



Published in final edited form as:

ACS Appl Mater Interfaces. 2025 May 14; 17(19): 28523–28531. doi:10.1021/acsami.5c02147.

Mesoporous Pd@Pt Nanoparticle Label/Lateral Flow Immunoassay Integrated with a 3D-Printed Smartphone Reader for Detection of Wood Smoke Biomarkers

Zhansen Yang[#],

School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99164, United States

Xinyi Li[#],

School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99164, United States

Yonghao Fu,

School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99164, United States

Yang Song,

School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99164, United States

Christopher D. Simpson,

Department of Environmental & Occupational Health Sciences, University of Washington, Seattle, Washington 98195, United States

Luke P. Naeher,

Department of Environmental Health Science, University of Georgia, Athens, Georgia 30602, United States

Yuehe Lin,

School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99164, United States

Dan Du

School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99164, United States; Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, Washington 99202, United States

Corresponding Authors: **Yuehe Lin** – School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99164, United States; yuehe.lin@wsu.edu, **Dan Du** – School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99164, United States; Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, Washington 99202, United States; annie.du@wsu.edu.

[#]Author Contributions

[#]Z.Y. and X.L. contributed equally to this work

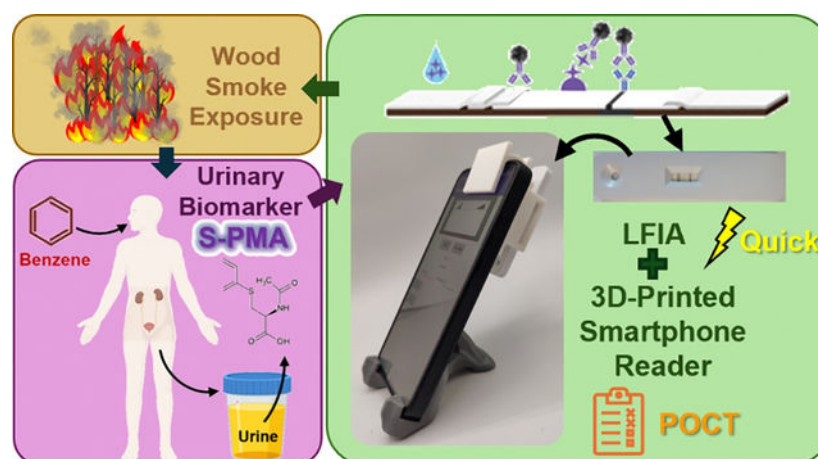
The authors declare no competing financial interest.

Complete contact information is available at: <https://pubs.acs.org/10.1021/acsami.5c02147>

Abstract

Wood smoke exposure poses significant health risks, particularly in occupations such as firefighting, where short-term exposure to high levels of pollutants is common. The biomonitoring of wood smoke-associated biomarkers is crucial for assessing human exposure. S-phenylmercapturic acid (S-PMA), a key metabolite of benzene, has been widely used as a reliable biomarker for this purpose. However, current S-PMA detection methods lack the speed, portability, and user-friendliness required for widespread, on-site applications. In this study, we propose a novel detection system that integrates mesoporous Pd@Pt nanoparticle-mediated lateral flow immunoassay (LFIA) with a 3D-printed smartphone-based reader for detecting S-PMA. Performance testing with S-PMA in phosphate-buffered saline and spiked urine samples yielded limits of detection of 0.5 ng/mL and 2.5 ng/mL, respectively. The use of mesoporous Pd@Pt nanoparticles as signal amplifiers for LFIA, along with the integration of a 3D-printed device for accurate image acquisition, significantly enhanced the system's sensitivity, achieving detection limits well below the threshold recommended by the American Conference of Governmental and Industrial Hygienists. With remarkable stability and reproducibility, our method provides a noninvasive, highly sensitive, rapid, portable, low-cost, and user-friendly solution for the on-site assessment of wood smoke exposure in occupational settings, laying the foundation for future innovations in real-time environmental exposure monitoring.

Graphical Abstract



Keywords

mesoporous Pd@Pt nanoparticles; lateral flow immunoassays; 3Dprinting; smartphone readers; wood smoke biomarkers

1. INTRODUCTION

Wood smoke contains numerous hazardous pollutants, including benzene, polycyclic aromatic hydrocarbons, and fine particulate matter, all of which are toxic and carcinogenic.^{1,2} Exposure to wood smoke can cause various health issues and has become a significant concern in occupational health, especially for firefighters, who experience high

levels of pollutant exposure over short periods.^{2–5} Current exposure assessment methods, such as subjective smoke density perceptions or prediction models based on meteorological data, are insufficient for accurately quantifying exposure levels data, are insufficient for accurately quantifying exposure levels and assessing their impact on human health.⁶ To address these limitations, directly measuring biomarkers associated with smoke exposure provides a more reliable and quantitative approach for assessing exposure levels.⁷

Benzene, a volatile organic compound (VOC) found in wood smoke, has drawn particular attention due to its association with severe health risks, including hematological disorders, leukemia, and other cancers following long-term or high-dose exposure.^{8,9} As an important metabolite of benzene in urine, S-phenyl mercapturic acid (S-PMA) has been identified as a sensitive biomarker for benzene exposure, even at low levels.^{8,10,11} Elevated levels of S-PMA in the urine of postshift firefighters highlight its relevance as a biomarker for wood smoke exposure.^{12,13} Given the significant contribution of wood smoke to benzene exposure in occupations like firefighting, S-PMA serves as a reliable marker for assessing human exposure to wood smoke.

Current S-PMA detection methods are primarily based on laboratory-based instrumental analysis, such as gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS).^{8,14–18} While highly sensitive and reliable, these techniques are costly, time-consuming, and require bulky instrumentation, complex sample pretreatment, and skilled professionals for operation. More recent methods, including those utilizing surface-enhanced Raman spectroscopy (SERS) or fluorescence modulation with metal–organic frameworks (MOFs),^{19,20} have been developed, but still lack the practicality for on-site use. Moreover, as S-PMA levels fluctuate rapidly after high-dose exposure, this further emphasizes the need for a portable and user-friendly on-site technique that provides quick and accurate detection of S-PMA for wood smoke exposure assessments.

Lateral flow immunoassays (LFIAs) are particularly promising for point-of-care testing (POCT) due to their portability, rapid results, selectivity, low cost, and ease of use.^{21,22} However, Au nanoparticle (NP)-based standard LFIAs often encounter challenges such as insufficient sensitivity and accuracy.^{21,23} To overcome these limitations, catalytic NPs with high peroxidase-like activity have been explored for their ability to generate stronger colorimetric signals via catalytic reactions, making them excellent candidates for signal amplification in biosensors.^{24–31} Among them, Pd@Pt NPs have garnered particular interest due to their bimetallic composition and distinctive mesoporous dendritic core–shell structure, which confer advantages such as facile synthesis, enhanced catalytic activity, increased surface area, and high stability. These mesoporous Pd@Pt NPs have been widely utilized for fabricating LFIAs with improved sensitivity and lower limits of detection (LOD) for various applications, including the detection of pathogens,^{32,33} pesticides,^{34–36} pharmaceuticals,³⁷ and protein biomarkers.³⁸

Moreover, quantifying LFIA results is still challenging. Recent advancements in smartphone-based platforms offer new opportunities for portable, low-cost, and user-friendly devices capable of performing on-site data acquisition.³⁹ Among these, 3D-printed smartphone-based devices are particularly appealing due to their rapid prototyping

capabilities and adaptability to various applications. Integrating smartphones, equipped with powerful calculation capabilities and high-resolution cameras, with 3D-printed devices, not only enables on-site data acquisition but also provides reliable and quick biological analysis in the field. While various smartphone-based LFIA systems have been reported,^{40–43} these devices are often tailored for specific functions. Thus, there is a need for a more flexible smartphone-based platform with broader applications that can accommodate diverse ambient conditions and smartphone configurations for on-site quantification and analysis based on LFIAs.

