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Smartphone-based rapid quantitative detection of serum creatinine

Performance validation and exploration of potential application in chronic kidney disease monitoring

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Abstract

Chronic kidney disease is a progressive condition, and serum creatinine (CR) levels are closely associated with the glomerular filtration rate, serving as a key indicator of renal function and disease progression. This study aimed to develop a smartphone-based colorimetric analysis system for the efficient and rapid quantification of serum CR and validate its performance to determine whether the method meets clinical testing standards. The CR standard solution was analyzed using a smartphone, and the R, G, and B values were plotted against the concentration. The precision, accuracy, detection limit, linear range, and clinically reportable range of the smartphone detection system were evaluated according to the National Committee for Clinical Laboratory Standards guidelines. Subsequently, 65 serum samples from healthy individuals and 26 serum samples from nephropathy patients were collected and tested using the smartphone system and an automated biochemical analyzer, respectively, to further validate the feasibility of the method. Among all the color channels, the G value showed the strongest correlation with CR concentration, and therefore was used to establish the standard curve. The validation of the assay system demonstrated that its precision and accuracy met clinical standards. The limit of blank, limit of detection, and limit of quantification were 29.95 μmol/L, 32.39 μmol/L, and 36.61 μmol/L, respectively. The linear range was 36.75 to 200.46 μmol/L, whereas the clinical reporting range spanned from 36.61 to 801.84 μmol/L. Furthermore, the results obtained from the 2 methods were statistically analyzed, revealing a strong correlation between the 2 sets of data. Smartphone-based serum CR testing meets clinical standards, and its portability and efficiency position it as a valuable tool for screening and monitoring chronic kidney disease.

Abbreviations: CKD = chronic kidney disease, CR = creatinine, GFR = glomerular filtration rate, LoB = limit of blank, LoD = limit of detection, LoQ = limit of quantification, NCCLS = National Committee for Clinical Laboratory Standards, RGB = *R* value, G value, and B value, TE = total error.

Keywords: chronic kidney disease, performance validation, serum creatinine, smartphone

1. Introduction

Chronic kidney disease (CKD) is a progressive decline in kidney function resulting from various factors and characterized by renal damage or reduced function lasting for at least 3 months. Clinically, CKD is primarily characterized by renal damage, which includes albuminuria, tubular injury, and a decline in glomerular filtration rate (GFR) that cannot be explained by other causes.[1,2] The prevention and treatment of CKD present significant challenges to global public health, as the incidence of the disease continues to rise. The causes of CKD are diverse, including diabetic nephropathy, hypertension, both primary and secondary glomerulonephritis, and tubulointerstitial disease.[3–5] In the early stages, patients may exhibit no discernible symptoms or may present with fatigue, nausea, vomiting, mild anemia, or edema. If left untreated, the disease progressively worsens and may eventually lead to severe renal failure.[6] At this stage, the GFR declines significantly, leading to the accumulation of metabolic waste products in the body. This leads to severe imbalances in the body's fluids and pH levels, jeopardizing the patient's life and health.[7,8] Therefore, early diagnosis and treatment are critical for improving the prognosis of CKD.

Serum creatinine (CR) is a widely used biomarker for assessing kidney function, as it is the end product of phosphocreatine metabolism in muscle tissue. Serum CR levels provide a reliable

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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estimate of GFR and are less influenced by factors such as diet, exercise, and hormonal changes, making it more specific than blood urea nitrogen (BUN). Therefore, serum CR levels can effectively monitor the progression of CKD.[9,10]

Currently, the primary clinical methods for determining serum CR levels include the Jaffe method, enzymatic methods, isotope dilution gas chromatography-mass spectrometry, and high-performance liquid chromatography.[11] However, each of these methods has certain limitations. For example, the Jaffe method exhibits low specificity, and substances such as vitamin C, glucose, and proteins can react with picric acid, forming red compounds and leading to false-positive results. Although the enzymatic method and high-performance liquid chromatography offer high specificity, they primarily rely on fully automated biochemical analyzers for detection. These analyzers are bulky, difficult to maintain, require professional operators, and have relatively long detection times, thus limiting their application in immediate detection scenarios. In recent years, emerging technologies, such as biosensing and microfluidic chips, have gradually been applied to the detection of serum CR. For instance, immunosensors that incorporate gold nanoparticles, boron-doped MXene, polyaniline, and anti-CR antibodies in an appropriate biocoupling reaction are capable of detecting CR within a linear dynamic range of 10 nM to 0.1 M.[12] SERS chips constructed using MOS and gold film-based SERS substrates have a detection limit as low as 5 μM.[13] Although these techniques enhance sensitivity to the nM level, their complex manufacturing processes and high equipment costs somewhat limit their scope of application. Against this backdrop, portable testing devices based on smartphones have gradually emerged as an important innovation in the medical field.[14–16] Leveraging their powerful computing and processing capabilities, highly integrated sensor modules, and diverse functions, smartphones offer significant technical advantages. The testing solution is not only cost-effective but also fast, user-friendly, and adaptable to various settings, significantly reducing the need for specialized skills from the operator. This technological innovation not only overcomes the spatial limitations of traditional laboratory testing but also paves the way for the intelligent transformation of medical services through real-time data transmission and cloud-based analysis. Currently, smartphone-based devices have successfully enabled the instant detection of key biochemical indicators in blood, such as serum protein, glucose, and albumin. This has significantly facilitated the development of intelligent, computerized healthcare services and provided patients with more accurate, personalized health management solutions.[17–19]

We hypothesize that a smartphone-based colorimetric system can accurately and efficiently measure serum CR levels for clinical testing. In this study, we strictly adhere to National Committee for Clinical Laboratory Standards (NCCLS) guidelines and conduct a comprehensive evaluation of the accuracy, precision, limit of detection (LoD), linear response range, and clinically applicable reporting range of this colorimetric system to ensure that its performance meets the stringent requirements of clinical practice. Additionally, we will collect real clinical samples to compare the performance of this new technology with that of traditional clinical biochemistry analyzers in serum CR detection and analyze whether the differences between the 2 are statistically significant using appropriate statistical methods. This study aims not only to validate the feasibility and accuracy of the smartphone-based colorimetric system for serum CR measurement, but also to offer a new, more convenient, and efficient solution for the early monitoring and management of CKD.

