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A smartphone-based rapid quantitative detection platform for lateral flow strip of human chorionic gonadotropin with optimized image algorithm

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ABSTRACT

Colloidal gold immunochromatographic test strip has been widely used as a rapid, simple and low-cost correct detection technology. However, its detection is often qualitative or semi-quantitative, which limits its clinical application to some extent. Herein, a portable test strip quantitative detection device based on smartphone to detect human chorionic gonadotropin (HCG) is developed. In experiment, a colloidal gold HCG detection strip based on antigen antibody immune response is constructed, and the quantitative results of three different image processing methods on the same strip detection are compared, including the threshold processing algorithm based on location information, the RGB color component extraction algorithm and the grayscale projection value processing algorithm, the results show that the last algorithm can realize the best recognition of the region of interest of strip. The mobile phone application software (App) based on this design shows that the detection limit of constructed colloidal gold HCG strip is 3 ng/mL with a linear range of 6–300 ng/mL. The detection result of real urine sample is consistent with the spiked concentration (R^2 = 0.988), indicating that the concentration of HCG can be accurately measured in urine with this method, presenting the potential for instant diagnosis.

1. Introduction

Human chorionic gonadotropin (HCG) is a glycoprotein hormone containing alpha (α-HCG) and beta subunits (β-HCG) secreted by placental syncytiotrophoblast [1,2]. The HCG content in blood and urine of pregnant female will increase hundreds of times after a certain period of pregnancy, which is one of the important hormones to maintain pregnancy [3,4]. At present, the early pregnancy test strip based on colloidal gold immunochromatography technology has been a popular HCG detection method, which can easily allow user to judge whether she is pregnant. The colloidal gold immunochromatography as a new type of immunoassay method developed in the early 1980s [5]. It combines the characteristics of immunoassay and chromatography technology, presenting the advantages of rapidity, simplicity, low cost, short operation time, and can be used for instant detection and on-site screening, ect [6–12]. It fully meets the requirements of early screening and detection for specific target. The early pregnancy test strip to detect HCG is based on a highly specific antigen–antibody immune response and capillary siphonage of test strip [13].

However, HCG level may vary significantly between individuals or even each pregnancy of the same person. In general, HCG level follows a typical range value [14], HCG level lower than 2 ng/mL is generally considered to be negative and unpregnant [15], and higher than 10 ng/mL can be confirmed as positive pregnancy [16]. At present, most early pregnancy test strips can achieve qualitative detection of HCG above 10 ng/mL. However, when the HCG level begins to rise in the early stage of pregnancy, the use of early pregnancy test strips can easily cause missed judgment. Therefore, the quantitative detection of low-level HCG is of help for early confirmation of pregnancy. At the same time, HCG in some cancer patients with malignant tumors will also be higher than the normal level [17], while HCG level will be lower than the normal level in ectopic pregnancy and threatened abortion [18]. In addition, in the presence of one or more mature follicles, the administration of HCG can trigger ovulation [19]. Since ovulation occurs between 38 and 40 h after HCG injection, the quantitative detection of HCG during this period is important to guide intrauterine insemination

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and fertility treatment. All in all, the quantitative detection of HCG is of importance in clinical practice. At present, the quantitative detection of HCG in hospital is mostly based on chemiluminescence method, it not only requires complex and expensive instruments, but also needs professional operation, which cannot meet the requirement in areas with limited resource [20,21]. Moreover, for patients who need regular and frequent testing, the cost of detection will also become a serious issue. Therefore, it is valuable to design a low-cost, easy-to-operate, and quantifiable portable detection device, which can not only detect HCG, but also could be applicable to other clinical markers, such as alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), etc.

At present, the portable detection technology based on smartphone has become a hot spot in the field of biosensor detection [22–26]. Due to smartphone has a large internal memory, high-quality camera and open source operating system, its microprocessor can easily achieve image acquisition, information processing, and result quantization. However, the test strip can only provide qualitative test result while the quantitative test is urgently needed, the smartphone as the quantitative analysis tool of test strip has also become the focus of researchers. This application can not only greatly reduce the development cost, but also be more conducive to the promotion and application of test strip. For example, Park et al established a highly pathogenic H5N1 virus fluorescence diagnostic test strip based on smartphone, which could provide high sensitivity measurement results within 15 min [27]; Roda et al developed a lateral flow immunochromatography technique based on chemiluminescence to detect salivary cortisol [28]; Cui et al developed a dual-modal imaging system based on smartphone for quantitative detection of HCG and carcinoembryonic antigen [29]; Yoon et al designed a smartphone-based device for quantitative measurement of thyroid stimulating hormone using an optimized Rayleigh/Mie scattering detection method [30].

