SUPPLEMENTARY INFORMATION

A smartphone-based immunochromatographic strip platform for on-site quantitative detection of antigenic targets

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1. Materials and methods

1.1 Preparation of BSA-AMP conjugates

N-Succinimidyl-S-acetylthioacetate (SATA) was first dissolved to a concentration of 15 mg/mL using dimethyl sulfoxide (DMSO), followed by BSA diluted to 1% (w/v) using a mixture of PBS (50 mM, pH 7.6) and EDTA-2Na (10 mM, pH 7.6). 1 mL of dissolved and mixed 15 mg/mL SATA solution was added into a 15 mL centrifuge tube containing 10 mL dissolved and mixed 1% (w/v) BSA solution. The reaction was shook for 30 min at room temperature and away from light. 830 μL hydroxylamine hydrochloride solution dissolved with PBS (50 mM, pH 7.6) and EDTA-2Na (10 mM, pH 7.6) was added to the reaction solution after reaction, and the reaction was carried out for 2 h by shaking at room temperature and away from light. The reacted liquid is dialyzed with a mixed solution of 10 mM PBS and 2 mM EDTA-2Na (pH 7.6) for 12 h, and the dialysate is replaced every 2 h.

1.2 Preparation of CNPs conjugates and CNPs-strips for SPICS testing.

In the competition method for testing of AMP. Briefly, the prepared carbon nanoparticles (CNPs) are washed 2 times with 0.01 M BB buffer and then resuspended in 0.01 M BB buffer. Subsequently, 10 μg of AMP antibody was added to 1 mL of 1 μg mL-1CNPs, respectively. The mixture was placed in a mixer for a 3-hour reaction at 40 rpm, followed by the addition of 150 μL of 10% BSA to block unreacted antibody binding sites. This was further incubated in the mixer at 40 rpm for 2 hours. After centrifugation at 10,000 rpm at 4℃ for 10 minutes, the supernatant was removed, and the pellet was suspended in 0.5 mL of dilution buffer (0.01 M, pH 7.6 Tris-HCl; containing 1% (w/v) BSA, 0.05% (w/v) Tween 20, 2% (w/v) sucrose, 2% (w/v) trehalose). The suspension was then sprayed onto the coupling pad using a three-dimensional scribing gold sprayer. On NC membranes, 0.6 mg/mL of AMP-BSA and 0.1 mg/mL of goat anti-mouse IgG were coated on the T and C lines, respectively. The sample pad was treated with a solution (ultrapure water; treating the sample pad with 10% Triton X-100 and 5% Tween 20) and left to dry overnight at 56℃. After

completing the immunochromatography, the assembled bands are tested in a transparent enclosure.

In the sandwich method for testing of HCG or PSA. Similarly, 40 μg of HCGmAb1 and 35 μg of PSA mAb1 were added to 1 mL of CNPs, respectively. After labeling, resuspend with dilution buffer (0.01 M, pH 7.6 Tris-HCl; containing 1% (w/v) BSA, 0.05% (w/v) Tween 20, 2% (w/v) sucrose, 2% (w/v) trehalose). The suspension was then sprayed onto the coupling pad using a three-dimensional scribing gold sprayer. 1.5 mg/mL HCGmAb2 or PSAmAb2 were coated on NC membranes to form the T line, and appropriate rabbit anti-chicken IgY polyclonal antibodies form the C lineage and form an independent quality control system with the CNPs-IgY pre-coated with conjugate pads. The sample pad was treated with a solution (0.2 M, pH 7.4 PBS; containing 0.5% (w/v) BSA, 1% S9, and 0.5% Tween 20) to remove non-specific reactivity and ensure buffering capacity on the NC membrane. The assembly was dried at 56℃ for 4 hours following the earlier described method. After completing the immunochromatography, the assembled bands are tested in a transparent enclosure.

1.3 Quantitative analysis of CNPs-Strips using both SPICS-reader and commercial immunochromatography instruments

In brief, evaluating CNPs-Strips results using an SPICS-reader is a straightforward process. Taking the CNPs-strip testing model as an example, 100 μL PBS samples containing different volumes of CNPs-IgY (0, 0.5, 1, 2, 4, 4, 8, 16, 32 μL) at concentrations of 2 μg mL^{-1} (0.5 mL reconstituted) were added to the strip filling wells. After the immune response is complete, the CNPs-Strips are placed in the SPICS-reader, and the light intensity value is read using a smartphone app. Simultaneously, the peak area values of the strip are scanned using a commercially available immunochromatographic quantitative analyzer.

