RESEARCH ARTICLE

Immune turbidimetric detection of SARS-CoV-2 antibodies based on smartphones

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Received: September 4, 2024; **accepted:** December 20, 2024.

Utilizing antibody levels to assess the progression of viral infections can also serve as a metric for evaluating vaccine efficacy. However, current quantitative detection methods of antibody concentration require specialized equipment. Given their widespread use, simplicity, and portability, smartphones offer a promising platform for immune analysis. Smartphone-based immune analysis methods can present a cost-effective, high-precision, and rapid solution for detecting SARS-CoV-2 antibodies. The SARS-CoV-2S protein (RBD) was conjugated with latex microspheres to create a reagent, to which the antibody sample was subsequently added. Image information of these reagents was captured using a mobile phone with spectrophotometer results serving as a reference for comparison. The study explored image colorimetric determination of turbidity by evaluating various background color cards and methods of incorporating black squares onto the background plate. Additionally, the impact of different color channels, light intensity, and color temperature on mobile phone detection was investigated. After determining the optimal parameters, the repeatability test was performed on three concentrations of 29.8 AU/mL, 49 AU/mL, and 166.9 AU/mL. The smartphone and spectrophotometer were used to analyze 30 unknown samples to examine the correlation between the test results of the two methods. The results showed that the sensitivity of smartphones in detecting SARS-CoV-2 antibodies was significantly improved by introducing black color blocks into the background plate. Mathematical model research showed that R channel parameters were more suitable for immunoturbidimetric detection with the best sensitivity and a detection limit of 0.09 AU/L. The repeatability test was conducted on samples with three concentrations. The repeatability relative standard deviations (RSDs) were 3.9%, 6.0%, and 11.1%, respectively, indicating good repeatability. The interference of light intensity and color temperature demonstrated no significant impact on the detection. The results measured by the two platforms were highly correlated (R^2 = 0.91), and the paired sample t test also showed no significant difference between the two methods. This study established a platform for antibody immunoturbidimetric detection using smartphones, presenting a novel avenue for smartphone applications in immune detection. The method held promise for widespread application in rapid detection of antibodies and antigens, thereby expanding the scope of smartphones in real-time detection methodologies.

Keywords: smartphone; immunoturbidimetry; SARS-CoV-2; antibody detection.

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Introduction

The rapid spread of the novel coronavirus (SARS-CoV-2) has precipitated a global health crisis. Effectively controlling its dissemination hinges upon timely detection and diagnosis of infected individuals. Current detection methods for the novel coronavirus encompass nucleic acid

detection [1], antigen detection [2], and antibody detection [3]. Nucleic acid and antigen detection methods directly identify the presence of the virus, whereas antibody detection relies on indirect identification. This method discerns infection by detecting antibodies produced by the body's immune response to the virus. In the early stages of the viral outbreak, the development of raw materials for antibody detection was expedited, rendering it the initial detection approach for the SARS-CoV-2 virus. Post-infection, the body generates specific antibodies, notably immunoglobulin M (IgM) and immunoglobulin G (IgG). IgM antibodies emerge earlier than IgG, yet IgG persists longer in the body, sometimes persisting for several months or more [4]. Consequently, using antibody levels in clinical practice to gauge viral infection progression also enables preliminary assessment of vaccine efficacy. Clinical studies and epidemiological observations have confirmed the potential for multiple infections posed by the new coronavirus, highlighting the utility of antibody testing in averting reinfection [5, 6].

There are many ways to detect SARS-CoV-2 antibodies. Vernet *et al*. developed a reproducible ELISA test protocol that was coated with SARS-CoV-2 S protein and could detect and quantify IgG antibodies [7]. However, this method required complex separation and cleaning operations, and a spectrophotometer for enzyme signal acquisition. Demey *et al*. used colloidal gold immunochromatography to detect SARS-COV-2 antibodies. This method was simple and fast, only performed qualitative detection, and could not achieve quantitative detection [8]. Li *et al*. combined time-resolved microspheres to achieve quantitative detection of SARS-COV-2 antibodies. However, due to the low precision of the immunochromatography method, the accuracy of the test results needed to be improved [9]. Further, Padoan *et al*. used chemiluminescence to detect SARS-COV-2 antibodies. This method had accurate results but required expensive chemiluminescence equipment and was not suitable for rapid on-site detection [10]. Smartphones are ideal for rapid on-site testing due to their portability, popularity, and powerful functions. Although there are reports on the use of smartphones to detect SARS-COV-2 antibodies, such as Li *et al*. combined smartphones with quantum dot lateral flow immunoassay test strips to achieve SARS-COV-2 antibody detection [11], this method was mainly used in immunochromatography research, which was limited by the chromatography test strip technology, resulting in unsatisfactory detection accuracy.