Some papers had been recently published on the subject about using image processing methods to achieve quantization. Yu et al used a smartphone to image the test area on strip with ImageJ and Matlab processing [31]. Jung et al evaluated the analytes concentration by peak area and intensity with the Matlab curve-fitting toolbox as well as an eighth-order polynomial function model [32]. Ruppert et al used a smartphone as a reading system and adopted statistical software R and ImageJ to perform image processing [33]. Di Nardo et al calculated the number of red pixels on the test line by RGB data evaluation of the strip image [34]. Qin et al concluded the advantages and shortages of different algorithms, which were middle or advanced algorithms in image processing, thus demanding professional programmer with excellent programming and mathematical modeling ability [35].

In this paper, a HCG test strip was constructed, which used the colloidal gold (AuNP) labeled HCG β subunit monoclonal antibody (Anti-β-HCG) as the gold-labeled probe (Anti-β-HCG-AuNP) to fix on the gold-label pad of test strip. At the same time, the monoclonal antibody of the α subunit (Anti-α-HCG) and the secondary antibody (goat anti-mouse polyclonal antibody IgG) were coated on test (T) line and control (C) line, respectively. When HCG was present in the test sample, the gold-labeled probe could form a sandwich structure through HCG with the antibody on T line, thus fixing the AuNP there for color development. After that, smartphone was used to take the image of test strip on the designed portable HCG detection device, and HCG in the actual urine sample was successfully measured by designed application software (App). For image processing methods to achieve quantization, this paper mainly used the gray-scale projection value algorithm to process rather than polynomial curve fitting, the background signal could be almost zero, and the flat response of control and test time could be obtained. The peak value calculation was used to estimate the concentration of the detection line instead of the peak area. In brief, this portable HCG detection device has an important reference value for simple, fast and efficient HCG quantitative detection.

2. Materials and methods

2.1. Apparatus

HM3030 XYZ platform dispenser (Shanghai Kinbio Technology, China) and ZQ2000 programmable strip cutter (Shanghai Kinbio Technology, China) were used to prepare test strips; UV-2550 spectrophotometer (Shimadzu, Japan) and HT7700 transmission electron microscope (Hitachi, Japan) for the characterization of gold nanoparticle and gold-labeled probe; other equipment used included a 5424 centrifuge (Eppendorf, Germany), DHG-90304 electrothermal thermostatic blast drying oven (Wuhan Yihengsujing, China) and ultrapure water meter (Millipore-Q, USA). Matlab R2018b software was used to image preprocessing. The experimental results were recorded, processed and displayed by smartphone Honor 10 (Huawei Technology, China).

2.2. Reagents and materials

Chloroauric acid ($HAuCl_4{\cdot}4H_2O$), trisodium citrate dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$), sodium chloride (NaCl), potassium carbonate (K_2CO_3), Tween 20, sucrose, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), and hydrochloric acid (HCl) were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China); bovine serum albumin (BSA), human HCG antigen, mouse anti-human α-HCG monoclonal antibody (Anti-α-HCG), mouse anti-human β-HCG monoclonal antibody (Anti-β-HCG), goat anti-mouse polyclonal antibody (IgG), Alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA) and thrombin were purchased from Shanghai Lingchao Biotechnology Co., Ltd. (Shanghai, China); CN140 nitrocellulose membrane (300 × 25 mm) was purchased from Sartorius (Gottingen, Germany); CB06 glass fiber membrane (300 × 200 mm), SX42 absorbent paper (300 × 200 mm) and SMNF31-25 PVC plastic adhesive backing (300 × 60 mm) were purchased from Shanghai Kinbio Biological Co., Ltd. (Shanghai, China). The water used in the laboratory was ultrapure water (18.2 MΩ·cm).

2.3. Preparation of colloidal gold

According to the reported method [36] with slight modification to prepare colloidal gold (AuNP) with an average diameter of 20 nm. Firstly, 100 mL $HAuCl_4$ (0.01%) aqueous solution was prepared and injected into a round bottom flask equipped with a reflux condensing device, and heated to boiling. Then 2 mL $C_6H_5Na_3O_7{\cdot}2H_2O$ (1%) was added to the flask quickly, and continued heating and refluxing under strong stirring. The color of solution gradually changed from colorless and transparent to black and finally became dark red, and then continued to stir and heated for 10 min to prepare 20 nm colloidal gold. After cooling to room temperature, it was filtered with 0.22 μm nylon membrane to remove large particle and stored in a refrigerator at 4 °C for later use.