2. Methods

2.1. Ethical statement

The collection of clinical serum samples for this study was approved by the Ethics Committee of the First Hospital of Shanxi Medical University (license number: KYLL-2024-180). The Ethics Committee waived the requirement for informed consent, as the serum samples used in this study were residual samples from hospital physical or patient examinations, for which informed consent could not be obtained, Appendix 1, Supplemental Digital Content, https://links.lww.com/MD/O945.

2.2. Main instruments and reagents

The MB100-4A Microplate Thermostatic Oscillator was manufactured by Hangzhou Allsheng Instruments Co., Ltd., and the Portable Smartphone Microplate Analysis System was developed by the School of Bioengineering at Taiyuan University of Technology. The CR detection kit, based on the principle of creatine oxidase, was provided by Ningbo Ruiyuan Biotechnology Co. The assay kit consisted of 3 reagents: Reagent 1, Reagent 2, and the Calibrator. Reagent 1 contained creatinase (2000 U/L), creatine oxidase (6000 U/L), peroxidase (5000 U/L), and N-ethyl-N-(2-hydroxy-3-propanesulfonic)-3-methylaniline (TOOS) (0.4 mmol/L). Reagent 2 consisted of CR enzyme (250,000 U/L) and 4-aminoantipyrine (1.0 mmol/L). The calibrator was a solution containing CR at a concentration of 177 μmol/L, with the value obtained through certified reference measurement procedures and metrologically traceable. Human serum samples were collected at the First Hospital of Shanxi Medical University. Additionally, the hospital's Beckman Coulter AU5800 fully automated biochemical analyzer was used as a reference standard for comparative analysis.

2.3. Development of a smartphone microplate analysis system

2.3.1. Design of hardware components. As shown in Figure 1, the system consists of highly integrated hardware modules, with the core components including a bottom light source, a 96-well microtiter plate (300 μL capacity), a pull-out drawer, a large-aperture convex lens, and a photo hole.[20] Due to variations in the image sensors of different smartphone models, there may be some impact on imaging quality. However, this impact is minimal compared to fluctuations in lighting conditions during shooting. Therefore, imaging deviations caused by sensor variations can be effectively eliminated by uniformly illuminating the sample with a light source of stable color temperature. To achieve this, we designed a surface light source that can uniformly provide transmitted light illumination, effectively eliminating interference from reflected light on the liquid surface. A microtiter array plate is installed on the top and bottom of the 96-well microtiter plate to ensure that light from the surface light source is illuminated from the bottom and emitted from directly above. The remaining parts of the microtiter plate are blocked by the array plate, physically shielding stray light from other directions and effectively isolating its interference on the image captured by the smartphone camera. The sample solution is reacted inside the 96-well microplate, while the pull-out drawer safely and reliably holds the microplate, making the operation more convenient and rapid. Additionally, the large-aperture convex lens can alter the light path direction, refract, and focus the light, effectively solving the problem of the limited light-gathering ability of smartphone lenses. This ensures the smartphone's accurate imaging of all 96 microholes, making the imaging results for each microhole consistent and unaffected by the edges. The device's housing is manufactured using 3D printing technology, which fixes and encloses all optical components, effectively avoiding interference from ambient light during imaging. A photo hole is provided on the top of the housing to facilitate imaging with smartphones.

2.3.2. The working principle of smartphone applications. The smartphone application was developed

using Java and digital image colorimetry. To ensure the stability and compatibility of the application, the widely available Android operating system was selected for development. The Huawei Enjoy 7 Plus smartphone was selected for debugging and installation. The user interface of the application is simple and intuitive, featuring 3 primary functional buttons: "Detect," "File," and "Quit" (as shown in Fig. 2A). These buttons direct the user to the detection interface, history interface, and exit program, respectively. Users first click "Detect" to enter the detection interface (Fig. 2B), align the red rectangular detection frame with the 96-well plate, and then the system automatically focuses. If the focusing effect is unsatisfactory, users can click the "Back" button to readjust and then click the "Photo" button to capture a clear image of the microplate. After capturing the image, the user clicks "Next," and the application will immediately start the automatic image processing and data analysis. The process is based on the scientific principle of digital image colorimetry, which automatically locates and captures a 25×25 pixel area in the center of the microtiter plate, accurately calculates the R value, G value, and B value (RGB) value of each pixel in the area, and then averages the values. Then, based on the color characteristics of the sample solution to be tested, the application converts the RGB values into chromaticity values such as H and Y. Using this data, we constructed an optimal standard curve based on the concentration values and chromaticity values of the standards and embedded it into the smartphone application. Therefore, when testing a solution of unknown concentration, the app automatically substitutes the chromaticity value of the solution into the standard curve and accurately calculates the concentration, providing users with intuitive and reliable test results (Fig. 2C).

2.4. Experimental procedure for the detection of serum CR based on a smartphone microplate analysis system

The experiments were conducted following the principles of the sarcosine oxidase assay. To minimize experimental error, the following standardized experimental procedure was adopted. First, 246 µL of serum sample was precisely mixed with 615 µL of R1 reagent in a 2 mL EP tube. The mixture was then incubated at 37°C for 5 minutes in a constant-temperature incubator to facilitate the enzymatic reaction. Then, 205 µL of R2 reagent was added to the EP tube and incubated at 37°C for an additional 10 minutes to complete the color development reaction. Once the reaction was complete, the mixture was transferred to a 96-well microplate, adding 260 µL to each well. The 96-well plate was then placed in a pull-out drawer, and a smartphone, positioned