This study intended to use homogeneous immunoturbidimetry to determine SARS-COV-2 antibodies to ensure excellent repeatability and accuracy. The turbidity after the immune response was collected using a smartphone, thereby realizing the detection of SARS-COV-2 antibodies using a smartphone. This research provided a simple, low-cost, and accessible on-site detection method for SARS-COV-2 antibodies detection.

Materials and methods

Turbidimetric reagent preparation

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), and carboxyl microspheres were mixed at a ratio of 1.15:1.15:1 and rotated at 20 rpm in a constant temperature and humidity chamber at 25°C and 75% humidity for 30 minutes to activate the microspheres. The activated solution was centrifugated at 10,000 rpm, 4℃, for 30 min to eliminate excess EDC and NHS followed by reconstitution with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4). The SARS-CoV-2S protein (RBD) (FAPON Biotech, Dongguan, Guangdong, China) was then added to the solution and vortexed mixing at 25°C for 120 minutes followed by centrifugation at 10,000 rpm for 30 min to remove excess free protein. To block blank sites on the microspheres, 1% bovine serum albumin (BSA) was added and vortexed mixing at 25°C for 120 minutes followed by centrifugation at 10,000 rpm for 30 min. The turbidimetric reagent was obtained by

reconstituting with the working solution (HEPES buffer pH 7.4, NaCl 10 g/L, BSA 5 g/L, choline chloride 0.1 g/L) and subjecting it to 10 minutes of ultrasonic dispersion treatment using an JY96-IIN Ultrasonic Homogenizer (Hunan Lichen Instrument Technology Co., Ltd., Changsha, Hunan, China)

Detection of SARS-CoV-2 antibody IgG

Humanized SARS-CoV-2 antibody IgG was obtained from GenScript Biotech (Piscataway, NJ, USA) to create six concentration samples of 0 AU/mL, 15.3 AU/mL, 29.8 AU/mL, 49 AU/mL, 104.3 AU/mL, and 166.9 AU/mL. The sample:turbidimetric detection reagent ratio of 1:10 was maintained. The mixed solution was placed in a clean cuvette, covered with a silicone stopper, and left to react for 10 minutes at room temperature before positioning it in the image collection area of an iPhone 13 (Apple, Cupertino, CA, USA) or a TU-19 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China) for detection. The setting for mobile phone camera included turning off the flash, setting auto white balance, and configuring all other parameters to auto, while the spectrophotometer was set at a wavelength of 546 nm. Each sample was tested three times. Four different color plates of white, red, green, and blue were used as the detection background of smartphone to conduct concentration and absorbance curve experiments. After acquiring the image, the characteristic absorbance and SARS-CoV-2 antibody concentration were used to establish the standard curve. A black box was also incorporated into the detection platform as the background light for gradient sample analysis (Figure 1).

Image acquisition and processing

The reaction solution was placed within the hexagonal frame at the image acquisition position. The distance between the mobile phone and the image acquisition location was fixed at 13 cm, and an LED screen positioned behind the image acquisition position ensured consistent lighting throughout the cuvette. The initial setting parameters of the LED screen were color temperature of 4,500K and light intensity of 18,000 Lux. Adobe Photoshop software (https://www.adobe.com/products/photoshop.html) was used to extract the R, G, and B values of the white background of the color-developed area and the blank area above it in the image. According to the method of previous research [12], the values were converted into characteristic absorbance for processing, which was calculated as follows.

$$Gray = 0.3R + 0.59G + 0.11B \quad (1)$$

$$A = -Log(Gray/Gray_0) \quad (2)$$

where Gray was the grayscale of the colored reaction area. $Gray_0$ was the grayscale of the blank area. The RGB values of the black background area were retrieved and transformed into grayscale values as well, which were denoted as Gray. Simultaneously, the RGB values of the blank area above the liquid level were acquired and converted into a grayscale value designated as $Gray_0$. The inverse relationship between the black background area and concentration was leveraged when computing the characteristic absorbance as below.