2.4. Preparation of gold-labeled probe Anti-β-HCG-AuNP

The gold-labeled β-HCG antibody was prepared according to the reference reported previously [37]. Firstly, the β-HCG antibody was diluted with a borate buffer of 50 mM, next added to 1 mL AuNP (pH 8.2), and shaken at room temperature for 30 min, and then added to a borate buffer with a final concentration of 10 mM. 2 M NaCl was added twice separately to make the final concentration of 100 mM. After centrifugation for three times, the Anti-β-HCG-AuNP complex was re-suspended and concentrated to 1/20 of its original volume (the re-suspension contained 50 mM borate borax saline buffer, 5% sucrose, 1% BSA and 0.25% Tween-20, pH 8.2), and stored it in a refrigerator at

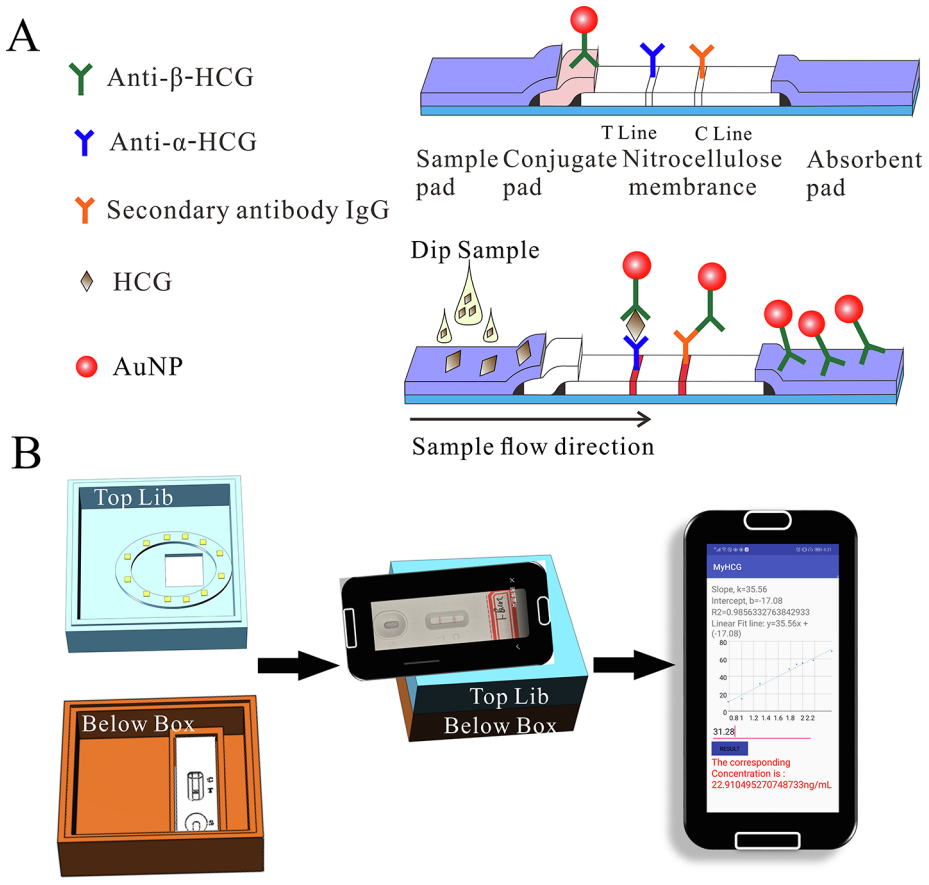

Fig. 1. Construction of HCG test strip (A) and 3D printed cassette for quantitative HCG signal by smartphone (B).

