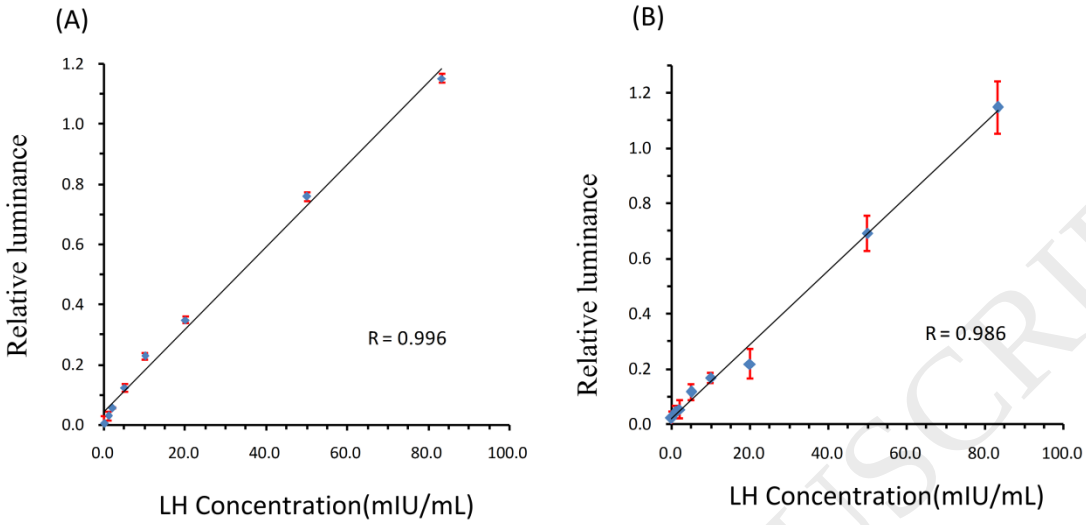


Figure 4. Relationship between the concentration of the LH and the relative luminance: (A) Linear curve of the proposed method with luminance compensation, and (B) linear curve of the proposed method without luminancecompensation