In this work, to overcome the challenges mentioned above, we developed a mesoporous Pd@Pt NP-mediated LFIA integrated with a 3D-printed smartphone reader for the on-site detection of S-PMA. Key features of this wood smoke detection system include: (1) a noninvasive, sensitive, and portable LFIA detection method for the urinary S-PMA biomarker; (2) synthesized mesoporous Pd@Pt NPs with high catalytic activity as signal amplifiers, enhancing the sensitivity and stability of the LFIA; (3) integration with a 3D-printed device and smartphone app for on-site data acquisition and biological analysis. This portable, sensitive, and low-cost LFIA, combined with a 3D-printed smartphone-based platform, enables rapid and reliable on-site assessment, advancing the field of POCT and facilitating large-scale screening for human exposure to wood smoke.

2. EXPERIMENTAL SECTION

2.1. Materials.

Pluronic F-127, potassium tetrachloroplatinate(II) (K_2PtCl_4), sodium tetrachloropalladate(II) (Na_2PdCl_4), L-ascorbic acid, acetone, potassium carbonate (K_2CO_3), sucrose, bovine serum albumin (BSA), 3,3',5,5'-tetramethylbenzidine (TMB), TWEEN 20, hydrogen peroxide (H_2O_2), rabbit antisheep IgG antibody and pH-indicator strips were purchased from Sigma-Aldrich (St. Louis, MO). Hydrochloric acid (HCl) was obtained from Lab Alley (Austin, TX). Phosphate buffered saline ($1\times$ PBS, pH 7.4) was obtained from Caisson Laboratories (Smithfield, UT). Sheep anti-S-PMA polyclonal antibody and S-PMA-BSA were purchased from Chemitrace (Kendal, UK). Nitrocellulose (NC) membrane was purchased from Sartorius (Göttingen, Germany). Backing cards were purchased from DCN Dx (Carlsbad, CA). Glass fiber pads and cellulose absorbent pads were purchased from Millipore (Burlington, MA). The lateral flow assay cassettes were purchased from Drummond Scientific Company (Broomall, PA). Ultrapure water, obtained from a Millipore Milli-Q water purification system, was used for experiments.

2.2. Synthesis of Mesoporous Pd@Pt NPs.

Mesoporous Pd@Pt NPs were synthesized following a previously published method.⁴⁴ Briefly, 100 mg of Pluronic F-127 was first ultrasonically dissolved in a mixed solution containing K_2PtCl_4 (9 mL, 20 mM), Na_2PdCl_4 (1 mL, 20 mM) and HCl (220 μ L, 6 M). Then, 10.0 mL of 100 mM L-ascorbic acid (AA) was added, and the mixture was subjected to continuous ultrasonic treatment at 35 °C for 4 h. The final product was centrifuged and washed five times with acetone and ultrapure water, respectively, before being dried at room

temperature. After resuspending in 25 mL of ultrapure water, the synthesized Pd@Pt NPs, with a concentration of 2 mg/mL, were stored at 4 °C for further use.

2.3. Preparation of Mesoporous Pd@Pt NP/Antibody Conjugates.

Mesoporous Pd@Pt NP/antibody conjugates were prepared according to a previously published method with slight modifications.²⁸ The pH of the Pd@Pt NP suspension (2 mg/mL) was first adjusted to 8.2–8.5 by adding 0.02 M K₂CO₃. Subsequently, 5 μ g of sheep anti-S-PMA polyclonal antibody was introduced into 1 mL of the adjusted NP suspension, followed by shaking incubation at room temperature for 60 min. Afterward, 10% (w/v) BSA was added, and the mixture was incubated with shaking for an additional 30 min. The conjugates were then collected by centrifugation at 9,000 rpm for 15 min and washed twice with 1 $\times$ PBS containing 1% BSA. Finally, the mesoporous Pd@Pt NP/antibody conjugates were resuspended in 100 μ L of PBS containing 2% BSA and 3% sucrose and stored at 4 °C for further use.

2.4. Preparation of Lateral Flow Immunoassays.

The LFIA consisted of five components: a sample pad, a conjugate pad, an NC membrane, an absorbent pad, and a backing card. The sample and conjugate pads, both made from glass fiber, were cut to specific sizes (300 $\times$ 20 mm for the sample pad and 300 $\times$ 5 mm for the conjugate pad), soaked in boric acid/borax buffer (pH 8.0), and then dried overnight at room temperature. The NC membrane was affixed to the backing card, and S-PMA-BSA (1.5 mg/mL) and rabbit anti-sheep IgG (1.0 mg/mL) were dispensed onto designated locations using an XYZ3050 Dispense System (BioDot Inc., Irvine, CA) to form the test and control lines, respectively. The membrane and backing card assembly were then dried at 37 °C for 3 h. Next, the sample pad, conjugate pad, and a cellulose absorbent pad were assembled onto the backing card, and the assembly was cut into 4 mm-wide individual strips using a CM4000 Guillotine Cutter (BioDot Inc., Irvine, CA). Subsequently, 2.0 μ L of Pd@Pt NP/antibody conjugates were applied to the overlapping region of the conjugate and sample pads. Finally, each test strip was placed into a lateral flow assay cassette.

2.5. Assay Procedure.

50 μ L of the sample to be detected was mixed with 50 μ L of PBST (mixed solution containing 0.1% TWEEN 20) running buffer and added to the conical sample port, where it migrated to the absorbent pad by capillary action. Once the black color appeared and stabilized on the test and control lines (approximately 10 min), the test strip was inserted into the cartridge slot of the 3D-printed device, and an image was captured using the smartphone. Next, 1.0 μ L of TMB solution (containing 0.03% H₂O₂) was applied to the test line, and the appearance of a blue colorimetric signal was observed. A second image was then captured using the same setup. The readout results of the test strip were provided by the “Strip Scan” app.

2.6. Image Capture and Analysis.

For LFIA optimization, test strip images were captured using a HUAWEI Mate 30 (equipped with a 40-megapixel main rear camera) and analyzed with ImageJ (NIH, MD) to determine

the peak area values of the test and control lines. To assess the detection performance of the proposed system (for S-PMA detection in PBST and urine samples), images of the test strips were captured and analyzed using our 3D-printed smartphone reader.

2.7. Characterization Methods.

TEM measurements were conducted using a FEI Tecnai G2 20 Twin, operating in bright field mode with a 200 kV LaB6 electron source, and images were captured using an integrated 4K Eagle camera. SEM imaging was performed using an Apreo VolumeScope SEM equipped with a high-resolution secondary electron detector, operating under high vacuum. For optimal resolution and detail, the SEM images were obtained at a 30 kV accelerating voltage, 0.8 nA beam current, 3.6 mm working distance, and a 35 μ s scan speed.