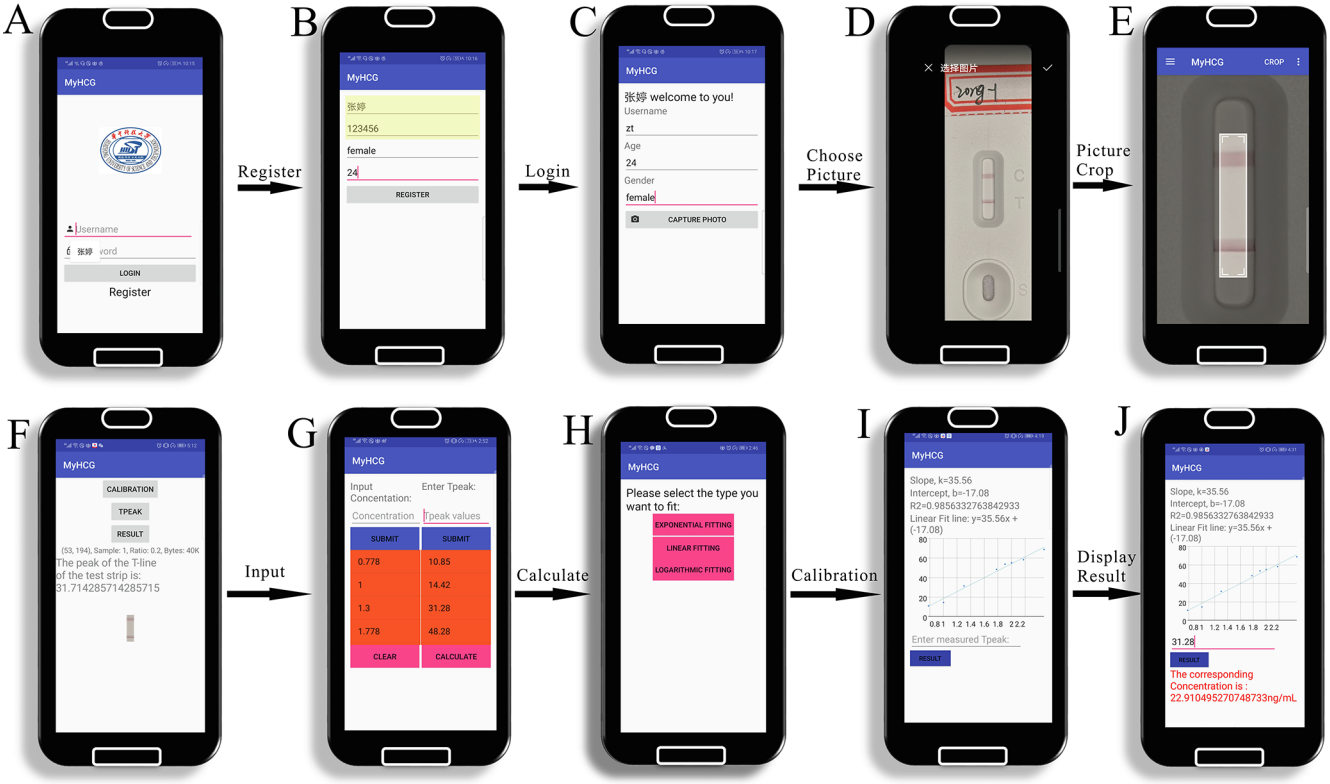

Fig. 2. Screenshots of My HCG in different stages of image analysis, user login registration interfaces (A, B); image acquisition interfaces (C, D); image crop interface (E); image processing interfaces (F ~ J).