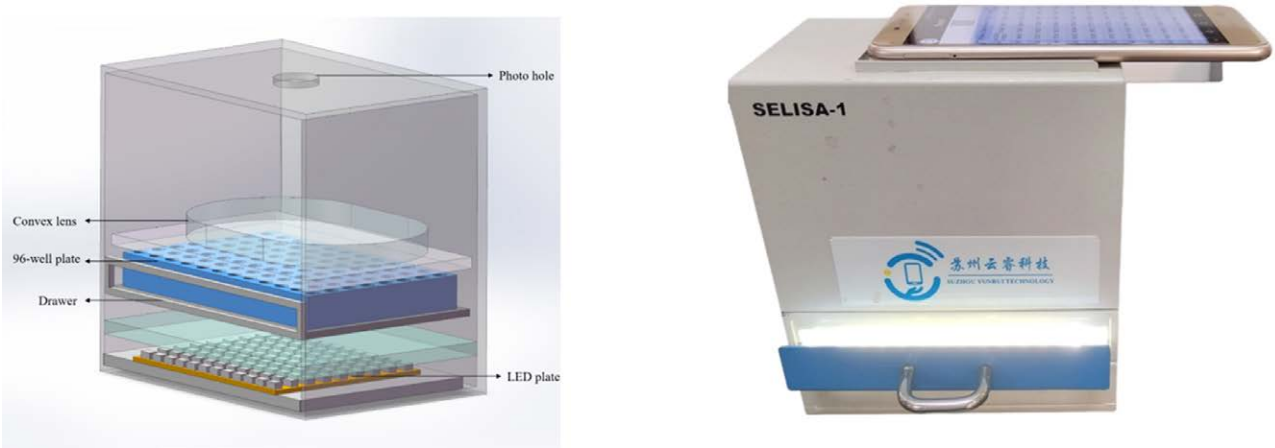

Figure 1. Smartphone-based microplate analysis system: schematic structure and physical representation.

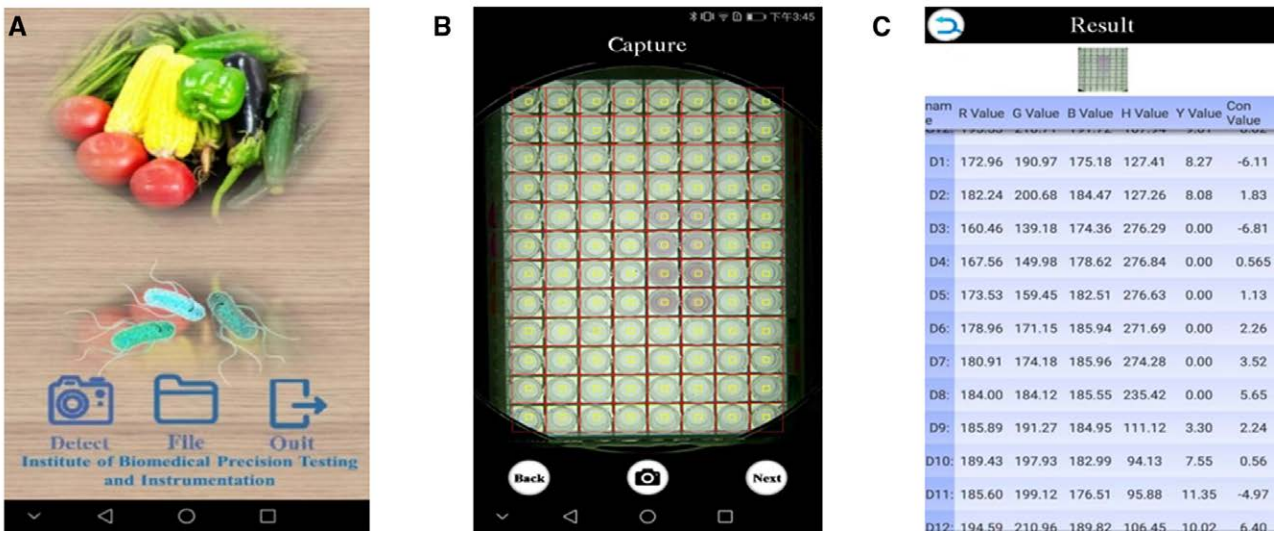

Figure 2. Interface display of the smartphone App detection process: (A) navigation interface, (B) photography interface, and (C) results interface.

in the top opening, photographed the 96-well plate through the photo hole. Once the photo is captured, the smartphone application automatically processes and analyzes the image data. The application utilizes the principle of digital image colorimetry to extract the RGB values of the reaction solution and display them on the interface. The application also calculates and displays the CR concentration in the serum sample, based on the standard curve generated from the color values and concentration values of a known standard. Prior to each test, users can perform baseline correction by testing calibrators to ensure that unknown samples are within the allowable experimental error, thus enhancing the reliability of smartphone-based test results.

2.5. Preparation of CR standard samples and establishment of standard curves

The CR standard in the assay kit had a concentration of 177 μmol/L and was diluted with distilled water to prepare 8 concentration gradients: 159.3 μmol/L, 141.6 μmol/L, 123.9 μmol/L, 106.2 μmol/L, 88.5 μmol/L, 70.8 μmol/L, 53.1 μmol/L, and 35.4 μmol/L. The standard and detection reagents were added to the 96-well microplate as described in Section 2.3, with 3 parallel determinations performed. Once the reaction was complete, the plate was placed in the drawer. The R, G, and B values (RGB values) of the reaction mixture were then determined using the smartphone application. The RGB values exhibit different responses to varying sample concentrations. Three curves were constructed by correlating the known concentrations of the standard with the measured R, G, and B colorimetric values. The curve with the highest correlation was selected as the final standard curve for CR. Finally, quantitative detection of unknown samples was performed using the standard curve.