For AMP sample testing, standards of different concentrations (0.5 ng/mL, 1 ng/mL, 2 ng/mL, 3 ng/mL, and 4 ng/mL) are added to fresh milk and then detected with CNPs-strips. The results are measured using an SPICS-reader, and the actual concentration of AMP is calculated based on a linear relationship.

For HCG or PSA sample testing, eight clinical HCG and PSA serum samples from the hospital that were determined by chemiluminescence assay. After CNPs-strips testing, the light intensity values are measured with the SPICS-reader, and the sample concentration is calculated based on a linear relationship.

2. Results

2.1 Light transmittance test of NC film with different thickness

Sartorius CN95 and CN140, Millipore HF90 and HF135, Whatman AE99, and Pall VIV90 were six commonly used NC films with different thicknesses, which were selected to test the light transmittance. The transmitted light intensity through the NC film was measured sequentially by setting the light source to any constant brightness, and the results were shown in Figure S10, indicating that the NC film thickness had no significant effect on the light projection.

2.2 Precision and accuracy of SPICS-reader

The feasibility of SPICS-reader system for the analysis of AMP-supplemented milk samples was verified. The results of the analysis are shown in Table S2, The recoveries in all of these samples ranged from 96.1-105%. And validating the feasibility of SPICS-reader system for the analysis of HCG and PSA in clinical serum samples. The results of the analysis are shown in Tables S3 and S4, the recoveries for all of these cases were 97.4-106.9% and 96.2-105.2%, respectively. This showed that the SPICS-reader system had excellent precision and accuracy.

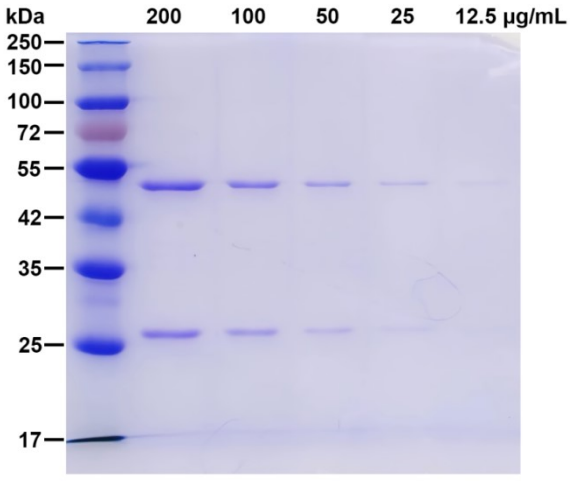

Figure S1. Identification of the monoclonal antibodies to ampicillin. Polyacrylamide

gel Coomassie Brilliant Blue staining assay prepared.

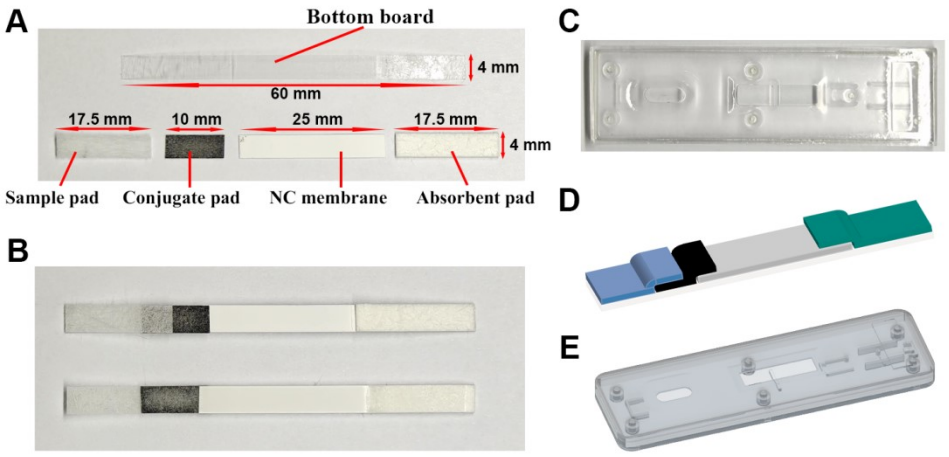

Figure S2. Schematic view of the modified CNPs-strip. (**A**) The composition of the test strip; (**B**) Test strips after assembly; (**C**) 3D printed transparent cassette; (**D**) the components of the modified CNPs-strip, and (**E**) the modified plastic housing.