$$A = 1/[-Log(Gray/Gray_0)] \quad (3)$$

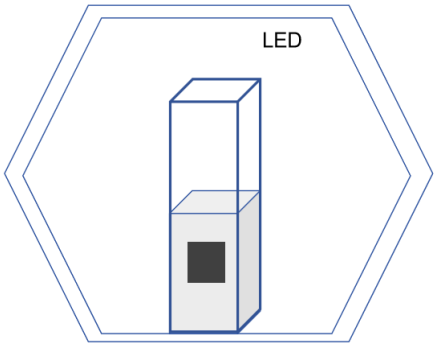

Figure 1. Immunoturbidimetric platform for smartphones.

Light intensity interference experiment

iPhone 13 was used as the detection device with a fixed color temperature of 4,500K and a

shooting distance of 13 cm to investigate the influence of light intensity. Five light intensities were simulated using an LED screen, and the light intensity was tested using a light meter, which were 7,300 Lux, 13,000 Lux, 18,000 Lux, 24,500 Lux, and 34,000 Lux. The influences of light intensity on the detection of six sample concentrations were investigated. Each sample was measured three times, and the average values were calculated.

Color temperature interference test

The light intensity was fixed at 18,000 Lux and the shooting distance was 13 cm to investigate the effect of color temperature on detection. The five color temperatures were tested as 3,300K, 3,900K, 4,500K, 5,000K, and 5,600K. The effects of light intensity on the detection of six sample concentrations were investigated. Each sample was measured three times. The average values were calculated.

Repeatability test and detection limit

iPhone 13 was used as the test phone. The LED screen was adjusted to a color temperature of 4,500K, a brightness of 18,000 Lux, and a shooting distance of 13 cm. Samples of three concentrations 29.8 AU/mL, 49 AU/mL, and 166.9 AU/mL were tested repeatedly with physiological saline as a blank sample. Each concentration sample was tested 20 times to obtain the test mean (AV) and standard deviation (SD). The detection limit was calculated as AV + 2SD.

Comparison experiment

30 unknown samples were used to compare the results obtained from a smartphone and a spectrophotometer, which covered the SARS-CoV-2 antibody detection concentration range. SPSS (IBM, Armonk, NY, USA) was employed to perform a paired sample t-test on the results of the two methods.

Results and discussion

Determination of the detection method

The results showed that, irrespective of the background color used, the gradient detected by the smartphone is significantly smaller compared to that detected by the spectrophotometer (Figure 2). Notably, when white light served as the background, no discernible linear gradient was observed. The application of red, green, and blue (RGB) lights as backgrounds resulted in minute linear gradients for each, rendering contrast item detection unattainable on smartphones. The immunoturbidimetric antibody determination operated on the principle that the presence of the target antibody induced latex microsphere agglomeration through antigen-antibody interactions, leading to turbidity changes. When assessed by a spectrophotometer, the absorbed light underwent significant changes in absorbance due to the aggregated microspheres. However, the alteration in image information induced by turbidity was less evident, particularly when the background was white light, where the turbidity itself appeared white, precluding effective differentiation. Turbidity influenced solution transmittance, causing objects behind the solution to appear lighter as turbidity increased. When the identified area corresponded to a black background, the color intensity diminished with increasing turbidity. The linear correlation between characteristic absorbance and sample concentration demonstrated a notable enhancement in the linear gradient of SARS-CoV-2 antibody detection on smartphone following the incorporation of a black background (Figure 3), therefore, the subsequent investigations extended the utilization of a black background as a reference in immunoturbidimetric antibody detection.