2.6. Precision verification experiment

Precision is defined as the degree of agreement between independent measurements obtained under specified conditions. The method's precision can be validated according to the NCCLS document EP5-A2. The initial step involves conducting a preliminary test, with the standard deviation serving as the fundamental standard deviation for precision evaluation. Subsequently, samples with high and low concentrations of 133.6 μmol/L and 93.5 μmol/L, respectively, were selected for duplicate measurements, following the same procedure. Each day was divided into 2 runs, with an interval of at least 2 hours between them. Testing was conducted over 20 consecutive days, resulting in 40 runs and 80 results for each assay concentration. If any result had an absolute variation exceeding 5.5 times the standard deviation of the baseline precision evaluation, it was considered an outlier and rejected, and the cause was identified for reanalysis. Precision was then evaluated using the following formula:

Standard deviation of the intra-run precision is as follows (Formula 1):

$$S_r = \sqrt{\frac{\sum_{i=1}^{1}\sum_{j=1}^{2}\left(X_{ij1} - X_{ij2}\right)^2}{4I}} \tag{1}$$

Standard deviation of between-run precision (Formulas 2 and 3):

$$A = \sqrt{\frac{\sum_{i=1}^{1}\left(\bar{X}_{i1\cdot} - \bar{X}_{i2\cdot}\right)^2}{2I}} \tag{2}$$

$$S_{rr}^2 = A^2 - \frac{S_r^2}{2} \tag{3}$$

Standard deviation of between-day precision (Formulas 4 and 5):

$$B = \sqrt{\frac{\sum_{i=1}^{1}\left(\bar{X}_{i\cdots} - \bar{X}_{\cdots}\right)^2}{I-1}} \tag{4}$$

$$S_{dd}^2 = B^2 - \frac{A^2}{2} \tag{5}$$

Standard deviation of total precision (Formula 6):

$$s_T = \sqrt{S_{dd}^2 + S_{rr}^2 + S_r^2} \tag{6}$$

where:

I = total number of days of operation, j = run, i = day i.

The coefficient of variation is calculated by dividing the standard deviation by the mean of all resultant concentrations, multiplying by 100, and then comparing it with the specified permissible error to determine if the precision is acceptable.

2.7. Validation of accuracy

The term 'accuracy' refers to the proximity of a single measurement to the true value. To verify accuracy, the NCCLS EP9-A2 document should be consulted, and a sufficient number of serum samples should be selected for double parallel testing using the method to be evaluated, typically analyzing 40 serum samples over a minimum period of 5 days. The precision of the method was then evaluated by comparing the results obtained from the smartphone assay with those from the Beckman Coulter AU5800 automated biochemical analyzer in the laboratory. The acceptable limit of the method was calculated as 4 times the mean of the absolute differences between the 2 determinations for each sample. Outliers beyond this limit were identified, examined for potential causes, and removed from the dataset. To assess the appropriateness of the distribution range of X values, the correlation coefficient (r) was used to estimate the relationship, calculated using the following formulas (7–9):

$$r = \frac{\sum_i^N\left(\overline{x_j} - \bar{x}\right)\left(\overline{y_j} - \bar{y}\right)}{\sqrt{\sum_i^N\left(\bar{x}_j - \bar{x}\right)^2}\sqrt{\sum_i^N\left(\bar{y}_j - y\right)^2}} \tag{7}$$

$$\bar{x} = \frac{\sum\sum x_{ij}}{2N} \tag{8}$$

$$\bar{y} = \frac{\sum\sum y_{ij}}{2N} \tag{9}$$

if $R \geq 0.975$ or $r^2 \geq 0.95$, it can be concluded that the range of values for X is appropriate. The dataset was then subjected to linear regression to determine the slope (b) and intercept (a), leading to the linear regression equation $\hat{Y} = bX + a$. Once all the above conditions have been met, the standard error of the estimate is calculated using Formula 10. The anticipated bias (B_c) at a designated medical decision level (X_c) was subsequently calculated as $\hat{B}_c = a + (b-1)X_c$. Finally, the 95% confidence interval for the anticipated bias (true bias at X_c) was calculated using Formula 11:

$$S_{y\cdot x} = \sqrt{\frac{\sum\sum\left(y_{ij} - \hat{Y}_{ij}\right)^2}{2N-2}} \tag{10}$$

$$\left[\hat{B}_{c,low}, \hat{B}_{c,high}\right] = \hat{B}_c \pm 2S_{y\cdot x}\sqrt{\frac{1}{2N} + \frac{\left(X_c - \bar{x}\right)^2}{\Sigma\Sigma\left(x_{ij} - \bar{x}\right)^2}} \tag{11}$$

The confidence interval for the expected bias is compared with the specified acceptable bias to determine if the assay's accuracy passes validation.

2.8. Evaluation of the detection capability of smartphone-based microplate analysis systems

The detection capability of the method was validated following the EP17-A2 guidelines, which include the limit of blank (LoB), LoD, and the limit of quantification (LoQ).

The experimental design for determining LoB and LoD is as follows: 5 samples are selected and divided into 2 batches each day, with each sample repeated 4 times per batch, for a total of 3 days of testing. This ensures that at least 60 results are obtained for each batch. The key difference between the 2 designs is that LoB uses blank samples, while LoD uses low-concentration samples for testing. The results of all blank samples are ordered from lowest to highest, and the 95th percentile is calculated to determine the rank position (rank position = 0.5 + B × 0.95, where B is the total number of results). The result at the rank position is taken as the LoB. If the rank position is not an integer, interpolation between the 2 nearest rank positions is performed, and the larger value from the 2 batches is selected as the final LoB. LoD is calculated using formulas (12–14):

$$SD_L = \sqrt{\frac{\sum_{i=1}^{J}(n_i - 1)\,SD_i^2}{\sum_{i=1}^{J}(n_i - 1)}} \tag{12}$$

$$C_P = \frac{1.645}{1 - \left(\frac{1}{4\,(L - J)}\right)} \tag{13}$$

$$LoD = LoB + C_p * SD_L \tag{14}$$

where L = the result of all low-level samples and J = the number of low-level samples. The larger of the batch results is then selected as the LoD.

LoQ, refers to the lowest sample concentration that can be accurately and consistently measured. Four independent low-concentration samples of known concentrations were selected and divided into 2 batches. Each batch was tested 3 times over a span of 3 days, and the mean, standard deviation, and bias were calculated for each low-concentration sample. The total error (TE) was calculated using the Westgard model (TE = |Bias| + 2 standard deviation), and the TE was compared to the specified allowable total error. The lowest concentration yielding acceptable results was selected as the LoQ for that batch, and the higher of the 2 batches' results was taken as the final LoQ for the assay procedure.