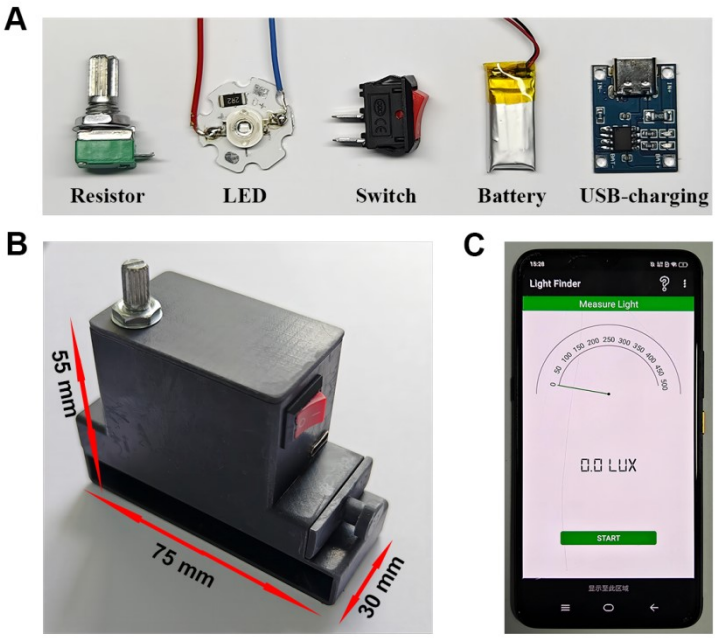

Figure S3. Structural composition of SPICS device. (**A**) Main components of light signal reading device; (**B**) The SPICS-device appearance drawing; (**C**) Smartphone data display interface.