Optimization of quantitative parameters

The quantitative mathematical model optimization study examined the chromaticity values of the R, G, and B channels and converted them into Gray for quantitative analysis. The results showed that the employment of the R channel for quantification yielded the highest gradient, whereas the use of the B channel resulted in the smallest gradient (Figure 4). This

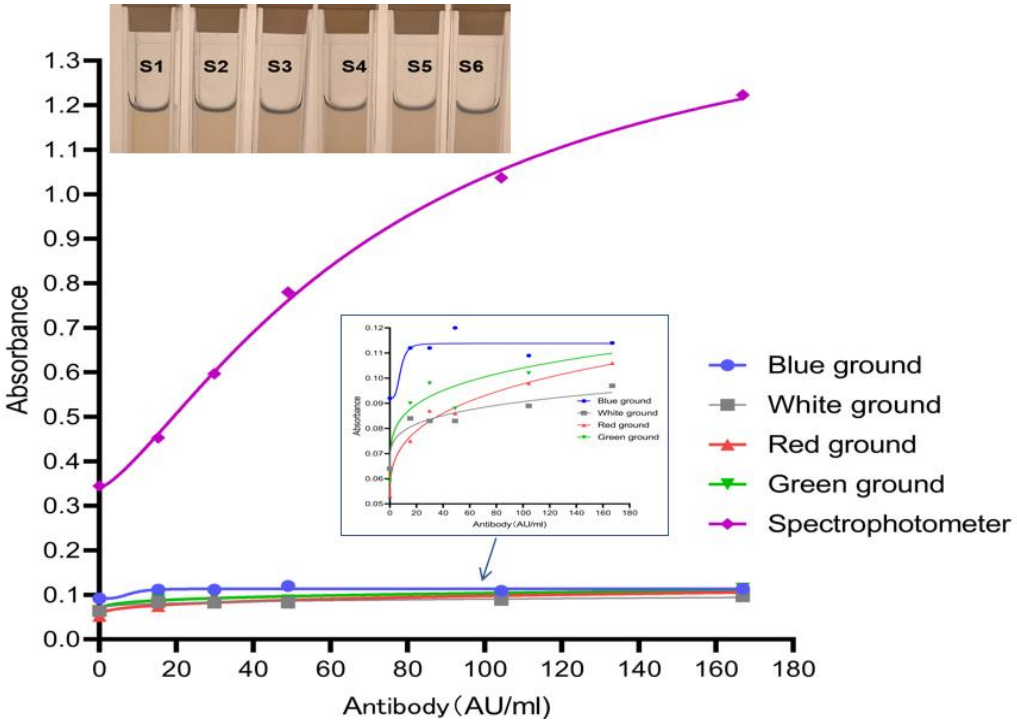

Figure 2. Effects of 4 test backgrounds on the gradient of SARS-CoV-2 antibody.

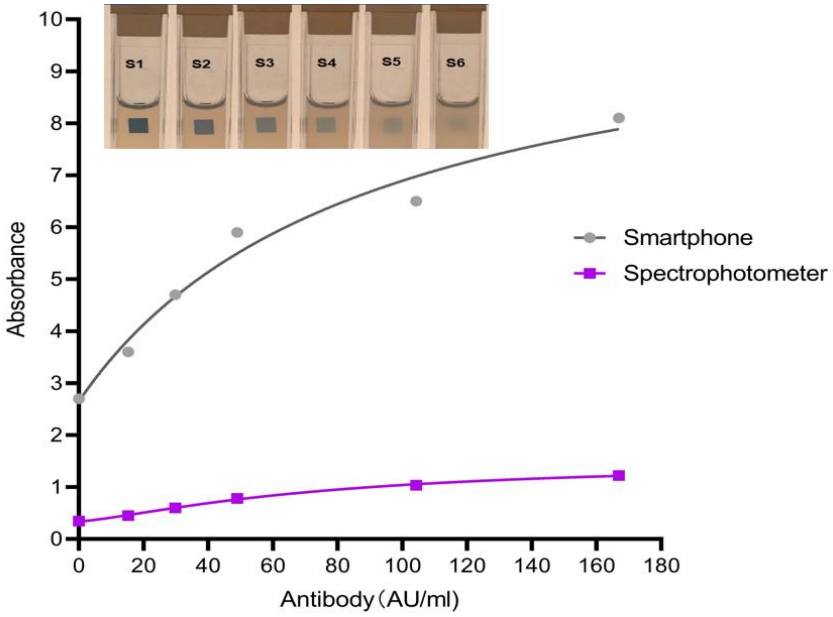

Figure 3. Comparison of gradients obtained from mobile phone and spectrophotometer tests.

observation aligned with the principle of complementary color development, where the R value corresponded to red, primarily absorbing short wavelengths, while the B value corresponded to blue, primarily absorbing long wavelengths. In the context of turbidity detection, aggregated microspheres were more likely to block short wavelengths, rendering short

wavelength detection more sensitive compared to long wavelength detection.