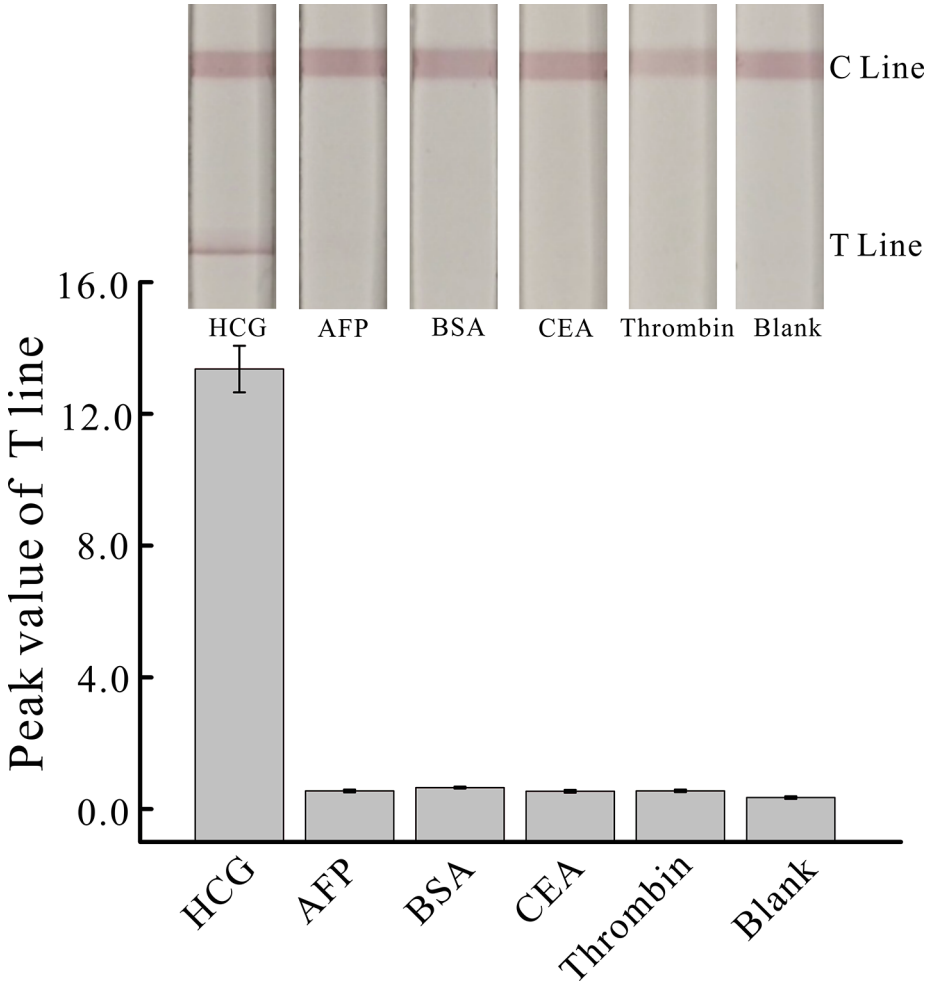

Fig. 3. Specificity of the test strip for HCG detection; HCG: 10 ng/mL, other substances: 1000 ng/mL.

4 °C until use. The gold-labeled probe was characterized by UV–vis absorption spectrum and TEM (Fig. S1), and the preparation of gold-labeled probe was optimized (Fig. S2).

2.5. Preparation of chromatography test strip

The HCG chromatography test strip was composed of four parts: sample pad, gold- labeled conjugate pad, nitrocellulose membrane and absorbent pad (Fig. 1A). The sample pad and gold-labeled conjugate pad were soaked in buffer solution (1% BSA, 5% sucrose and 0.5% Tween-20, 10 mM PB buffer solution, pH 7.4) for 30 min according to the reference [38], and then dried at 37 °C for 1 h and stored for future use. Gold-labeled probe (Anti-β-HCG-AuNP) was sprayed on the gold-labeled binding pad by XYZ film dispenser, the concentration of probe was 5 μL/cm. And the gold-labeled conjugate pad was dried at 37° C for 1 h after the spraying was completed. The α-HCG antibody and IgG were diluted to the corresponding concentration with coating solution (0.9% NaCl, 0.3% sucrose and 0.2% BSA, 10 mM PB buffer, pH 7.4), the T line and C line (5 mm interval) were formed by spraying 1 μL/cm them respectively on the nitrocellulose membrane by XYZ film dispenser, and then dried at room temperature for 1 h. Finally, the sample pad, gold-label conjugate pad, nitrocellulose membrane and absorbent pad were adhered to the corresponding position of PVC plastic adhesive backing, and the overlap between each two parts was 1–2 mm, so as to ensure good contact between the parts in the detection process. Using a programmable strip cutter to cut a 3.0 mm width test strip and then kept for use.

2.6. Design of cassette for chromatography strip

Because the quantitative detection of test strip in this work was based on the color density of the captured image, the color interpretation was susceptible to be interfered by the external environment. Therefore, in order to avoid serious interference of the external ambient light on test strip detection, we designed and used a 3D printing technology to make a cassette (Fig. 1B), which contained two parts of top lib and below box. The main function of top lib was to ensure that the camera of the mobile phone could aim at the test strip and capture photos. The main function of below box was to fix the position of test strip and ensure the uniformity of the photo. There was a test strip slot for fixing strip, and also contained an independent light source, while a ring LED light was selected, and placed on the top of the cassette to allow the test strip to receive light uniformly. A simple switch circuit device was used to control the light source and provided power for the light source unit. The battery could be selected as the power source, or be directly powered through the external USB interface on the circuit board. A camera window was open on the top of cassette as a lighting camera window of smartphone camera. The image could be collected by aligning the camera at the detection window, which could be also adapted to various smartphone types. The specific dimensions of the top lib and below box were shown in Fig. S3.