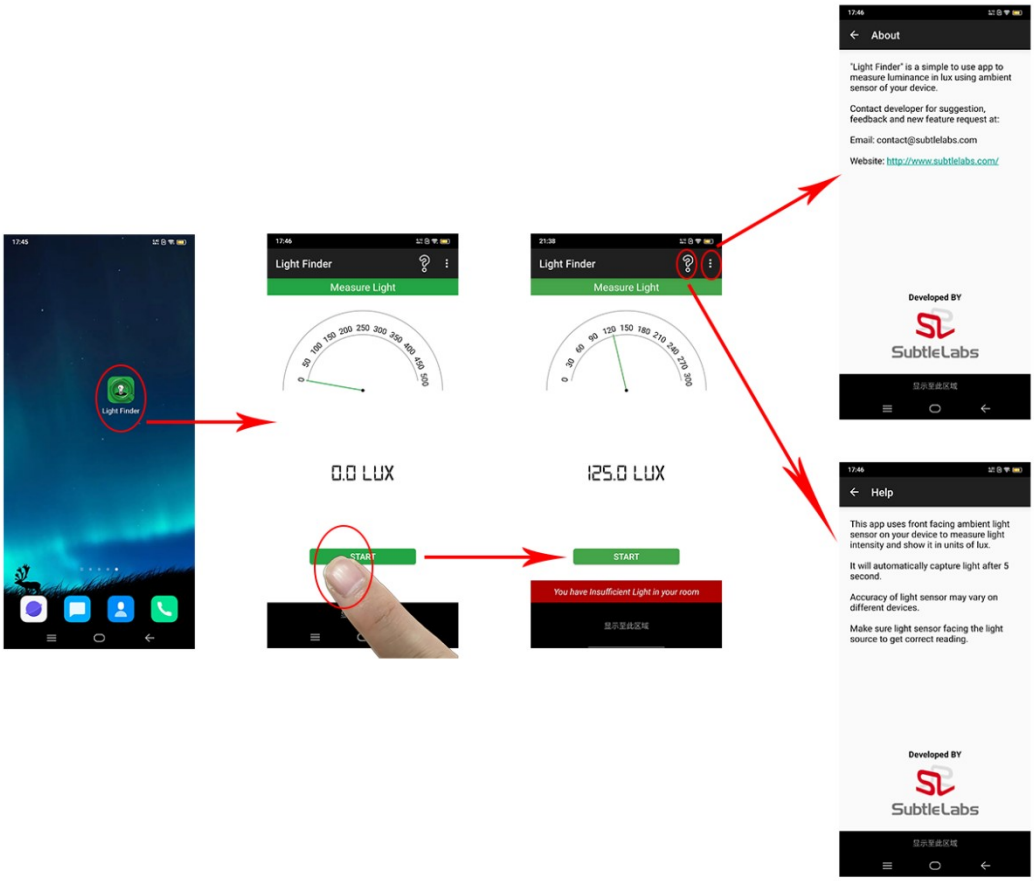

Figure S4. Detailed operating instructions for the photometer APP

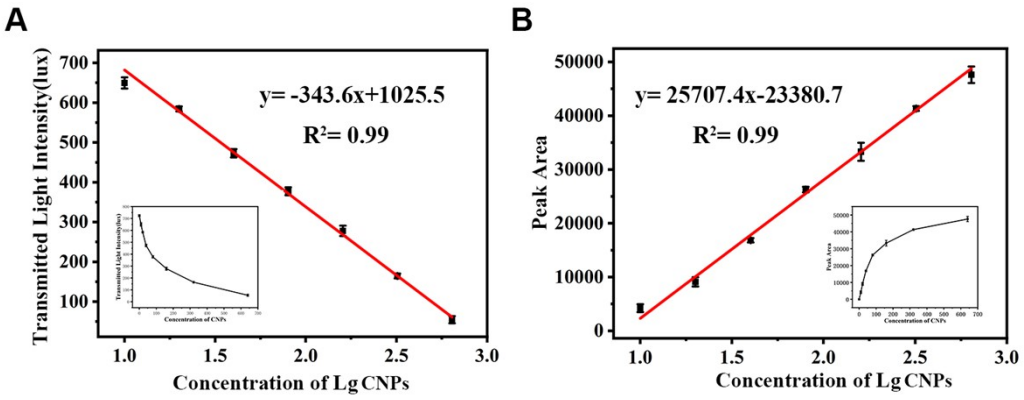

Figure S5. Performance of the SPICS-reader. (**A**) CNPs-strips analysis results obtained by SPICS-reader; (**B**) CNPs-strips detected by commercial analytical instruments. Each value represents the average of three independent experiments (n = 3).

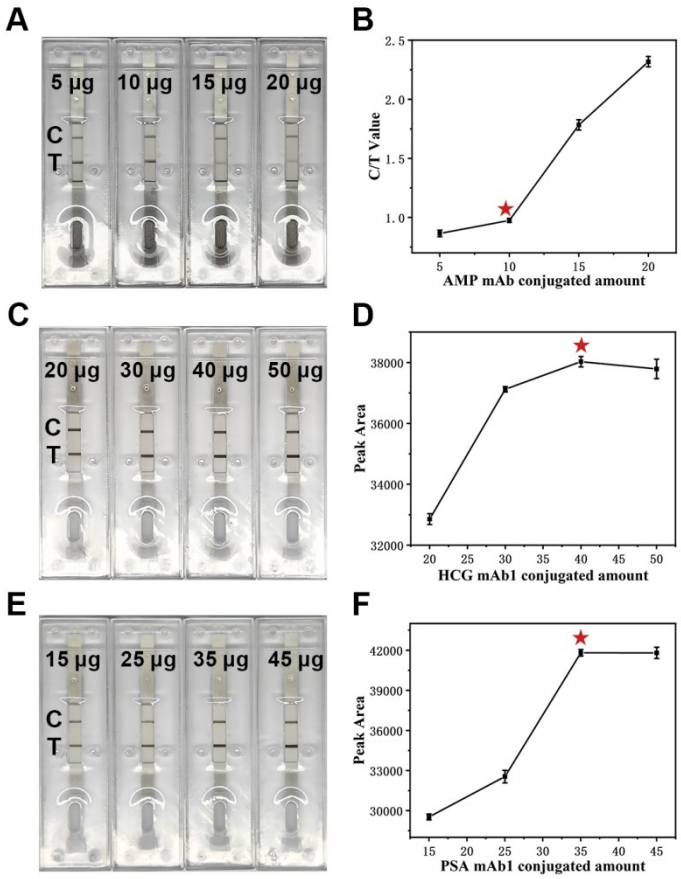

Figure S6. Optimization of the amount of the CNPs conjugates. (**A/B**) The AMP-strips; (**C/D**) The HCG-strips; (**E/F**) The PSA-strips. Each value represents the average of three independent experiments (n = 3).