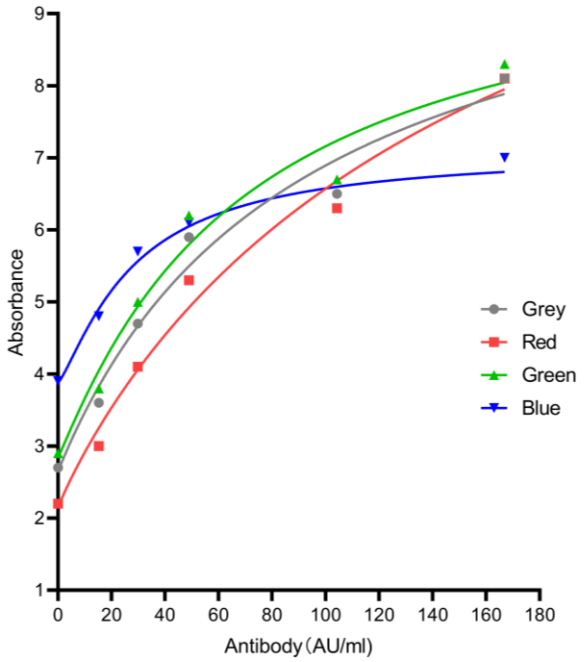

Figure 4. The characteristic absorbance of each color component against the concentration of SARS-CoV-2 antibodies.

Anti-interference research

In smartphone detection, commonplace interference factors encompassed light intensity and color temperature. This study systematically investigated the influence of these factors on various mathematical models to assess the effect of light intensity on samples with different concentrations. The results showed that light intensity exhibited no discernible impact on the detection of low-concentration samples (Figure 5A). However, for high-concentration samples, a positive correlation trend was evident between the detection signal and light intensity. The effects of color temperatures on the detection results showed that the color temperature had no effect on the detection of low-concentration samples. When detecting high-concentration samples, there was no obvious correlation between the detection signal intensity and the color temperature (Figure 5B). The overall impacts of light intensity and color temperature on detection were minimal, which was attributed to the fact that all data models extracted information from both color and blank areas. The influence of light on these areas remained consistent and tended to offset each other, thereby mitigating any substantial impact on the detection process.

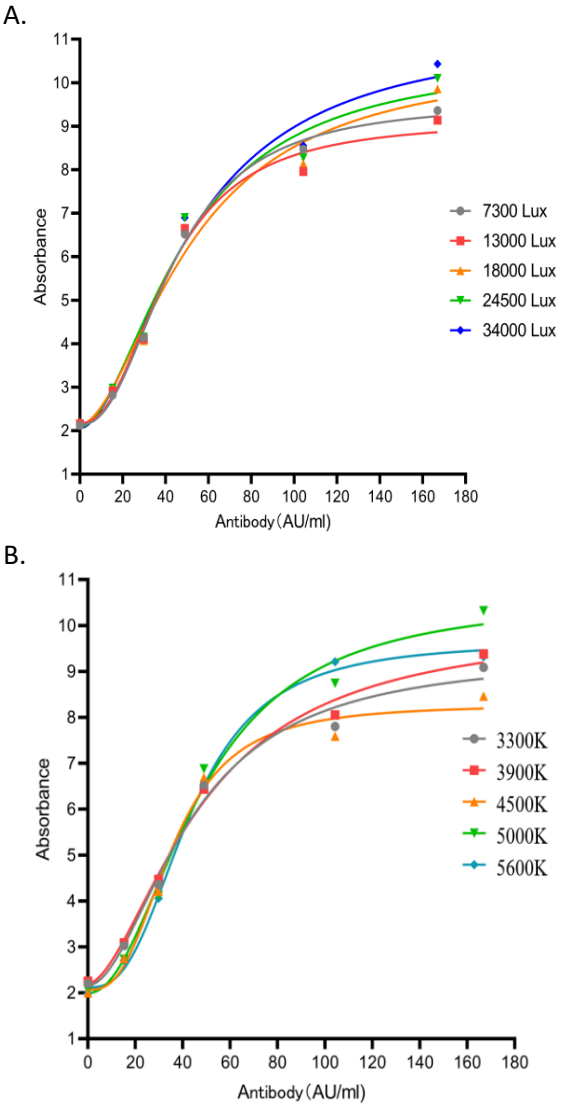

Figure 5. The influence of light intensity (A) and color temperature (B) on the detection of SARS-CoV-2 antibody.