2.9. Establishing the linear range of this detection system

High- and low-concentration samples are prepared in specific ratios to create 9 equally spaced concentrations, in accordance with the NCCLS document EP6-A2, covering the anticipated measurement range. Each concentration is tested in triplicate, preferably within a single day. Subsequently, regression analysis is performed to assess the linearity of the assay by comparing expected values with actual measurements. The ideal regression equation is Y = bX + a, where b = 1 and a = 0. If the b value lies between 0.95 and 1.05 and the a value is close to 0, the method can be considered to demonstrate satisfactory linearity across both the low and high concentration ranges. Therefore, the linear range of the assay procedure is determined between the lower and upper concentration limits. If the b value exceeds 1.05 or falls below 0.95, a statistically significant difference may be present.

2.10. Determine the clinical reportable range of the method

The clinically reportable range refers to the range of analyte concentrations that can be achieved by diluting, concentrating, or otherwise preprocessing the sample to expand the analytical measurement interval. The upper limit of this range is determined through a maximum dilution experiment. This is achieved by selecting 3 high-concentration samples, each within 1/3 of the upper limit of the measurement interval. Each sample is subsequently diluted at least 5 times, with the concentration of each dilution measured in duplicate. Next, the ratio of the actual measured concentration to the theoretical concentration is calculated to obtain the dilutional recovery. Additionally, the maximum dilution is determined, ensuring that the recovery remains within the specified range of 90% to 110%. Multiplying the upper limit of the analytical measurement range by this maximum dilution gives the upper limit of the clinical reportable range. Finally, the clinical reportable range is determined by combining the limits of quantification.

2.11. The method was further validated and statistically analyzed via clinical serum samples

The CR concentrations of 65 serum samples from healthy individuals and 26 serum samples from patients, provided by the First Hospital of Shanxi Medical University, were measured using a smartphone microplate analysis system and the hospital laboratory's fully automated biochemical analyzer, respectively. A comparison was made between the smartphone test results and the hospital measurements to determine whether a significant difference existed between the 2 datasets. Furthermore, linear regression analysis was conducted to calculate the correlation coefficient, thereby evaluating the degree of agreement between the 2 assays.

3. Results

3.1. Establishment of the CR standard curve

The CR assay kit uses the creatine oxidase method as the detection principle. An increase in CR concentration results in a color change from colorless to red, with higher concentrations leading to darker shades. The smartphone colorimetric analysis system was then used to detect the CR standard at 8 concentration levels, thus establishing the relationship between the R, G, and B values and their concentrations. Figure 3 shows that the G value decreases with increasing CR concentration, demonstrating a strong correlation ($R^2 = 0.9932$). As a result, the G value and the concentration of the standard solution can be used to construct the standard curve for CR, represented by the equation $Y = -0.4251X + 212.5967$.

3.2. Verification results of precision experiments

Standard deviations of 3.98 and 4.64 were obtained from the pre-experiment for samples with high and low CR concentrations, respectively, and were used as the basis for assessing precision. A comparison of all the experimental data with 5.5 times the standard deviation of the pre-experiment data shows that all the data meet the requirements. The coefficients of variation for intra-run, between-run, between-day, and total precision were subsequently calculated using formulas 1 to 6 (Table 1). According to the CLIA '88 quality control requirements, intra-batch precision CV% ≤ 1/4TEa (3.75%), inter-batch and inter-day precision CV% ≤ 1/3TEa (5%), and total precision CV% ≤ 1/2TEa (7.5%) fall within the clinically acceptable range. The permissible total error (TEa) for CR is 15%. Therefore, the coefficients of variation for all the following results fall within the set target range, indicating that the precision experiments for serum CR testing using smartphones were validated.

3.3. Verification results of accuracy

After testing 40 serum samples, an within-group outlier analysis was conducted, which did not identify any outliers. A range verification of the distribution of the X values was subsequently performed, showing that the correlation coefficient ($R = 0.985$) > 0.975 met the expected criteria. Therefore, the X values exhibit a sufficient range of distributions for analysis via linear regression to obtain the regression equation Y = 4.3599 + 0.9347X. Finally, 2 medical decision levels were selected for analysis: CR concentrations of 110 μmol/L and 30 μmol/L. The calculations indicate that the assay results fall within the acceptable range of bias (Table 2). Therefore, the accuracy of the smartphone detection system for serum CR has been fully verified.

3.4. Assessment results of detection capabilities

Given that there were a total of 60 blank samples, the corresponding rank position was 57.5. The LoB was determined by sorting the 60 data points and calculating the average of the 57th and 58th results. The larger of the 2 batches of results, 29.95 μmol/L, was then selected as the LoB. Subsequently, 5 low-concentration samples were tested, yielding a LoD value of 32.39 μmol/L based on the formula. Subsequently, 4 known low-concentration samples (33.41 μmol/L, 36.75 μmol/L, 40.09 μmol/L, and 43.43 μmol/L) were tested, yielding the lowest concentrations of 36.61 μmol/L and 36.53 μmol/L for the first and second batches, respectively, while meeting the allowable total error of 15% (Table 3). The highest value, 36.61 μmol/L, was chosen as the LoQ from the 2 batches of results. In conclusion, the LoB, LoD, and LoQ for serum CR detected by the smartphone were 29.95 μmol/L, 32.39 μmol/L, and 36.61 μmol/L, respectively. These results demonstrate that the assay exhibited satisfactory analytical sensitivity and met the requirements for clinical testing.

3.5. Experimental results for the linear range

Serum samples with CR concentrations of 36.75 μmol/L and 200.46 μmol/L were mixed in specific ratios (0.875L + 0.125H, 0.75L + 0.25H, 0.625L + 0.375H, 0.5L + 0.5H, 0.375L + 0.625H, 0.25L + 0.75H, 0.125L + 0.875H) to prepare 7 intermediate samples with concentrations of 57.21 μmol/L, 77.68 μmol/L, 98.14 μmol/L, 118.61 μmol/L, 139.07 μmol/L, 159.53 μmol/L, and 180.00 μmol/L. In addition to the original low- and high-concentration samples, a total of 9 samples were tested. A regression analysis of the mean measured values against the expected concentrations (Fig. 4) yielded the following regression equation: Y = 0.9888X + 1.1555, where the slope (b) is between 0.95 and 1.05, and the correlation coefficient (R^2) is 0.9998. These results indicated a statistically significant linear relationship between the assay measurements and concentrations in the range of 36.75–200.46 μmol/L.