CNPs-AMPmAb conjugates; CNPs-HCGmAb1 conjugates; CNPs-PSAmAb1 conjugates

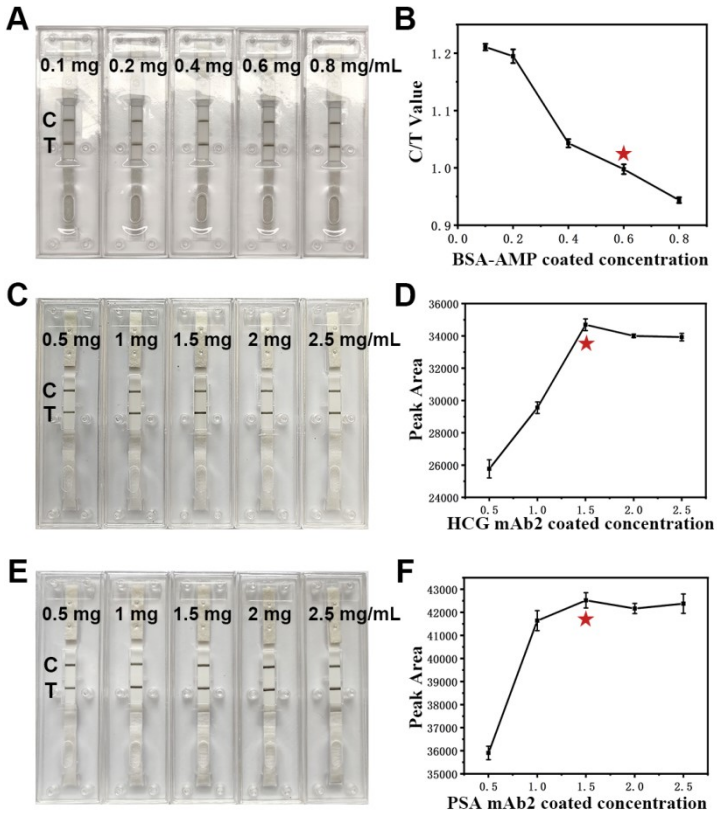

Figure S7. Optimization of NC membrane coated concentration. (**A/B**) The AMP-strips; (**C/D**) The HCG-strips; (**E/F**) The PSA-strips. Each value represents the average of three independent experiments (n = 3).

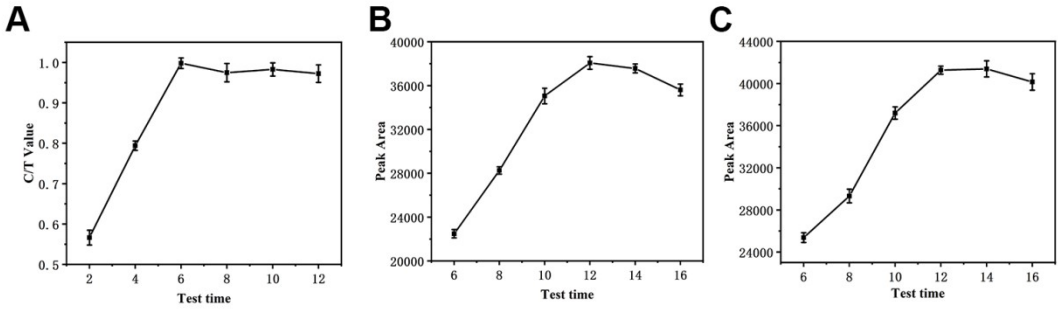

Figure S8. Optimization of strips detection time. (**A**) The AMP-strips; (**B**) The HCG-strips; (**C**) The PSA-strips. Each value represents the average of three independent experiments (n = 3).