2.7. The development of smartphone application software (App)

The mobile App that analyzed images and provided users with quantitative results was compiled and debugged in Android Studio

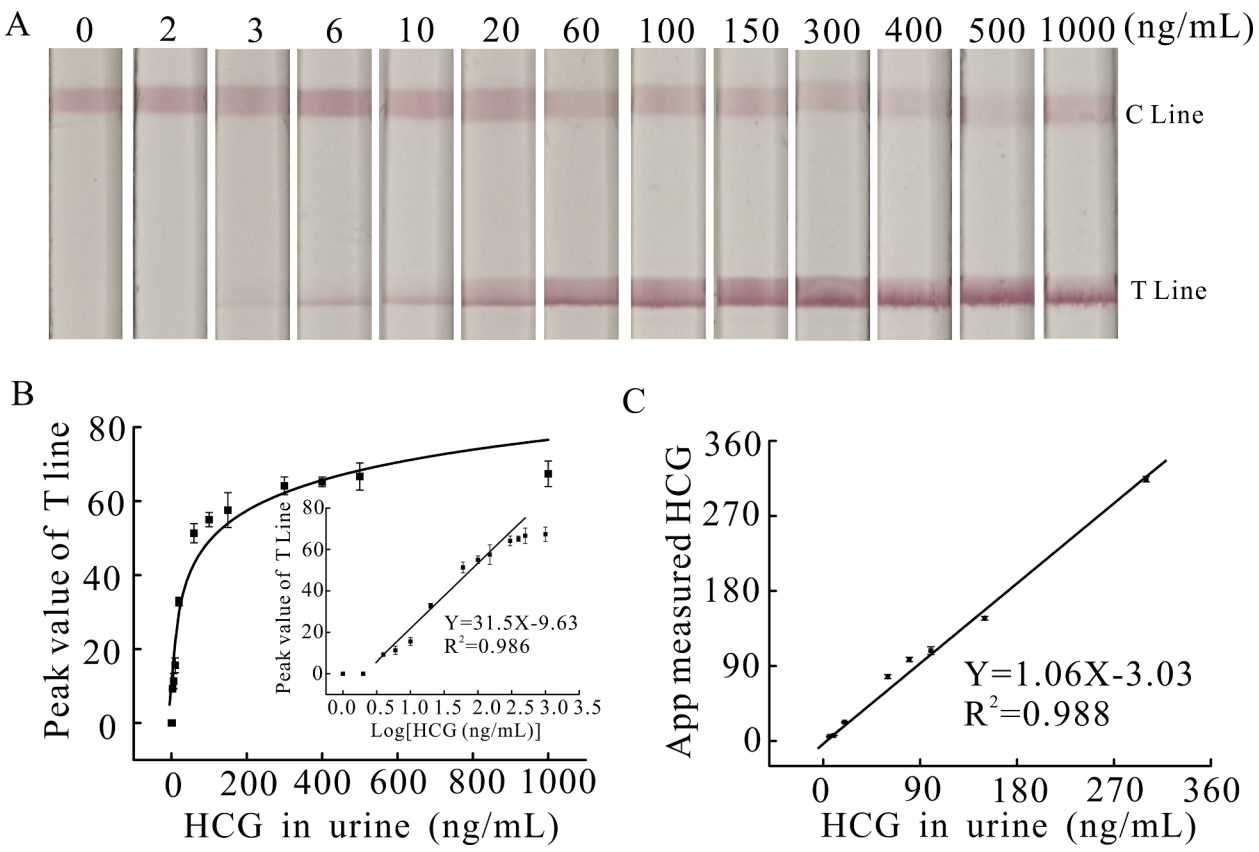

Fig. 4. The testing results of strip for HCG with different concentrations (A) and relationship between gray intensity and concentration (B); the relationship between smartphone App's test results and actual spiked samples (C).

software using Java programming language. The quantification of HCG signal was based on the change of T line signal with the change of HCG concentration, which was realized by processing the signal intensity of T line.

3. Results and discussion

3.1. The sensing strategy of the test strip

The HCG test strip was constructed by colloidal gold chromatography (Fig. 1A), the basic step of which used to ensure the gold-labeled probe (Anti-β-HCG-AuNP) on the gold-labeled conjugate pad, and at the same time the α-HCG antibody and secondary antibody IgG were coated on the T and C lines, respectively. When HCG was added, it would first bind to the gold-labeled antibody on the gold-labeled pad, and the complex would continue to move to T line of test strip through capillary action, with the fixed α-HCG monoclonal antibody to form a sandwich structure, while the excess gold-labeled probe continued to be chromatographically combined with the secondary antibody IgG on C line, thus fixing the gold particles on T and C lines to show color. When no HCG in the sample, the gold-labeled probe could not form a sandwich structure with α-HCG monoclonal antibody on T line. At this time, the gold-labeled antibody could only bind to the secondary antibody IgG on C line. In this case, the test strip only showed color at C line.

3.2. Image processing algorithm for test strip quantitative result

Since the test strip could only provide qualitative test result (positive or negative) or semi-quantitative information about the concentration of the analyte, it could not meet the simple, fast and efficient quantification requirements. Therefore, smartphone was used to complete the quantification work. Because the T line signal intensity of test strip was proportional to the content of substance to be detected, this enabled us to quantify its content by analyzing T line signal. In order to quantitative analysis, three different image algorithms were used to compare the quantitative results of T line and C line images of the same test strip.