3. RESULTS AND DISCUSSION

3.1. Mechanism of S-PMA Detection.

The proposed S-PMA detection system, as illustrated in Scheme 1, integrated LFIA with a 3D-printed smartphone reader. The LFIA functions as the core component of the S-PMA detection and operates on the principle of competitive assays. In the absence of S-PMA in the test sample, the applied Pd@Pt NP/antibody conjugates migrate along the NC membrane to the test line. There, they bind to S-PMA-BSA, forming Pd@Pt NP/antibody-S-PMA-BSA complexes, which result in a visible black line. The unbound Pd@Pt NP/antibody conjugates continue migrating to the control line, where they bind to rabbit anti-sheep IgG, forming another black line. When S-PMA is present in the test sample, it competes with S-PMA-BSA on the test line for binding to the Pd@Pt NP/antibody conjugates. This competition reduces the formation of Pd@Pt NP/antibody-S-PMA-BSA complexes at the test line, resulting in a gray line instead of a black one. Upon addition of TMB and H₂O₂, the peroxidase-like catalytic activity of the Pd@Pt NPs produces a strong blue color, enhancing the colorimetric signal. The quantification of LFIA results was achieved using the proposed 3D-printed smartphone reader, where the 3D-printed device ensured vibration resistance, accurate positioning, and improved contrast for image capture, while the smartphone app enabled on-site data analysis.

3.2. Characterization of Mesoporous Pd@Pt NPs and Pd@Pt NP/Antibody Conjugates.

To evaluate the morphology of the synthesized Pd@Pt NPs, we performed scanning electron microscopy (SEM) and transmission electron microscopy (TEM), as shown in Figure 1B. The Pd@Pt NPs exhibited a spherical, hyperbranched, mesoporous nanostructure with a uniform size of 45–50 nm, which was evident in both SEM and TEM images. This distinctive concave morphology resulted from the formation mechanism of mesoporous bimetallic Pd@Pt NPs, described as a surfactant-assisted self-assembly process, as illustrated in Figure 1A. In this process, the Pluronic F-127 surfactant first formed spherical micelles, with hydrophobic poly(propylene oxide) (PPO) positioned internally and hydrophilic poly(ethylene oxide) (PEO) oriented externally, serving as structural templates for the nanoparticles. With the introduction of ascorbic acid (AA) as a reducing agent, a Pd-dominated core initially formed, followed by the subsequent deposition of Pt, resulting in a

core-shell structure. This sequential growth was driven by the faster reduction rate of the Pd precursor compared to that of the Pt precursor. The high lattice match between Pd and Pt led to the epitaxial growth of Pt, promoting the formation of the uniform concave morphology. Additionally, the presence of HCl during synthesis optimized the pH conditions for Pt growth, resulting in the highly branched Pt structures in the shell. Simultaneously, F127 micelles, in coordination with metal-aqua complexes, served as structure-directing agents.⁴⁵ From the TEM image (Figure 1B), the Pt dendrites (2–3 nm in size) that construct the outer shell, supported by the Pd inner core, were distinctly visible. This unique mesoporous, dendritic, flower-shaped core-shell structure provides a large surface area and numerous active sites for catalytic reactions, contributing to the high catalytic performance of the Pd@Pt NPs.

Following antibody conjugation, TEM (Figure 1C) image shows that the NPs preserved their spherical, mesoporous, hyperbranched morphology and consistent size, suggesting that the conjugation process did not compromise the structural integrity of the particles. Additionally, high-contrast TEM image revealed a faint halo around the particles, indicating successful antibody attachment to the Pd@Pt NP surface. The absence of structural deformation or aggregation in the TEM image further confirmed the stability of the Pd@Pt NPs after conjugation.

3.3. Optimization of the LFIA.

To achieve optimal LFIA performance, several key parameters were optimized (as shown in Figure 2), including the amount of conjugated antibody in the preparation of Pd@Pt NP/antibody conjugates, the volume of Pd@Pt NP/antibody conjugates, the concentration of S-PMA-BSA on the test line, and the pH of the phosphate-buffered saline containing 0.1% Tween-20 (PBST) running buffer. The amount of conjugated antibody and the volume of Pd@Pt NP/antibody conjugates influence the binding efficiency of the detection probe to the target, thereby affecting the performance of the LFIA. As shown in Figure 2A, the LFIA signal increased and reached its maximum at 5 μg under both conditions (with or without TMB and H_2O_2), then decreased at 6 μg , indicating that the optimal result was achieved by adding 5 μg of S-PMA antibody to 1 mL of Pd@Pt NPs (2mg/mL). As presented in Figure 2B, for varying volumes of Pd@Pt NP/antibody conjugates, the signal increased up to 2.0 μL and remained stable (with TMB and H_2O_2 added), indicating saturation of the blue colorimetric signal. Without TMB and H_2O_2 added, the signal decreased at 2.5 μL . Therefore, 2 μL of Pd@Pt NP/antibody conjugates was determined to be optimal for both conditions. The S-PMA-BSA on the test line competes with the target for binding sites on the Pd@Pt NP/antibody conjugates. The concentration of S-PMA-BSA affects the signal modulation of LFIA detection and, thus, determines the detection range. As shown in Figure 2C, the signal reached its maximum at 1.50 mg/mL under both conditions (with or without TMB and H_2O_2). Therefore, 1.5 mg/mL of S-PMA-BSA was selected for optimal results. For LFIA detection, the PBST running buffer is critical for minimizing nonspecific interactions. The pH of PBST can also affect the binding efficiency of the detection probe to the target, as well as the peroxidase-like catalytic activity of Pd@Pt NPs, thereby influencing the overall performance of the proposed LFIA. A pH close to physiological conditions (6.5–7.9) is generally ideal for optimal binding. As shown in Figure

2D, signal variation was minimal for both conditions (with or without TMB and H₂O₂), and the optimal result was obtained at pH 7.1. In summary, 5 μ g of conjugated antibody, 2 μ L of Pd@Pt NP/antibody conjugates, 1.50 mg/mL of S-PMA-BSA, and PBST running buffer at pH 7.1 were selected for subsequent experiments.

3.4. Design of the 3D-Printed Smartphone Reader.

To enhance image contrast and enable quantification of the test strip results, we designed a 3D-printed smartphone-based lens device, as shown in Figure 3. The device consisted of three main components: a smartphone clamp, a major block housing strip cartridge slots, and a lens holder with a convex lens. These components were designed in Fusion 360 (CAD software) and fabricated using a commercial 3D printer (EinStar3D, Co., Shenzhen, China).

The choice of white polylactic acid (PLA) filament and the hollow design of the major block facilitated optimal ambient light transmission for image capture. To accommodate smartphones with varying thicknesses and camera positions, an adjustable phone clamp was employed. Instead of a holder with specific dimensions for specific smartphone model, this phone clamp design can spread the applicability of the 3D-printed device. The designed phone clamp was expected to suit majority of the manufactured smartphones in the market. An easily removable and adjustable lens holder with a convex lens was placed in front of the strip cartridge slot within the major block. This additional lens expands the field of view (FOV) when capturing images at close distances using a smartphone, which enhances image contrast by improving resolution and reducing scattered ambient light.

Figure 3B shows the images taken with (right) and without (left) the lens device, demonstrating the improved contrast when using the 3D-printed apparatus. Notably, optical lenses with different focal lengths can be selected and installed on the lens holder, and its y and z positions can be adjusted to fit various smartphone camera setups. Additionally, the 3D-printed smartphone-based lens device enables image capture with vibration resistance, making it suitable for on-site applications that require stable and accurate measurements under diverse ambient conditions. The entire 3D-printed device is compact, with dimensions under 6 cm in length, 4 cm in width, and 4 cm in height, and costs less than \$2.

Images of the test strips were captured and analyzed using our 3D-printed smartphone reader, as demonstrated in Figure 3C and 3D. A previously developed Android strip reader app, “Strip Scan”,⁴⁰ was employed to generate quantitative results from the captured images. The typical interface of the app is shown in Figure 3D, where the “FIRST” and “SECOND” peak area values were extracted to quantify the test strip results.