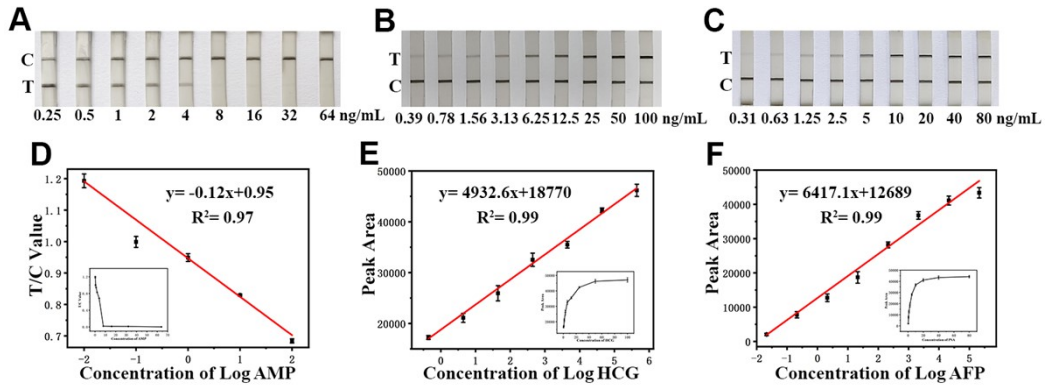

Figure S9. CNPs-strips were analyzed using conventional commercial image analysis instruments. (**A/D**) The AMP-strips; (**B/E**) The HCG-strips; (**C/F**) The PSA-strips. Each value represents the average of three independent experiments (n = 3).

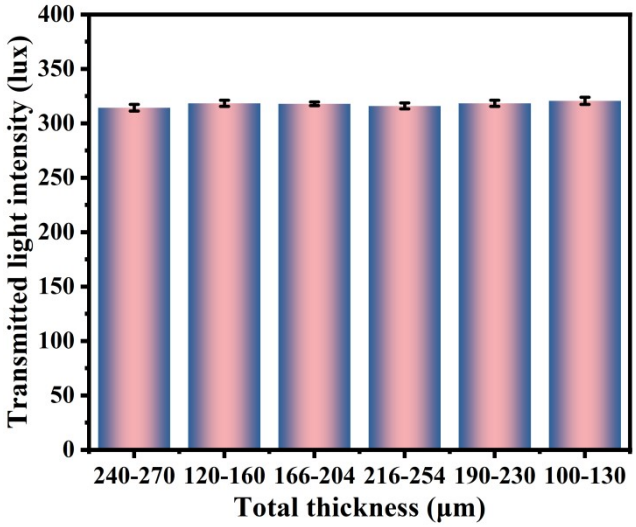

Figure S10. Examination for light transmission of NC films with different thickness. Sartorius CN95 (240-270 μm) and CN140 (120-160 μm), Millipore HF90 (166-204 μm) and HF135 (216-254 μm), Whatman AE99 (1000-130 μm), and Pall VIV90 (190-230 μm). Each value represents the average of three independent experiments (n = 3).

Table S1. Cost of each component of SPICS-reader and strip.

Sensor device		CNPs-strip	
Materials	**Cost ($)**	**Materials**	**Cost ($)**
3D printing consumables	0.18	Plastic housing	0.2
LED	0.33	NC membrane	0.04
Lithium battery	0.6	Antibody	0.22
switch	0.14	Conjugate pad	0.005
USB-charging	0.12	Sample pad	0.011
Others	0.1	PET bottom	0.015
Total	1.47	Total	0.491

Table S2. Detection of AMP in milk samples using SPICS-reader.

Sample number	Spiked Concentration (ng/mL)	SPICS mean	S.D.	CV (%)	Recovery (%)
1	0.5	0.48	0.05	9.95	96.1
2	1	1.05	0.11	10.19	105.0
3	2	2.06	0.11	5.17	102.8
4	3	3.02	0.11	3.63	100.5
5	4	3.98	0.14	3.63	99.5

Mean: Average of AMP concentrations in milk samples test by the SPICS (n=3). S.D.: Standard deviation of AMP concentration in milk samples test by the SPICS (n=3). Coefficient of variation (CV) = (S.D. / Mean)×100%. Recovery (%) = (Results of SPICS / Spiked concentration)*100%.

Table S3. Detection of HCG in human serum samples using SPICS-reader.