3.2.1. Threshold processing based on location information

The test strip could be analogized to an M × N numerical matrix in the process of image quantification, and the T and C lines could be compared to the sub-matrices in the entire image matrix (Fig. S4A). Each element in the matrix was an image unit. The test strip was actually a rectangle of length (L) × width (W). The L was analogous to M in the matrix while W to N. The length L of test strip could be represented by Eq. (1), then the image unit of test strip in unit length was expressed by Eq. (2). Supposed the distance from C line to the top of test strip was constant as L1, and the width of C line of test strip was L2 (a constant when the experimental conditions were consistent), and the exact coordinate position of C line could be known by Eq. (3), and similarly, the Eq. (4) could get T line information. After determining the precise position information of C and T lines, according to the difference of grayscale intensity between C/T lines and background area, the threshold segmentation algorithm was used after converting the original image to grayscale image by Matlab software, and the binary image with only foreground and background was obtained, and the white of foreground represented C and T lines regions. After the regions of interest (ROI) of test strip was segmented, it was numerical multiplied with the original gray image to obtain the gray intensity values of C and T lines (Fig. S4A). The analysis of test results of a series of HCG standard solutions continued to be performed (Fig. S4B). By analyzing the relationship between the average gray level of T line region and HCG concentration (Fig. S4C), it could be found that there was a good linear relationship between the grayscale value and the logarithm of

analyte concentration (R^2 = 0.953).

$$L = L1 + L2 + L3 + L4 + L5 \quad (1)$$

$$Pixel_{unit\,distance} = M/L \quad (2)$$

$$C_{position} = [L1,\ L1 + L2] \times Pixel \quad (3)$$

$$T_{position} = [L1 + L2 + L3,\ L - L5] \times Pixel \quad (4)$$

3.2.2. Component extraction based on RGB color model

The RGB (red, green and blue) color model was derived from the abbreviation of the three primary colors [39]. And the colors of three channels of RGB could be superimposed by different proportions to produce 256 × 256 × 256 = 16777216 possible colors, which included almost all the colors that human vision could perceive. The difference of color between the background and positive test result was greatly different, which showed red at T line on the test strip. Therefore, it was possible to extract only the area of obvious red channel by processing RGB three components, so as to realize T line region recognition. Firstly, the R, G, and B components of the original image were extracted to obtain R1, G1 and B1 by Matlab software. It was found that Eq. (5) could obtain the region with a significant red channel (Fig. S5A). After obtaining the binary image (BI) containing only the foreground and background, further carried out image morphology processing and extraction of connected region, noise reduction, and then multiplied the original image by numerical multiplication to obtain the gray intensity values of T and C lines. Also after processing the series of detection results, the average grayscale of T line region was obtained (Fig. S5B). Analyzing the gray value and HCG concentration (Fig. S5C), it was found that the grayscale value and the logarithm of concentration also showed a good linear relationship (R^2 = 0.980).

$$BI = R1 > 1.06 \times G1 \,\&\, R1 > 1.09 \times B1 \quad (5)$$

where the BI is the binary image, R1, G1, and B1 separately represent the R, G, and B components of the original image.

3.2.3. Gray projection value processing based on test strip

Because C and T lines of test strip had a large difference in grayscale intensity with the background area, C and T lines could also be located and distinguished from the background area by grayscale projection. Using Matlab software to perform grayscale projection calculation on the original image, the average pixel intensity of each row a_i could be calculated, and a one-dimensional array $[a_0\ a_1\ a_2 \ldots\ a_i \ldots a_m]$ was obtained. Each element in the one-dimensional pattern was the line projection value of test strip. The obtained projection curve was further filtered and denoised by sliding window algorithm (Fig. S6). It was observed that the baseline of the projection curve was smooth and consistent, indicating that the background of test strip was basically the same (Fig. S7A). The two peaks were the C and T lines respectively, and they represented the gray intensities of C and T lines. It was found that for blank sample, only one peak of the C line appeared, and both the T line signal and the background signal were almost 0 (Fig. S8). In order to obtain quantitative information, Matlab software was also used to process a series of HCG standard solutions to obtain the corresponding peak intensities (Fig. S7B). It could be seen from the analysis of the peak and HCG concentration (Fig. S7C) that the logarithm of the peak and concentration had a good linear relationship (R^2 = 0.999).