3.5. Performance of S-PMA Detection.

To evaluate the performance of the proposed detection system, varying concentrations of S-PMA in PBST were tested and compared to results from Au NP-based standard LFIAs using previously optimized parameters. Figures 4A, 4B, and 4C depict the LFIA detection of S-PMA at concentrations of 1000, 100, 10, 1, 0.1, 0.01, and 0 ng/mL (from left to right), utilizing Pd@Pt NPs, Pd@Pt NPs with the addition of TMB and H₂O₂, and Au NPs, respectively. Quantitative results were obtained by capturing images using the 3D-printed

device, cropping the test zones, and analyzing them with a previously developed smartphone app that employed a novel algorithm to extract the peak area values of the test lines.

Due to the principle of competitive LFIA, the signal, represented by the peak area value of the test line, is expected to be inversely proportional to the concentration of S-PMA. Figure 4D shows the relationship between the peak area values and the log-transformed concentrations of S-PMA (0, 0.01, 0.1, 1, 10, 100, and 1000 ng/mL) in PBST for all three LFIAs, which confirmed the inverse correlation. The linear detection range for the standard LFIA using Au NPs as signal labels was 1–1000 ng/mL ($y = -1595.6x + 4836.6$, $R^2 = 0.98$). Similarly, the LFIA using Pd@Pt NPs without TMB and H_2O_2 showed a comparable linear range of 1–1000 ng/mL ($y = -1709.6x + 7927.5$, $R^2 = 0.977$). Notably, the addition of TMB and H_2O_2 extended the linear range to 0.1–1000 ng/mL ($y = -1301.1x + 8474.6$, $R^2 = 0.9818$), with the lower boundary decreased by 1 order of magnitude.

We defined the limit of detection (LOD) as the concentration of S-PMA at which the signal intensity equals the mean signal intensity at 0 ng/mL minus three times the standard deviation. The LOD of the Pd@Pt NP-mediated LFIA, in the absence of TMB and H_2O_2 , was calculated to be 6.96 ng/mL, which is lower than the calculated LOD of the Au NP-based LFIA (9.72 ng/mL). With the addition of TMB and H_2O_2 , the LOD decreased to 0.50 ng/mL, showing a one-order-of-magnitude reduction. This significant enhancement in sensitivity was attributed to the peroxidase-like catalytic activity of mesoporous Pd@Pt NPs, which acted as signal amplifiers in LFIA detection. Because of the peroxidase-like property of Pd@Pt, it can produce an amplified colorimetric signal in the presence of TMB + H_2O_2 , which is the sum of the inherent colorimetric signal of the nanozyme and the catalytic product, consequently, enhancing the sensitivity of LFIA.²⁴

3.6. Detection of S-PMA in Urine Samples.

To demonstrate the proof-of-concept and potential application for the practical screening of human exposure to wood smoke, human urine samples were spiked with varying known concentrations of S-PMA and subsequently analyzed using the proposed detection system. Figure 5 shows the relationship between the peak area values and the logarithm of S-PMA concentrations (0.5, 1, 2, 4, 20, 100, and 500 ng/mL) in urine samples. The linear range for the LFIA, in the absence of TMB and H_2O_2 , was 2–500 ng/mL ($y = -3241.7x + 9140.2$, $R^2 = 0.9936$). When TMB and H_2O_2 were added, the linear range expanded to 0.5–500 ng/mL ($y = -3331.8x + 9715.8$, $R^2 = 0.9948$). Notably, the calculated LOD for the Pd@Pt NP-mediated LFIA with TMB and H_2O_2 was 2.50 ng/mL, which is considerably lower than the LOD of the LFIA without TMB and H_2O_2 (7.05 ng/mL).

The biological exposure index (BEI) for urinary S-PMA levels of 25 $\mu\text{g/g}$ creatinine is suggested by the American Conference of Governmental Industrial Hygienists (ACGIH) as biomonitoring equivalent to an exposure of 0.5 ppm benzene in the air.⁴⁶ Since typical creatinine concentrations in human urine range from 80 to 200 $\mu\text{g/dL}$,⁴⁷ the LOD of our proposed detection system for S-PMA is substantially lower than the ACGIH recommended standard. This highlights the practical significance of our method for assessing human exposure to wood smoke.

Additionally, we calculated the coefficient of variation (CV) for the peak area of ten urine samples spiked with 20 ng/mL and another ten spiked with 100 ng/mL S-PMA, as shown in Table 1. The CV values were relatively low under both conditions (with and without TMB + H₂O₂) and for both S-PMA concentrations (20 ng/mL and 100 ng/mL), demonstrating the excellent stability and reproducibility of our method.

4. CONCLUSIONS

In summary, we have developed a novel detection system that integrated LFIA with a smartphone-based platform, enabling the highly sensitive detection of S-PMA in urine and facilitating rapid, on-site evaluation of human exposure to wood smoke. The performance of the proposed detection system was evaluated by testing S-PMA in PBST and spiked urine samples, achieving LODs of 0.5 ng/mL and 2.5 ng/mL, respectively. The enhanced sensitivity, compared to standard Au NP-based LFIA, was attributed to the use of mesoporous Pd@Pt NPs as signal amplifiers, leveraging their excellent peroxidase-like catalytic properties. Furthermore, this performance is competitive with reported mass spectrometry methods (LOD = 0.2–50 ng/mL).^{8,14–18} This noninvasive urinary S-PMA detection method offers significant advantages over conventional techniques in terms of speed, portability, cost-effectiveness, and ease of use. By integrating a 3D-printed smartphone reader, including a custom-designed 3D-printed device and an accompanying app, it ensures accurate data acquisition and on-site quantification. The proposed detection approach demonstrates excellent sensitivity, which is well below the ACGIH-recommended standard, along with remarkable stability and reproducibility. The intrabatch difference remains below 10%, while the interbatch difference stays below 15%, both of which are within the acceptable range for biological assays. Overall, this detection system offers significant advantages and holds great potential for practical application in monitoring occupational exposure to wood smoke. Moreover, it provides valuable insights into advancing POCT across various fields, including environmental health monitoring, occupational safety, and biomarker-based diagnostics. By offering a robust and user-friendly approach, it paves the way for broader adoption in public health screening and real-time environmental exposure assessments.

ACKNOWLEDGMENTS

This work was supported by the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health (CDC/NIOSH) Grant No. 1 R01OH012579–01-00. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC. We thank the WSU Franceschi Microscopy & Imaging Center for providing access to the SEM and TEM instruments.

REFERENCES

- (1). Jaffe DA; O'Neill SM; Larkin NK; Holder AL; Peterson DL; Halofsky JE; Rappold AG Wildfire and Prescribed Burning Impacts on Air Quality in the United States. *J. Air Waste Manage Assoc* 2020, 70 (6), 583–615.
- (2). Cherry N; Aklilu Y; Beach J; Britz-McKibbin P; Elbourne R; Galarneau J-M; Gill B; Kinniburgh D; Zhang X Urinary 1-Hydroxypyrene and Skin Contamination in Firefighters Deployed to the Fort McMurray Fire. *Ann. Work Expo Health* 2019, 63 (4), 448–458. [PubMed: 30753267]