3.6. Results of the clinically reportable range experiments

The 3 high-concentration samples (148.49 μmol/L, 170.76 μmol/L, and 193.04 μmol/L) were diluted at various levels (1.5-, 2-, 2.5-, 3-, and 4-fold), and their diluted concentrations were measured using the smartphone detection system. The measured concentrations and dilution recoveries were calculated, showing that recovery remained within acceptable limits even at a 4-fold dilution (Table 4). The clinically reportable concentration range was established by combining the upper limit of the linear range with the LoQ, resulting in a range of 36.61 to 801.84 μmol/L.

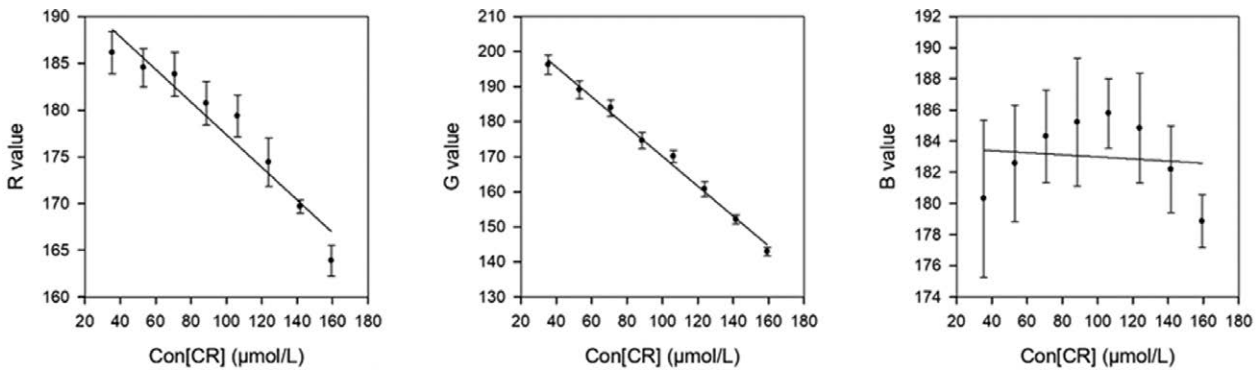

Figure 3. Changes in R, G, and B values with increasing creatinine (CR) concentration.

Table 1

Results of the experimental evaluation of creatinine precision.

Concentration	Intra-run CV%	Between-run CV%	Between-day CV%	Total CV%	Conclusion
CR_H	1.72%	1.92%	1.64%	3.06%	Acceptable
CR_L	2.73%	3.33%	2.48%	4.97%	Acceptable

Table 2

Expected bias at 2 medical decision levels.

Project name	$S_{y \cdot x}$	Xc (μmol/L)	$\hat{B}_c$	$\hat{B}_{c,low}$	$\hat{B}_{c,high}$	Allowed error	Conclusion
CR	5.4941	110	-2.8231	-4.1055	-1.5407	8.25	Acceptable
	5.4941	30	2.4009	-0.4809	5.2827	2.25	Acceptable

Table 3

Results of the limit of quantification (LOQ) determination of serum creatinine.

	Mean value of measured concentration (μmol/L)		SD		Bias		TE%	
Theoretical concentration (μmol/L)	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
33.41	32.60	32.00	1.58	2.25	-0.81	-0.60	11.91%	15.25%
36.75	36.61	36.53	1.46	1.26	-0.15	-0.08	8.34%	7.09%
40.09	40.66	41.03	2.23	1.41	0.57	0.37	12.53%	7.97%
43.43	44.20	43.73	1.62	1.87	0.77	-0.47	9.25%	9.68%

SD = standard deviation.

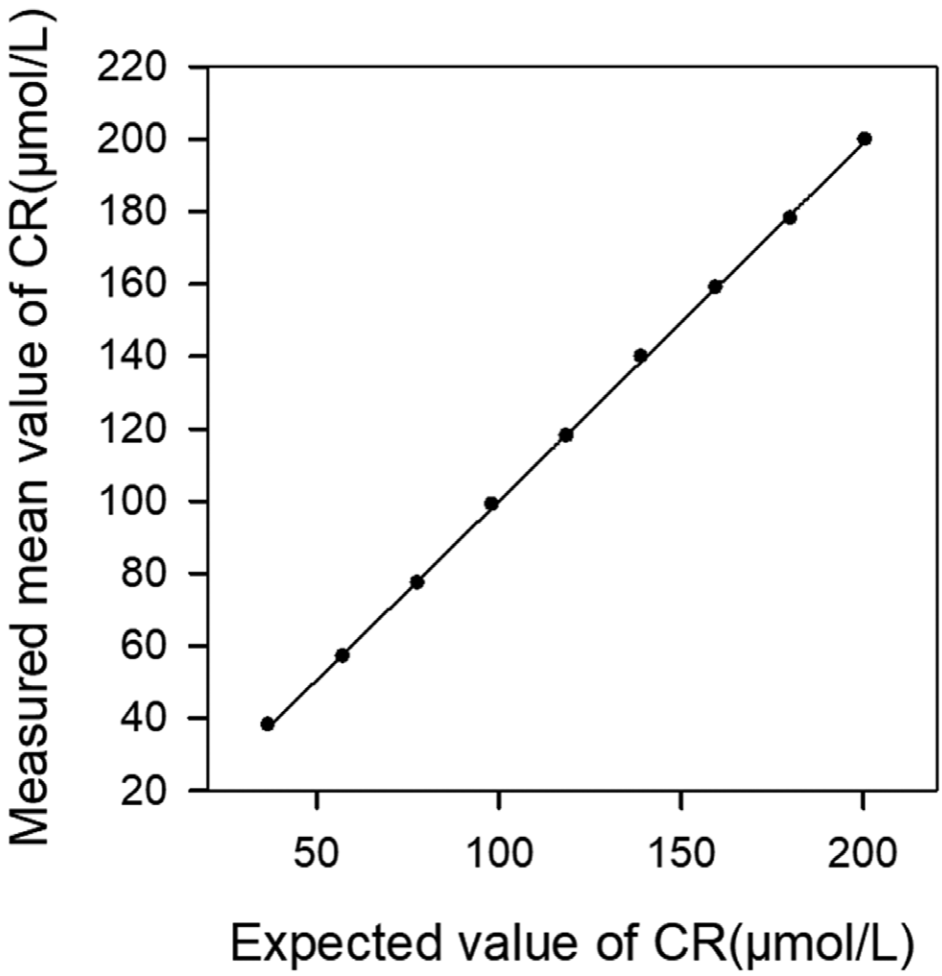

Figure 4. Linear regression analysis results.