3.3. Mobile application development

The above experimental results showed that all three image algorithms could quantitatively analyze detection results of test strip. The threshold processing algorithm based on position information was relatively simple, but it was necessary to determine the relative position of T line and C line in advance, so the consistency requirement of the test strip preparation was strict. The RGB channel extraction algorithm was the most direct method for extracting color channels, however, the relationship between the RGB three channels required continuous debugging to obtain the ROI. The method of processing the grayscale projection value based on the test strip was simple and intuitive, and had good stability and portability, so we ultimately chose it to develop a smartphone App "My HCG" to analyze the test strip image and provided users with HCG quantification results (Fig. 2). The program adopted the Java programming language and was compiled and debugged in Android Studio. The process of using the App was to first open a login and registration interface (Fig. 2A), required the user to register and then log in (Fig. 2B) to ensure user's information security. After that, user needed to obtain the image to be processed by clicking the "Load Image" button to activate the camera to take picture or directly capture picture from the mobile phone gallery (Fig. 2C, D). After obtaining test strip image, user could select the "Crop" button to crop the image (Fig. 2E), remove the invalid area and reduce the amount of processed data. Then the image processing button "TPeak" was selected to calculate the peak of T line (Fig. 2F), which was the quantized signal value of HCG concentration. After obtaining the T line peak value, one could select the "Calibration" button to manually enter the HCG concentration corresponding to the test strip image (Fig. 2G). After collecting images of a series of standard concentration HCG test strips and entering their concentrations, user could click "Calculate" to select the appropriate data type (exponential fitting, linear fitting or logarithmic fitting) to obtain the working curve (Fig. 2H). After finising the optimal fit curve (Fig. 2I), when user measured the unknown concentration of HCG, he could just take a photo of its test strip and process the image to get the peak value, clicked the button "Result" and the concentration of HCG could be displayed on the screen (Fig. 2J), providing the user with intuitive data result.

3.4. Specificity

After the App was programmed, the specificity of test strip was examined. In experiment, several common tumor markers and proteins such as AFP, CEA, BSA and thrombin were selected as interferents, and the concentration of them was 100 times of HCG concentration. Experiments showed that these proteins had almost no signal on the HCG test strip and therefore did not interfere the detection (Fig. 3). However, the test strip still produced a sensitive response to HCG at such low concentration, and a red visible band to the naked eyes appeared on T line, indicating that the prepared test strip had good specificity.

3.5. Quantification of test strip for actual sample detection

Above all, a smartphone was used to analyze the test strip results of actual samples. In experiment, 5 urine samples were collected randomly, mixed and diluted 10 times with 10 mM PBS buffer (pH 7.4), and then different concentrations of HCG standard solutions were added in the physiological range (2–1000 ng/mL). Each concentration was tested using strip for 5 parallels to obtain an average of peak of T line. The results showed as HCG concentration was increased, more HCG was captured on T line, and the corresponding gold-labeled probe was also increased, thereby gradually deepening the T line color (Fig. 4A). There was a good linear relationship between the peak of T line and the logarithm of the concentration from 6 to 300 ng/mL (R^2 = 0.986). After the HCG concentration was higher than 300 ng/mL, the peak increase of T line slowed down (Fig. 4B), indicating that the binding of gold-labeled probe to HCG tended to be saturated. The measured results of the mobile phone App were very consistent with the HCG content (Fig. 4C), indicating that the test strip biosensor had a

high accuracy (R^2 = 0.988) and could be used for HCG detection in actual sample. The detection limit of test strip was 3 ng/mL (S/N = 3), which was better than the detection limit of 10 ng/mL for commercial early pregnancy test strip. The above experimental results showed that the App could directly display accurate reading of HCG concentration in urine real time, thus having the potential for instant diagnosis.

4. Conclusion

In this work, we designed a portable detection device that could quantitatively analyze HCG colloidal gold test strip based on smartphone. It possessed the characteristics of low cost and easy operation. The testing result obtained by App was in good agreement with the actual content of HCG. The minimum detection limit was lower than the qualitative detection limit of commercial test strip, indicating that the App had good accuracy and could perform quantitative analysis of test strip.

CRediT authorship contribution statement

Ting Zhang: Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Hai-Bo Wang:** Data curation, Formal analysis, Methodology, Supervision, Validation. **Zi-Tao Zhong:** Data curation, Formal analysis, Supervision, Validation, Writing - review & editing. **Chao-Qing Li:** Supervision, Validation, Writing - review & editing. **Wei Chen:** Supervision, Validation, Writing - review & editing. **Bo Liu:** Supervision, Validation, Writing - review & editing. **Yuan-Di Zhao:** Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Funding acquisition, Resources, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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