- (3). Valière M; Petit P; Persoons R; Demeilliers C; Maître A Consistency between Air and Biological Monitoring for Assessing Polycyclic Aromatic Hydrocarbon Exposure and Cancer Risk of Workers. *Environ. Res.* 2022, 207, 112268. [PubMed: 34695431]
- (4). Williamson-Reisdorph CM; Tiemessen KG; Christison K; Gurney S; Richmond D; Wood K; Quindry TS; Dumke CL; Quindry JC Cardiovascular and Blood Oxidative Stress Responses to Exercise and Acute Woodsmoke Exposure in Recreationally Active Individuals. *Wilderness Environ. Med.* 2022, 33 (1), 17–24. [PubMed: 34887190]
- (5). Adetona O; Reinhardt TE; Domitrovich J; Broyles G; Adetona AM; Kleinman MT; Ottmar RD; Naeher LP Review of the Health Effects of Wildland Fire Smoke on Wildland Firefighters and the Public. *Inhal Toxicol* 2016, 28 (3), 95–139. [PubMed: 26915822]
- (6). Reinhardt TE; Ottmar RD Smoke Exposure at Western Wildfires 2000, DOI: 10.2737/PNW-RP-525.
- (7). Barros B; Oliveira M; Morais S Urinary Biohazard Markers in Firefighters. *Advances in Clinical Chemistry* 2021, 105, 243–319. [PubMed: 34809829]
- (8). Rosting C; Olsen R Biomonitoring of the Benzene Metabolite S-Phenylmercapturic Acid and the Toluene Metabolite s-Benzylmercapturic Acid in Urine from Firefighters. *Toxicol. Lett.* 2020, 329, 20–25. [PubMed: 32380125]
- (9). Barros B; Oliveira M; Morais S Biomonitoring of Firefighting Forces: A Review on Biomarkers of Exposure to Health-Relevant Pollutants Released from Fires. *Journal of Toxicology and Environmental Health, Part B* 2023, 26 (3), 127–171.
- (10). Fustinoni S; Consonni D; Campo L; Buratti M; Colombi A; Pesatori AC; Bonzini M; Bertazzi PA; Foà V; Garte S; Farmer PB; Levy LS; Pala M; Valerio F; Fontana V; Desideri A; Merlo DF Monitoring Low Benzene Exposure: Comparative Evaluation of Urinary Biomarkers, Influence of Cigarette Smoking, and Genetic Polymorphisms. *Cancer Epidemiology, Biomarkers & Prevention* 2005, 14 (9), 2237–2244.
- (11). Scheepers PTJ; de Werdt L; van Dael M; Anzion R; Vanoirbeek J; Duca RC; Creta M; Godderis L; Warnakulasuriya DTD; Devanarayana NM Assessment of Exposure of Gas Station Attendants in Sri Lanka to Benzene, Toluene and Xylenes. *Environ. Res.* 2019, 178, 108670. [PubMed: 31472361]
- (12). Lovreglio P; Carrieri M; Barbieri A; Sabatini L; Fracasso ME; Doria D; Iavicoli S; Drago I; D'errico MN; Imbriani M; Violante FS; Bartolucci GB; Soleo L Applicability of Urinary Benzene to Biological Monitoring of Occupational and Environmental Exposure to Very Low Benzene Concentrations. *G Ital Med. Lav Ergon* 2011, 33 (1), 41–46.
- (13). Carrieri M; Spatari G; Tranfo G; Sapienza D; Scapellato ML; Bartolucci GB; Manno M Biological Monitoring of Low Level Exposure to Benzene in an Oil Refinery: Effect of Modulating Factors. *Toxicol. Lett.* 2018, 298, 70–75. [PubMed: 30086327]
- (14). Milagres VG; de Paiva MJN; d'Auriol-Souza MM; Larentis AL; Costa-Amaral IC; André LC Comparison Study on Extraction Techniques Used to Assess S-phenylmercapturic Acid as Low Benzene Exposure Biomarker Based on Gas Chromatography Coupled with Mass Spectrometry. *Sep Sci. Plus* 2023, 6, 2300031.
- (15). Dugheri S; Mucci N; Cappelli G; Bonari A; Campagna M; Arcangeli G; Bartolucci G New Fully Automated Gas Chromato-graphic Analysis of Urinary S-phenylmercapturic Acid in Isotopic Dilution Using Negative Chemical Ionization with Isobutane as Reagent Gas. *Journal of Mass Spectrometry* 2020, 55, e4481. [PubMed: 31770470]
- (16). Melikian AA; Qu Q; Shore R; Li G; Li H; Jin X; Cohen B; Chen L; Li Y; Yin S; Mu R; Zhang X; Wang Y Personal Exposure to Different Levels of Benzene and Its Relationships to the Urinary Metabolites S-Phenylmercapturic Acid and Trans, TransMuconic Acid. *Journal of Chromatography B* 2002, 778 (1–2), 211–221.
- (17). Schwedler G; Murawski A; Schmied-Tobies MIH; Rucic E; Scherer M; Pluym N; Scherer G; Bethke R; Kolossa-Gehring M Benzene Metabolite S-PMA and Acrylamide Metabolites AAMA and GAMA in Urine of Children and Adolescents in Germany - Human Biomonitoring Results of the German Environmental Survey 2014–2017 (GerES V). *Environ. Res.* 2021, 192, 110295. [PubMed: 33065072]
- (18). Tevis DS; Willmore A; Bhandari D; Bowman B; Biren C; Kenwood BM; Jacob P; Liu J; Bello K; Hecht SS; Carmella SG; Chen M; Gaudreau E; Bienvenu J-F; Blount BC; De Jesús VR

Large Differences in Urinary Benzene Metabolite S-Phenylmercapturic Acid Quantitation: A Comparison of Five LC-MS-MS Methods. *J. Anal. Toxicol.* 2021, 45 (7), 657–665. [PubMed: 33025019]