3.7. Validation of the results with clinical serum samples

A total of 91 clinical serum samples were collected to assess the feasibility of serum CR testing using a smartphone. The data obtained from the smartphone were compared with the results from the Beckman Coulter AU5800 automated biochemical analyzer, yielding a t value of 1.3205 based on the t test. According to the t distribution table, a probability range of $0.1 < P < .2$ was derived. Since P was greater than the preestablished significance level ($\alpha = 0.05$), the difference between the 2 assays was not statistically significant. Additionally, correlation analyses were performed. Due to the limited number of patient samples and the unequal distribution of concentrations, only the results from healthy individuals within the normal concentration range were selected for regression analysis. The results yielded a regression equation of $Y = 0.9662X + 2.2140$, with a correlation coefficient of $R = 0.9949$ and a coefficient of determination (R^2) = 0.9898. Histogram analysis was conducted for the patient samples outside the normal concentration range (Fig. 5). As illustrated in Figure 5, the results from the smartphone colorimetric analysis system and the automated biochemical analyzer showed a high degree of concordance in detecting serum CR, both in the normal population and in patients. These findings further support the suitability of the smartphone assay for routine clinical testing.

4. Discussion

CKD is a progressive, long-term condition categorized into 5 stages globally based on the glomerular filtration rate. In the early stages of CKD, there are usually no discernible symptoms. Consequently, treatment focuses primarily on addressing the underlying cause of CKD, aiming to alleviate symptoms,

Table 4

Results of dilution measurements of 3 high-concentration samples.

Sample	Theoretical concentration (μmol/L)	Dilution factor	Result 1	Result 2	Mean value	Measured concentration (μmol/L)	Recovery
1	148.49	1.5	99.95	98.91	99.43	149.14	100.44%
	148.49	2	73.57	77.69	75.63	151.27	101.87%
	148.49	2.5	61.44	58.05	59.74	149.36	100.58%
	148.49	3	51.46	47.93	49.70	149.09	100.41%
	148.49	4	37.04	38.78	37.91	151.65	102.13%
2	170.76	1.5	111.19	112.81	112.00	168.00	98.38%
	170.76	2	84.80	82.68	83.74	167.47	98.08%
	170.76	2.5	67.60	69.55	68.58	171.44	100.40%
	170.76	3	56.59	56.52	56.56	169.67	99.36%
	170.76	4	41.61	40.36	40.98	163.93	96.00%
3	193.04	1.5	128.83	129.09	128.96	193.44	100.21%
	193.04	2	95.26	96.09	95.68	191.35	99.13%
	193.04	2.5	80.68	76.47	78.57	196.43	101.76%
	193.04	3	64.71	65.34	65.02	195.07	101.05%
	193.04	4	46.31	48.38	47.35	189.38	98.11%

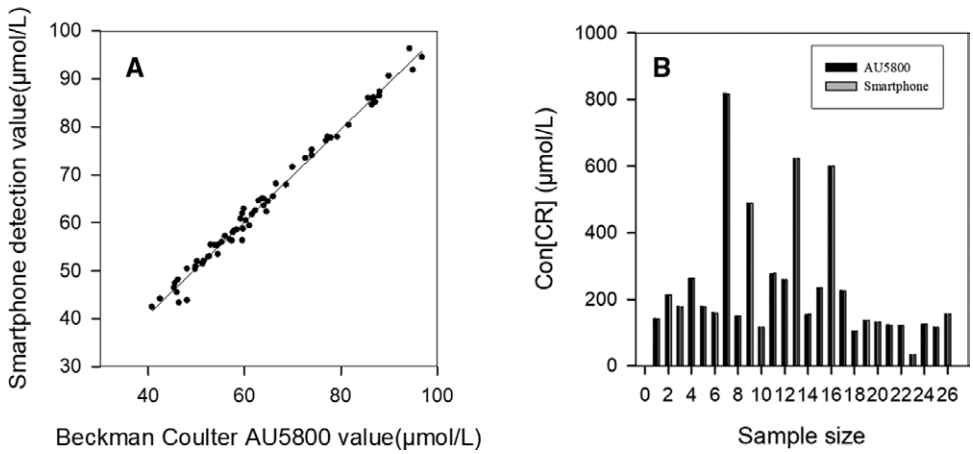

Figure 5. Correlation analysis results: (A) normal and (B) patient.

preserve renal function, and slow disease progression. Once CKD reaches stage 5, end-stage renal disease, patients usually require dialysis and may be considered for renal replacement therapy. At this stage, patients face a significantly higher risk of mortality.[21,22] Therefore, identifying biomarkers for early diagnosis and monitoring the progression of CKD is crucial, as this enables appropriate treatment and improves patient outcomes. After excluding the effects of exogenous factors and exercise, serum CR levels are primarily influenced by the glomerular filtration rate. A decline in kidney function and glomerular filtration rate leads to a significant rise in serum CR, with the increase accelerating as the severity of injury intensifies.[23–25] In pregnant women, the traditional method of calculating glomerular filtration rate is no longer applicable due to physiological changes in kidney function, making serum CR an important indicator for assessing pregnancy-associated CKD.[26,27] Therefore, quantifying serum CR concentration to reflect the glomerular filtration rate allows for assessing CKD progression, which is crucial for monitoring the disease.