Sample number	CLIA (mIU/mL)	SPICS mean	S.D.	CV(%)	Recovery (%)
1	35.5	34.59	1.12	3.23	97.4
2	11.3	11.95	0.82	6.85	105.7
3	15	16.04	0.81	5.07	106.9
4	44.5	43.97	1.22	2.77	98.8
5	83.7	85.97	1.21	1.41	102.7
6	64	65.40	2.16	3.31	102.2
7	1.45	1.51	0.18	12.18	104.3
8	3.13	3.17	0.30	9.32	101.2

Mean: Average of HCG concentrations in serum samples test by the SPICS (n=3). S.D.: Standard deviation of HCG concentration in serum samples test by the SPICS (n=3). Coefficient of variation (CV) = (S.D./Mean)×100%. Recovery (%) = (Results of SPICS / Results of CLIA)*100%.

Table S4. Detection of PSA in human serum samples using SPICS-reader.

Sample number	CLIA (ng/mL)	SPICS mean	S.D.	CV(%)	Recovery (%)
1	63.44	62.21	1.62	2.60	98.1
2	11.46	11.66	0.76	6.49	101.8
3	25.7	24.84	1.19	4.79	96.7
4	7.74	7.45	0.77	10.31	96.2
5	10.6	11.15	0.88	7.92	105.2
6	6.76	6.93	0.63	9.11	102.4
7	14.63	14.40	0.85	5.90	98.4
8	5.07	5.21	0.58	11.14	102.8

Mean: Average of PSA concentrations in serum samples test by the SPICS (n=3). S.D.: Standard deviation of PSA concentration in serum samples test by the SPICS (n=3). Coefficient of variation (CV) = (S.D./Mean)×100%. Recovery (%) = (Results of SPICS / Results of CLIA)*100%.

Table S5. Comparison of the performance between different biosensors for the detection of the antigenic targets.

Detection technique	Antigenic target	Limit of detection	Linearity	Total analysis time	References
SPICS	AMP	0.23 ng/mL	0.25-4 ng mL	7 min	This work
Smartphone-colorimetric sensing	AMP	12 ng/mL	0.05-100 μg/mL	10 min	[1]
Electro-optical platform	AMP	0.5 μg/mL	0.5–600 μg/mL	10 min	[2]
Dual fluorescence –colorimetric	AMP	2 ng/mL	5–100 ng/mL	1 h	[3]
Fiber optic nanoplasmonic biosensor	AMP	0.74 ng/mL	/	15 min	[4]
SPICS	HCG	0.30 mIU/mL	0.39-100 mIU/mL	13 min	This work
Au@Polydopamine immunochromatographic	HCG	1.59 mIU/mL	2-10 mIU mL	25 min	[5]
plasmonic thermal sensing device	HCG	2.8 mIU/mL	35-700 mIU/mL	/	[6]
Fluorescent immunochromatographic	HCG	4.7 mIU/mL	10–5000 mIU/mL	15 min	[7]
Smartphone-lateral flow strip	HCG	3 ng/mL	6–300 ng/mL	/	[8]
SPICS	PSA	0.28 ng/mL	0.31-80 ng/mL	15 min	This work
Cellulose-binding protein LFIA	PSA	0.25 ng/mL	0.25–2.5 ng/mL	20 min	[9]
Photoelectrochemical biosensor	PSA	/	0.08-50 ng/mL	＞12 h	[10]
Bioluminescent immunoassay	PSA	0.4 ng/mL	1-20 ng/ mL	15 min	[11]
Au–Se bonded nanoprobe	PSA	/	1-40 ng/mL	50 min	[12]

Table S6. Comparison of the performance between different grayscale scanning methods and smartphone immunochromatography methods.

Detection technique	Total analysis time	Price （Analyzer included）	Operational complexity*	References
SPICS	7/13/15 min	Low	+	This work
Smartphone-Based Electrochemical lateral-flow device	12 min	High	++	[13]
Polydopamine-Coated Gold lateral-flow	30 min	High	++	[14]
Cube^{TM} quantitative reader	20 min	High	+	[15]
Fluorescent lateral flow platform	20 min	High	+	[16]
Cube Reader	20 min	High	+	NUMED
Colloidal gold analyzer AGS1000	20 min	High	++	Guangzhou Lanbo Biotechnology Co., Ltd
YR-200 Portable Gold Mark Reader	20 min	High	++	Bioeasy Biotechnology, Inc.
Immunoassay Analyzer CT3	/	High	++	Empirica Medical Company

* +, simple; ++, relatively complex.

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