- (19). Wei H; Zou W; Feng R; Liu L; Zhang M; Meng X; Chen W; Jia Q; Wang C Point-of-Care Testing of Benzene Metabolite S-Phenylmercapturic Acid Using Salt-Induced Phase Separation Combined with Nanoparticle-Based Surface-Enhanced Raman Spectroscopy. *ACS Appl. Nano Mater.* 2024, 7 (14), 16237–16244.
- (20). Li W; Li D; Yang Y; Su S; Qie S; Jia Y; Hu M Identification of S-Phenylmercapturic Acid Using Heterometallic Zn-Eu MOF as a Fluorescence Sensor. *J. Mol. Struct.* 2025, 1321, 139974.
- (21). Atta S; Zhao Y; Sanchez S; Seedial D; Devadhasan JP; Summers AJ; Gates-Hollingsworth MA; Pflughoeft KJ; Gu J; Montgomery DC; AuCoin DP; Zenhausem F; Vo-Dinh T Plasmonic-Enhanced Colorimetric Lateral Flow Immunoassays Using Bimetallic Silver-Coated Gold Nanostars. *ACS Appl. Mater. Interfaces* 2024, 16 (40), 54907–54918. [PubMed: 39342509]
- (22). Du D; Wang J; Wang L; Lu D; Lin Y Integrated lateral flow test strip with electrochemical sensor for quantification of phosphorylated cholinesterase: biomarker of exposure to organo-phosphorus agents. *Anal. Chem.* 2012, 84, 1380–1385. [PubMed: 22243414]
- (23). Li Y; Chen L; Li R; Zhao X; Shi M; Zhang G; Li F Long-Term Stable Eu₂O₃-Loaded Dendritic Mesoporous Silica Nanoprobes with Coordination-Enhanced Photoluminescence for Ultrasensitive Lateral Flow Immunoassay. *ACS Appl. Mater. Interfaces* 2024, 16 (45), 61645–61654. [PubMed: 39480967]
- (24). Panferov VG; Liu J Optical and Catalytic Properties of Nanozymes for Colorimetric Biosensors: Advantages, Limitations, and Perspectives. *Adv. Opt Mater.* 2024, 12, 2401318.
- (25). Niu X; Cheng N; Ruan X; Du D; Lin Y Review—Nanozyme-Based Immunosensors and Immunoassays: Recent Developments and Future Trends. *J. Electrochem. Soc.* 2020, 167 (3), 037508.
- (26). Xu M; Zhao S; Lin C; Li Y; Zhang W; Peng Y; Xiao R; Huang Z; Yang Y Dual-Mode Lateral Flow Immunoassay Based on “Pompon Mum”-Like Fe₃O₄@MoS₂@Pt Nanotags for Sensitive Detection of Viral Pathogens. *ACS Appl. Mater. Interfaces* 2024, 16 (9), 11172–11184. [PubMed: 38388390]
- (27). Shu R; Liu S; Wang M; Yin X; Cheng Y; Wang J; Zhang D Antibody-mimic nanozyme mediating sandwich lateral flow immunoassay with bulk and parallel catalysis for Salmonella typhimurium detection. *Chem. Eng. J.* 2025, 506, 159862.
- (28). Xu X; Sun T; Zhou X; Liu Z; Zhang L Specific and enzyme-free monitoring of propiconazole pesticide residues in vegetables with a portable nanozyme-based paper sensor. *Food Chem.* 2025, 464, 141686. [PubMed: 39437679]
- (29). Ma K; Cao Y; Xuan C; Yang J; Li F; Zhe T; Luo Q; Wang L Ru nanoclusters engineer Prussian blue analog nanocubes for multiple active sites synergistic catalysis enabling high-performance bacterial detection. *Chem. Eng. J.* 2025, 507, 160553.
- (30). Xu Z; Tong C; Zhang T; Xu Z; Ma Y; Niu Z; Chen J; Zhang M; Shi F Rapid Detection of Brucellosis by Two-Signal Vertical Flow Immunofiltration Based on Three-Dimensional Flower-Like Au@Mn₃O₄ Nanozymes. *ACS Appl. Mater. Interfaces* 2025, 8, 6014.
- (31). Cheng X; Chen H; Li W; Tu Z; Wang Y; Wei H; Wang S; Liu L; Rong Z Nanozyme-Catalyzed Colorimetric Microfluidic Immunosensor for the Filtration Enrichment and Ultrasensitive Detection of Salmonella typhimurium in Food Samples. *Anal. Chem.* 2025, 97, 6454. [PubMed: 40043091]
- (32). Cheng N; Song Y; Zeinhom MMA; Chang Y-C; Sheng L; Li H; Du D; Li L; Zhu M-J; Luo Y; Xu W; Lin Y Nanozyme-Mediated Dual Immunoassay Integrated with Smartphone for Use in Simultaneous Detection of Pathogens. *ACS Appl. Mater. Interfaces* 2017, 9 (46), 40671–40680. [PubMed: 28914522]
- (33). Wang X; Zhang M; Pang X; Huang K; Yao Z; Mei X; Cheng N Comparative Study of Pd@Pt Nanozyme Improved Colorimetric N-ELISA for the Paper-Output Portable Detection of Staphylococcus Aureus. *Talanta* 2022, 247, 123503. [PubMed: 35649327]

- (34). Kwon EY; Ruan X; Wang L; Lin Y; Du D; Van Wie BJ Mesoporous Pd@Pt Nanoparticle-Linked Immunosorbent Assay for Detection of Atrazine. *Anal. Chim. Acta* 2020, 1116, 36–44. [PubMed: 32389187]
- (35). Majid Z; Zhang Q; Yang Z; Che H; Cheng N A Multi-Enzyme Cascade Response for the Colorimetric Recognition of Organophosphorus Pesticides Utilizing Core-Shell Pd@Pt Nanoparticles with High Peroxidase-like Activity. *Foods* 2023, 12 (17), 3319. [PubMed: 37685251]
- (36). Ruan X; Wang Y; Kwon EY; Wang L; Cheng N; Niu X; Ding S; Van Wie BJ; Lin Y; Du D Nanomaterial-Enhanced 3D-printed Sensor Platform for Simultaneous Detection of Atrazine and Acetochlor. *Biosens Bioelectron* 2021, 184, 113238. [PubMed: 33878594]
- (37). Kalambate PK; Li Y; Shen Y; Huang Y Mesoporous Pd@Pt Core-Shell Nanoparticles Supported on Multi-Walled Carbon Nanotubes as a Sensing Platform: Application in Simultaneous Electrochemical Detection of Anticancer Drugs Doxorubicin and Dasatinib. *Analytical Methods* 2019, 11 (4), 443–453.
- (38). Jiang T; Song Y; Du D; Liu X; Lin Y Detection of P53 Protein Based on Mesoporous Pt-Pd Nanoparticles with Enhanced Peroxidase-like Catalysis. *ACS Sens* 2016, 1 (6), 717–724.
- (39). Bui TH; Thangavel B; Sharipov M; Chen K; Shin JH Smartphone-Based Portable Bio-Chemical Sensors: Exploring Recent Advancements. *Chemosensors* 2023, 11 (9), 468.
- (40). Chen Y; Zhang M; Wang X; Wang X; Majid Z; Huang K; Xu W; Luo Y; Nan C A Portable 3D-Printed Pretreatment Device Combined with Graded Lateral Flow Assay for Detection of *S. Aureus*. *Sens Actuators B Chem.* 2023, 383, 133601.
- (41). Li Y; Zhang Q; Pang X; Luo Y; Huang K; He X; Yao Z; Li J-C; Cheng N Fe-N-C Nanozyme Mediated Bioactive Paper-3D Printing Integration Technology Enables Portable Detection of Lactose in Milk. *Sens Actuators B Chem.* 2022, 368, 132111.
- (42). Zhang Q; Wang W; Yang Z; Wang X; Xu W; Huang K; Luo Y; He X; Cheng N A Portable 3D-Printed Biosensing Device for Rapid Detection of Genetically Modified Maize MON810. *Sens Actuators B Chem.* 2021, 349, 130748.
- (43). Song G; Zhang J; Huang H; Wang X; He X; Luo Y; Li J; Huang K; Cheng N Single-Atom Ce-N-C Nanozyme Bioactive Paper with a 3D-Printed Platform for Rapid Detection of Organophosphorus and Carbamate Pesticide Residues. *Food Chem.* 2022, 387, 132896. [PubMed: 35421648]
- (44). Ataee-Esfahani H; Imura M; Yamauchi Y All-Metal Mesoporous Nanocolloids: Solution-Phase Synthesis of Core-Shell Pd@Pt Nanoparticles with a Designed Concave Surface. *Angew. Chem., Int. Ed.* 2013, 52 (51), 13611–13615.
- (45). Cheng N; Shi Q; Zhu C; Li S; Lin Y; Du D Pt-Ni(OH)₂ Nanosheets Amplified Two-Way Lateral Flow Immunoassays with Smartphone Readout for Quantification of Pesticides. *Biosens. Bioelectron.* 2019, 142, 111498. [PubMed: 31319328]
- (46). ACGIH. Threshold limit values for chemical substances and physical agents and biological exposure indices. American Conference of Governmental Industrial Hygienists, Cincinnati, OH 2013.
- (47). Barr DB; Wilder LC; Caudill SP; Gonzalez AJ; Needham LL; Pirkle JL Urinary Creatinine Concentrations in the U.S. Population: Implications for Urinary Biologic Monitoring Measurements. *Environ. Health Perspect.* 2005, 113 (2), 192–200. [PubMed: 15687057]