Fully automated biochemical analyzers are now widely used in clinical laboratories to measure biochemical markers. These devices have been shown to enhance efficiency and accuracy by automating processes and integrating data.[28,29] However, the large size and complex maintenance needs of these devices pose challenges for their use in resource-limited settings. In contrast, portable detection devices, with their compact design and ease of use, can meet a wide range of needs, especially in resource-limited settings. The rapid development of smartphone technology in recent years has not only transformed communication, but also profoundly impacted healthcare. This transformation has led to the combination of portable detection devices and smartphones, which is a major innovation in medical testing. This combination greatly improves testing convenience and efficiency, while also indicating that portable detection devices will have broad applications and play a crucial role in future medical diagnosis and health monitoring. For instance, the University of Cincinnati developed a handheld testing system combining chemiluminescent ELISA technology with a smartphone analyzer to detect infectious diseases like malaria.[30] Similarly, the smartphone microplate analysis system used in this study integrates portable detection devices with a smartphone. It has been used to efficiently quantify human serum albumin and infectious disease biomarkers, including C-reactive protein, serum amyloid A, and procalcitonin. Statistical analysis confirmed its reliability, showing no significant difference in results between the device and an automated biochemistry analyzer in a clinical laboratory.[20]

Based on this, the study adhered strictly to standardized operating procedures, and through precise control of reagent dosage and reaction temperature, the serum CR levels were innovatively determined using the smartphone detection system. Regarding methodological validation, the study strictly followed the NCCLS document's requirements, and the results demonstrated that the assay system's precision and accuracy met clinical trial standards while showing good analytical stability. Additionally, through comprehensive evaluation of detection limit, linear range, and reportable range, it was confirmed that the system possesses high sensitivity and a wide detection range. During

the clinical serum sample validation phase, a rigorous testing protocol was followed, and the results showed that the developed smartphone analysis system was highly consistent with the measurements from an automated biochemical analyzer, fully validating its clinical application value and detection accuracy. Although inherent differences in image sensor characteristics across devices and individual bias from manual operation may occur, these potential influences were effectively controlled within clinically permissible error ranges by implementing standardized procedures, calibrating the instrument before each test, and providing standardized training to users. Regarding reagent management, CR test kits remain stable for 12 months at 2 to 8°C, protected from light, and stable for 1 week at the same temperature after opening. For optimal performance, the kit is stored at 4°C when not in use, and repeated freezing and thawing of serum samples is avoided. Furthermore, the assay system's long-term reliability was ensured through a quality control program involving monthly standard calibration and preventive maintenance. This study provides strong evidence for the feasibility of smartphone testing technology in clinical testing and lays a solid foundation for its promotion in early screening and long-term monitoring of CKD.

As society progresses and people's health awareness significantly increases, the medical model is undergoing a profound transformation. The rise of the personalized healthcare model indicates that healthcare services are shifting from the traditional hospital-centered, disease-treatment model to a patient-centered approach focusing on prevention and health management. This shift not only demands that medical services become more integrated into patients' daily lives but also drives the development of medical devices and technologies towards greater convenience and personalization to meet diverse patient needs.[31–33] Compared with other serum CR testing devices on the market, the smartphone analysis system in this study is fast, compact, portable, low-cost, easy to operate, and suitable for use in various fields such as community healthcare and home self-testing, significantly improving the accessibility and convenience of serum CR testing.[34,35] This not only aids in the early detection and effective management of CKD but also provides a new impetus to global health.

Despite the method's good performance in serum CR determination, some limitations and challenges remain. First, the method currently applies only to the detection of biochemical indicators using endpoint methods, and does not yet comprehensively cover indicators that require continuous monitoring. This means future studies must further explore and optimize the method to broaden its scope and achieve accurate detection of additional biochemical markers. Second, although this study has provided preliminary validation of serum CR detection in CKD patients, the sample size and diversity need improvement. To better assess the clinical value of this technique, we plan to expand the sample size in the future to include more patients of different ages and disease stages, further validating its accuracy and reliability in the early diagnosis and monitoring of CKD. This will help us gain deeper insights into the technology's performance across different patient groups, providing strong support for its broader clinical application. Additionally, the experimental procedure requires reactions to be carried out in a thermostatic incubator, which may be difficult to implement in resource-limited settings. Therefore, we will explore the possibility of conducting the reaction at room temperature to further improve the technique's convenience and utility. Finally, current research primarily focuses on technology validation; therefore, to achieve the crucial shift from laboratory validation to clinical application, a multidimensional usability assessment system must be established. This includes inviting interdisciplinary healthcare professionals to conduct full-process simulations and create a dynamic feedback mechanism to continuously optimize the testing process based on clinical usage data, improving clinical acceptance. By implementing the multidimensional optimization pathway described above, we expect to systematically overcome the limitations of current technology and advance the progress of this assay toward clinical translational goals, including covering more indicators, adapting to more scenarios, and benefiting more people.

In conclusion, despite the limitations and challenges of the smartphone analysis system for serum CR testing, its portability, ease of use, and low cost demonstrate great potential for primary care and a wide range of users. The system can alleviate the problems of insufficient equipment and a shortage of professional technicians in primary care, improve the efficiency and quality of healthcare services, and enable self-monitoring and better healthcare management via smartphones. Looking ahead, we are committed to continuously optimizing and improving the technology, expanding it to cover more biochemical indicators, and providing convenient, efficient solutions for the prevention, diagnosis, and treatment of chronic diseases, while promoting sustainable development in global healthcare and leading the industry toward an intelligent, personalized future.

5. Conclusions

In this study, we preliminarily validated the smartphone-based serum CR detection method, which meets clinical standards across key performance indicators and is highly consistent with results from the laboratory automated biochemical analyzer, fully demonstrating the method's reliability and accuracy. Therefore, this method demonstrates significant advantages and potential in monitoring patients with CKD, offering a convenient and efficient tool for patient monitoring. However, it is important to acknowledge the limitations of the current methodology. To enhance its clinical value, future studies should further optimize the technique, expand its scope, increase the sample size, and stratify by CKD stage to comprehensively assess the method's applicability and accuracy across various disease stages and populations. These efforts are expected to lay a solid foundation for the widespread application of smartphone testing technology in CKD management and offer patients more personalized and accurate medical services.

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