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

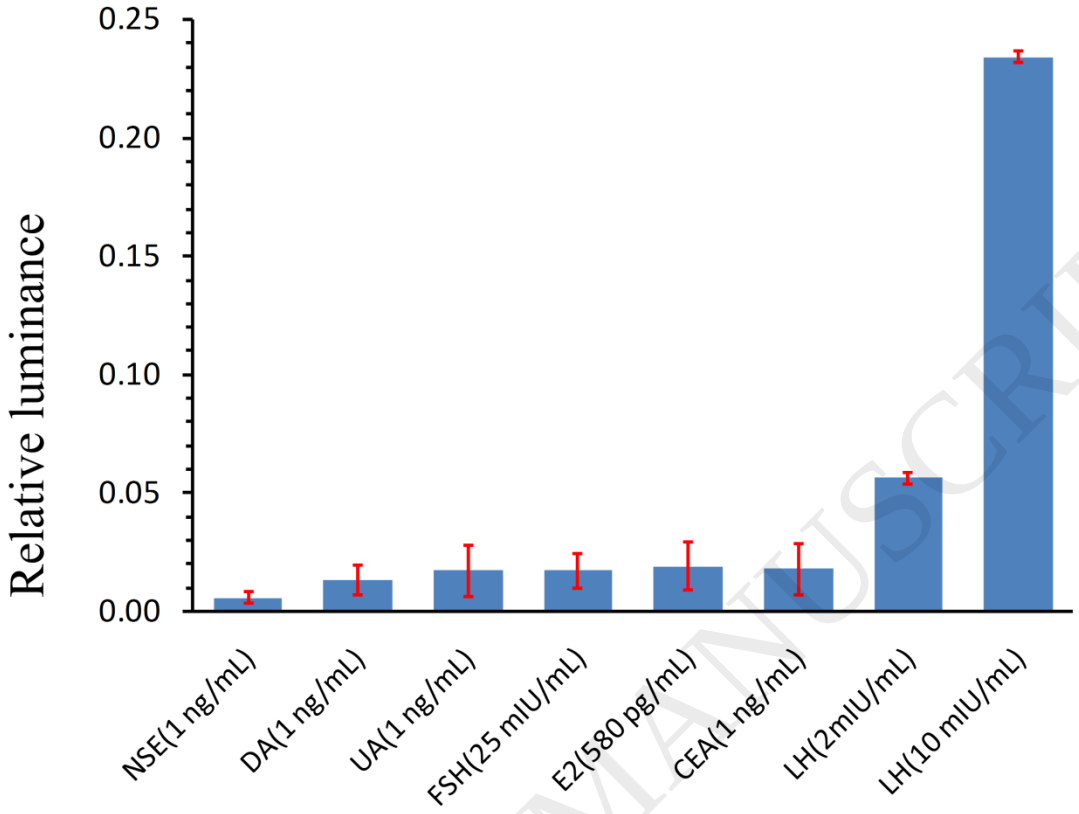


Figure 5. Selectivity of the proposed method

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

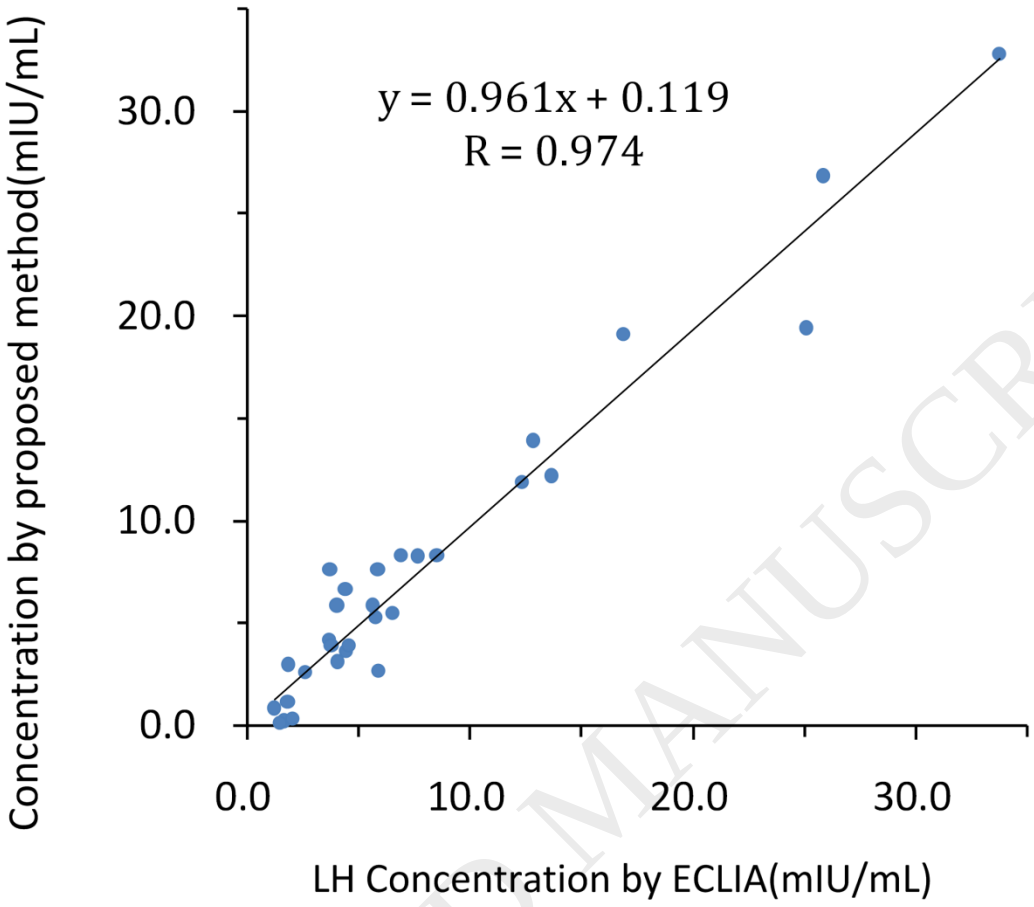


Figure6. Correlation between the ECLIA method and the proposed method

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

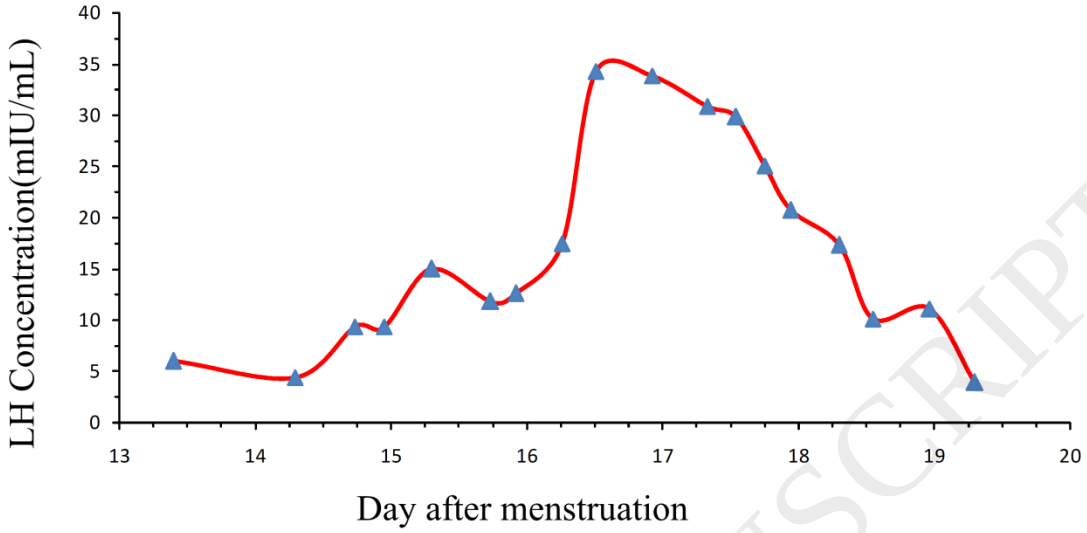


Figure 7. Variation of the LH level in the urine of a female volunteer during ovulation.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1. Relative standard deviation of the two methods

Environment	A		B	
	Relative luminance	RSD	Relative luminance	RSD
Bright environment	0.679	4.26%	0.733	8.04%
Dim environment	0.686		0.781	
Flash-lamp environment	0.733		0.856	

Table 2. Repeatability of proposed method

No.	CV of Each GICS	CV of All GICSs
1	4.26%	7.45%
2	0.69%	
3	5.62%	
4	6.06%	
5	5.30%	

ACCEPTED MANUSCRIPT

Table 3. Reproducibility between different types of smart phones

NO.	Concentration (mIU/mL)	Relative luminance			RSD
		A	B	C	
1	2.5	0.117	0.120	0.135	7.78%
2	10.0	0.282	0.256	0.298	7.72%
3	25.0	0.595	0.582	0.611	2.37%
4	50.0	0.883	0.880	0.908	1.71%