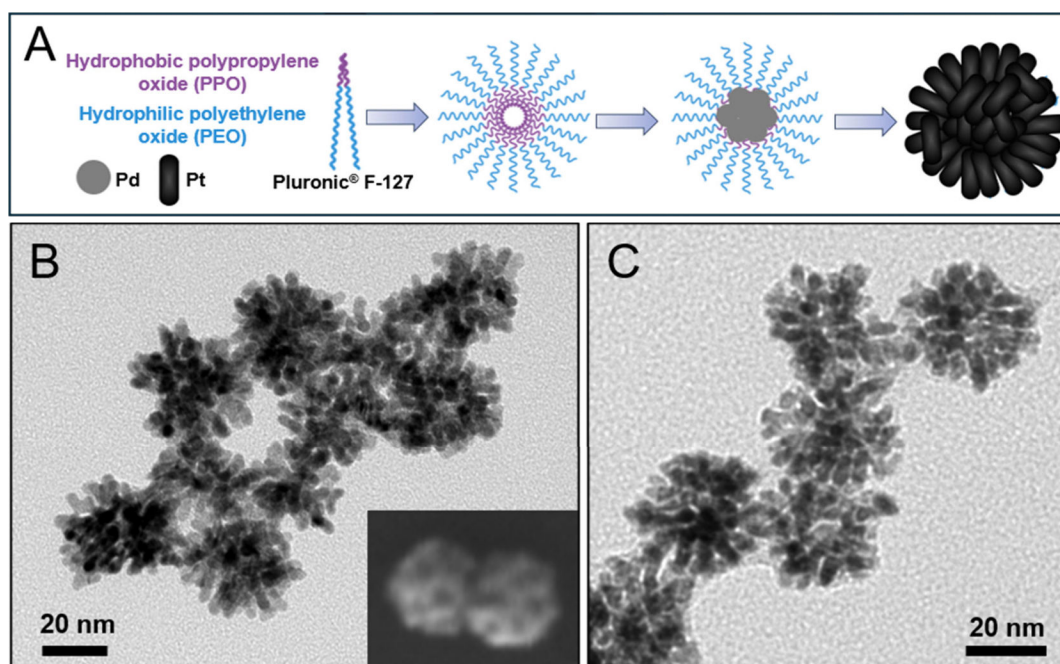


Figure 1. Characterization of mesoporous Pd@Pt NPs and Pd@Pt NP/antibody conjugates. (A) Schematic of mesoporous Pd@Pt NPs synthesis process. (B, C) TEM images of the synthesized mesoporous Pd@Pt NPs and their antibody conjugates, respectively. The inset in (B) shows the SEM image of the synthesized mesoporous Pd@Pt NPs.

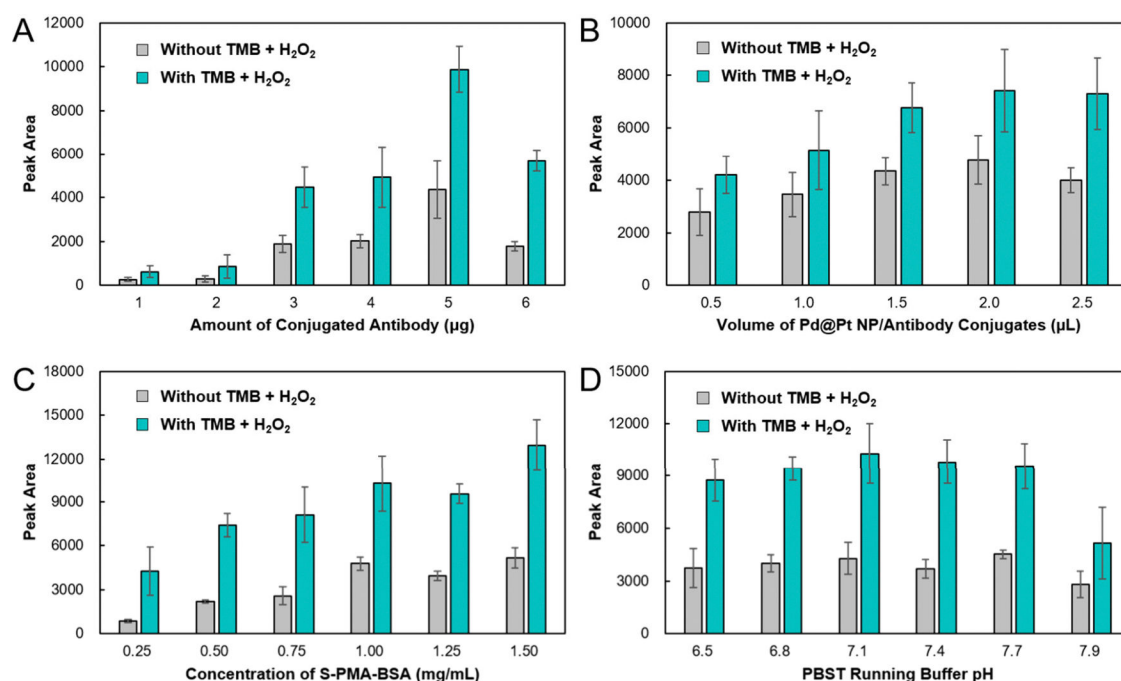


Figure 2.

Optimization of the LFIA. (A) Effect of varying amounts (1, 2, 3, 4, 5, and 6 μg) of conjugated antibody in the preparation of Pd@Pt NP/antibody conjugates. (B) Effect of varying volumes (0.5, 1.0, 1.5, 2.0, and 2.5 μL) of Pd@Pt NP/antibody conjugates. (C) Effect of varying concentrations (0.25, 0.50, 0.75, 1.00, 1.25, and 1.5 mg/mL) of S-PMA-BSA dispensed on the test line. (D) Effect of varying pH (6.5, 6.8, 7.1, 7.4, 7.7, and 7.9) of PBST running buffer.

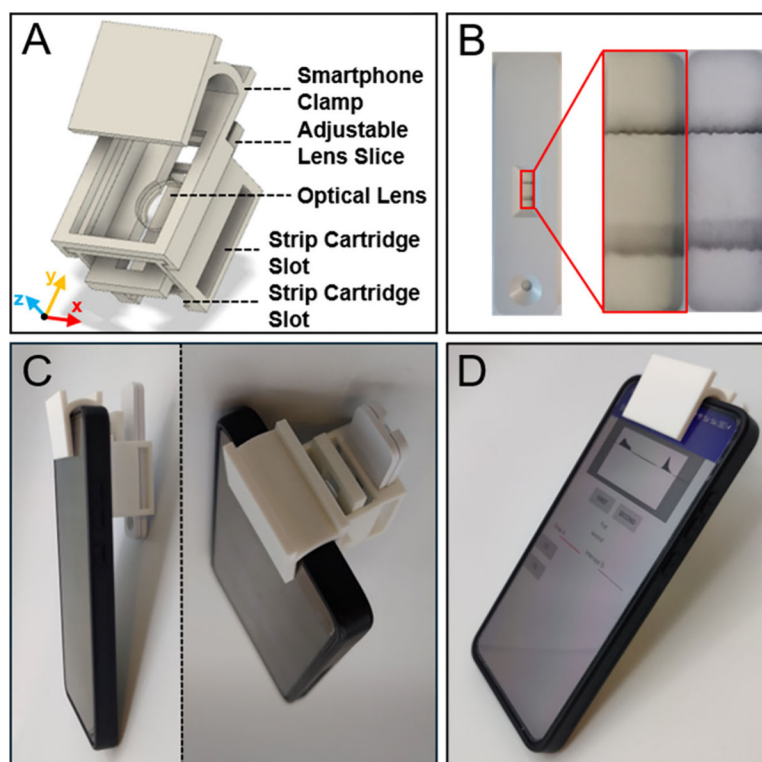


Figure 3. Design and advantages of the 3D-printed smartphone reader. (A) Designed lens device (in Fusion 360 software) includes a smartphone clamp, an adjustable lens holder with an optical lens, and two cartridge slots for test strips. (B) Enhanced contrast of image captured with lens device (right) as compared to bare image (left) captured by smartphone alone. (C, D) Demonstrated image acquisition and analysis using HUAWEI Mate 30 with the 3D-printed lens device and “Strip Scan” app.