Repeatability and detection limit

Repeatability is the premise for accurate and reliable test results. During the testing with a smartphone, the repeatability relative standard

deviations (RSDs) for three distinct sample concentrations of 29.8 AU/mL, 49 AU/mL, and 166.9 AU/mL were 3.9%, 6.0%, and 11.1%, respectively, with of them falling below 15%, thus affirming commendable repeatability (Table 1). The detection limit was determined as 0.09 AU/L for this proposed method.

Table 1. Reproducibility of three concentrations of human antibody against SARS-CoV-2 detected by smartphone.

	S3 (AU/mL)	S4 (AU/mL)	S6 (AU/mL)
Mean	27.62	54.98	184.13
SD	1.08	3.31	20.40
RSD	3.9%	6.0%	11.1%

Comparison with spectrophotometer

The same samples were tested using a smartphone and a spectrophotometer. The correlation coefficient between the results obtained through the two detection methods was 0.9162, signifying a robust correlation (Figure 6).

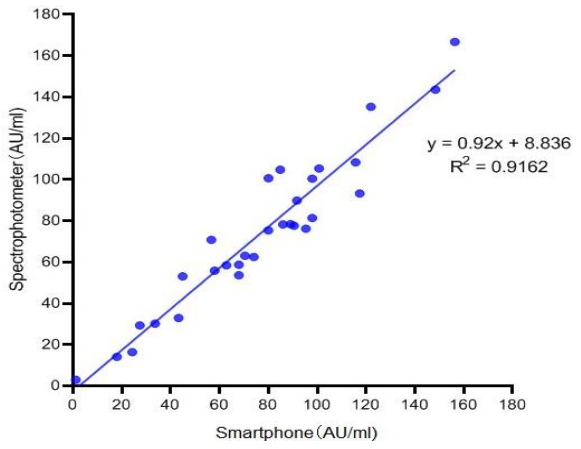

Figure 6. Comparison of test results between smart phone and spectrophotometer methods.

The comparison results showed that the contents of 30 unknown samples detected by smartphone and spectrophotometer were 76.82 ± 36.54 AU/mL and 73.89 ± 38.03 AU/mL, respectively, with the paired samples correlations shown that the Pearon correlation coefficient of the two methods was r = 0.957 and $P < 0.001$, indicating that the results of the two methods were highly correlated. The paired sample t test analysis results showed that the average value detected using a smartphone was 2.92 AU/mL, which was higher than that of a spectrophotometer with the 95% CI from -1.19 to 7.03. However, there was not a statistically significant between two groups (t = 1.455, $P > 0.05$), indicating that the two methods were consistent.

This research employed a smartphone and utilized the immunoturbidimetric method with a black dot box background as a control for SARS-CoV-2 antibody detection. The results exhibited commendable repeatability and linearity, demonstrating high consistency with spectrophotometer results. This study established a robust theoretical foundation for the method's promotion and application. Exploring the data model revealed the R channel signal's suitability for turbidimetric detection, given its maximal detection gradient. Presumably, the R channel's correspondence to short wavelengths enhanced signal blockage by aggregated microspheres. Subsequent smartphone-based antibody detection studies may prioritize investigating data models linked to the R channel. The method developed by this study for SARS-CoV-2 antibody detection demonstrated notable resistance to interference with negligible impacts from light intensity or color temperature on detection outcomes. This method introduced a novel approach for employing smartphones in immune detection of protein substances with broad applicability to the swift detection of antibodies, antigens, and other proteins. While this study employed transmission turbidimetry, it was worth noting that scattering turbidimetry offered heightened sensitivity, presenting a potential avenue for future research in smartphone-based methods.

Acknowledgements

This research was financially supported by the Natural Science Foundation of Hunan (Grant No. 2023JJ60207 and 2024JJ9053), Hunan Provincial Department of Education Scientific Research Project (Grant No. 23C0625 and 24C0863), Hunan Environmental Biology Vocational and Technical College Youth Fund Project (Grant No. QN2023-03).

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