Table 4. Reproducibility of the proposed method using different GICSs

No.	Standard concentration (mIU/mL)	GICS A		GICS B	
		Proposed method (mIU/mL)	Relative error (%)	Proposed method (mIU/mL)	Relative error (%)
1	5.0	4.69	6.20%	4.56	8.80%
2	10.0	10.48	-4.80%	10.84	-8.40%
3	20.0	20.47	-2.35%	21.19	-5.95%
4	25.0	25.10	-0.40%	25.5	-2.00%
5	30.0	27.62	7.93%	28.32	5.60%

ACCEPTED MANUSCRIPT

Table 5 Relative standard errors for urine assays

No.	Proposed method (mIU/mL)	ELISA (mIU/mL)	Relative error (%)
1	42.18	42.55	-0.88%
2	32.21	32.25	-0.12%
3	24.57	26.06	-5.72%
4	14.61	13.70	6.63%
5	6.97	6.58	5.84%

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Author	Detection method	Detection instrument	Sample	Detection object
Jiang [17]	Reflective optical detection	Reflective fiber optical module	Serum	AFP
Sumonphan [18]	Image processing	Commercial scanner	Standard solution	HIV
Wu [19]	Image processing	Optical image reader	Serum	PSA
David [29]	Image processing	Smartphone-based fiber optical module	Serum	TSH
Preechaburana [30]	Image processing	Smartphone	Standard serum	NT-proBNP
Present work	Image processing	Smartphone	Serum ,	LH

Table 6. Comparison of different quantitative detection methods

ACCEPTED MANUSCRIPT