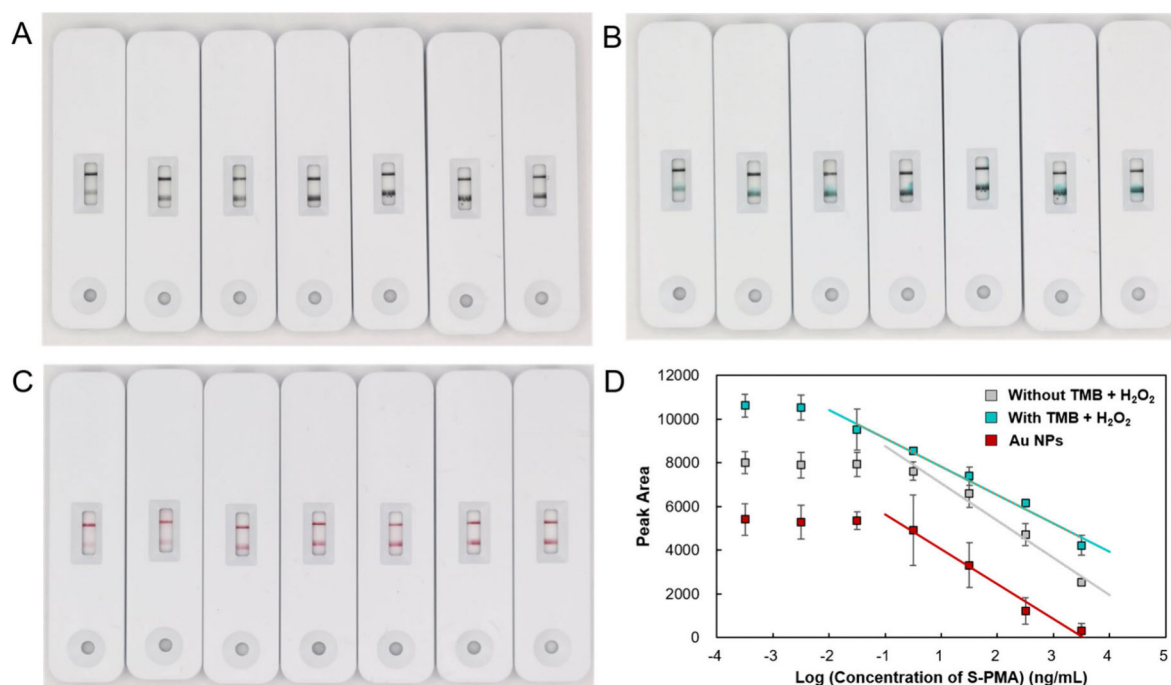


Figure 4.

Sensitivity of S-PMA detection in PBST. (A, B, C) Photographs of the Pd@Pt NP-mediated LFIA (without TMB and H₂O₂), Pd@Pt NP-mediated LFIA (with TMB and H₂O₂), and Au NP-based LFIA, respectively, for the detection of S-PMA at various concentrations (1000, 100, 10, 1, 0.1, 0.01, and 0 ng/mL, from left to right). (D) Calibration curve of peak area values versus the logarithm of S-PMA concentrations in PBST. Data points for the 0 ng/mL concentration of S-PMA were plotted on the leftmost to illustrate its corresponding peak area value, as 0 ng/mL is not meaningful in the log scale. Error bars represent standard deviations of three measurements.

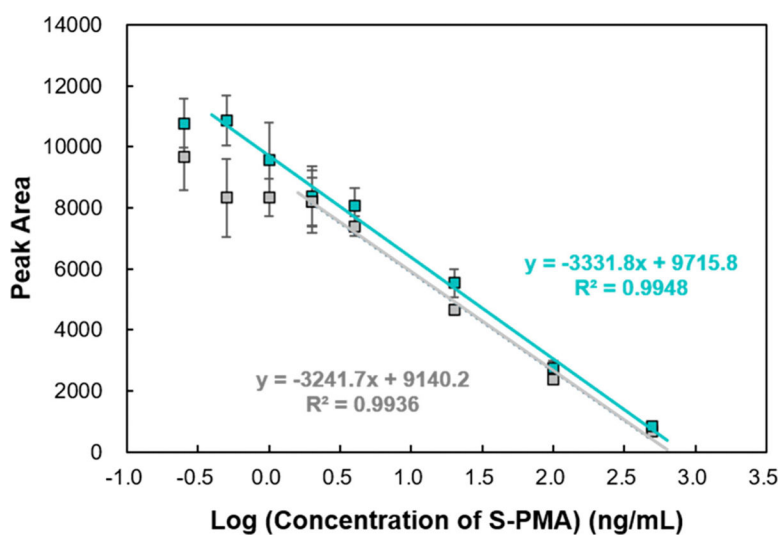


Figure 5.

Sensitivity of S-PMA detection in urine samples. Calibration curves of peak area values versus the logarithm of S-PMA concentrations (0.5, 1, 2, 4, 20, 100, and 500 ng/mL) in urine samples. Data points for the 0 ng/mL concentration of S-PMA were plotted on the leftmost to illustrate its corresponding peak area value, as 0 ng/mL is not meaningful in the log scale. Error bars represent standard deviations of three measurements.

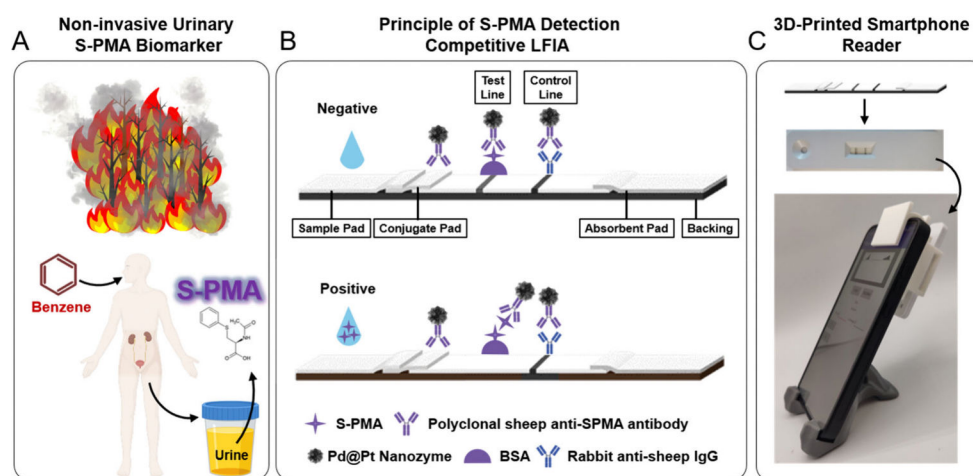
**Scheme 1.**

Illustration of the Proposed S-PMA Detection System: (A) Detecting Urinary S-PMA Biomarker for Assessing Wood Smoke Exposure; (B) Competitive LFIA Using Mesoporous Pd@Pt NPs as Signal Simplifier; (C) Integrated 3D-Printed Smartphone Reader for Data Acquisition and Analysis

Table 1.
Coefficient of Variation (CV) for Peak Area Values in S-PMA Detection from Urine Samples

S-PMA Concentration	Condition	CV for Peak Area
20 ng/mL	Without TMB+H ₂ O	8.61%
	With TMB+H ₂ O	6.18%
100 ng/mL	Without TMB+H ₂ O	6.39%
	With TMB+H ₂ O	9.42%