Supplementary Information

Fast, Sensitive and Quantitative Point-of-Care Platform for the Detection of Drugs of Abuse in Urine, Serum and Whole Blood

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The supplementary information includes:

Methods

Methods

A. Comparison of aldehyde- and epoxy- modified glass surface for antibody coating.

We compared the performance of the aldehyde- and epoxy- modified glass surface by using anti-cocaine antibody. After the bottom plate underwent surface treatment, 2 μL of the mouse anti-cocaine antibody (~10 μg/mL) was added to the ELISA wells and incubated overnight at 4°C. After washing three times with PBS buffer containing 0.05% Tween, the wells were blocked with 5% BSA in PBS for 1h. Then 2 μL of the FITC-conjugated, anti-mouse IgG secondary antibody was added to the wells and fluorescence images were taken. We also scratched antibody-coated surface with a pipette tip to confirm the coating performance.

B. Conjugation of platinum nanoparticles (PtNPs) to drug-BSA.

PtNPs were prepared using a method similar to previously reported methods.[1] The size distribution of PtNPs was characterized by dynamic light scattering (DLS; Malvern Zetasizer Nano Series, Malvern, UK). The average size of the PtNPs synthesized was ~28 nm (Figure S5a).

Immunoassay-based drug detection is always using competitive ELISA, in which the drug-derivate competes with the target drug to bind to the coated antibody on the surface. In conventional ELISA, drug-HRP is always used as the drug-derivate. Here, we change HRP to PtNP, which can efficiently catalyze H_2O_2 to generate oxygen.[2] To adapt PtNPs to the detection of drugs of abuse, we conjugated PtNPs to the drug-BSA derivate. Drug-BSA is commercially available in Fitzgerald (MA, USA) for all the target drugs we tested. Twenty microliter drug-BSA solution (1 mg/mL) was mixed with 180 μL PtNP solution (1 mg/mL, dissolved in PBS) and incubated with shaking at 800 rpm for 2h. After that, bovine serum albumin (BSA) was added to a final concentration of 1% to block the surface of the PtNPs, and the unconjugated

antibody was removed using a centrifugal filter. Finally, the drug-BSA-PtNPs were suspended in 200 μL of 10 mM PBS, pH 7.4, containing 0.1% Triton X-100, 5% sucrose, and 1% BSA.[3] During the conjugation, BSA bound to the surface of PtNPs by the cleavage of the sulfur-sulfur bond in disulfides through oxidation.[4] This conjugation is based on covalence, so it is very stable. We used DLS to measure the average diameter of cocaine-BSA, PtNP and and PtNP-conjugated cocaine-BSA.

C. **Device reuse.**

To reuse the device, we detach the device to two plates and use wipers to clean the oil/ reagents with ethanol/isopropanol after the assay. Then the two plates are treated with ultrasonic (put the two plates in a beaker with Millipore water) for 2 min. The devices are then thoroughly washed with Millipore water and dried with nitrogen gas.

D. Comparison of catalytic capability between PtNPs and HRP.

For PtNPs, 2 μL H_2O_2 (35%, w/v) and 2 μL PtNPs (0.1 nM) were mixed on a glass slide by using pipette; then time-lapse imaging of bubbles induced by oxygen gas was conducted. For HRP, luminol (35 mM, dissolved in 0.1 M sodium carbonate buffer, pH11.0) were mixed with 35% (w/v) H_2O_2 at 1:1 volume-to-volume ratio; then 2 μL luminol-H_2O_2 solution and 2 μL HRP (1 nM) were mixed on a glass slide by using pipette; and then time-lapse imaging of bubbles induced by nitrogen gas was conducted. Though the concentration of PtNP was 10-fold lower than that of HRP, the produced bubbles from PtNPs/ H_2O_2 were much more than that from HRP/ luminol-H_2O_2.

E. Antibody selection and cross talk check. We purchased at least two antibodies (one monoclonal antibody and one polyclonal antibody) for each drug and the antibody crossing

reactivity for the targeted drugs was validated using conventional ELISA before applied to the on-chip assay. Those antibodies without cross reactivity were chosen for the on-chip assay.

F. Evaluation of potential hemolysis.

To evaluate the influence of potential hemolysis, we spiked cocaine to the blood obtained from a drug-free volunteer to make a sample with a concentration of 300 ng/mL. We then injected the plasma (obtained through centrifugation of the blood sample) on the top end of the device (pre-coated with cocaine antibody and the drug derivate), and injected the plasma (obtained through microfiltration of the same blood sample) to the bottom end. During the loading, we didn't find apparent hemolysis (based on the color of the plasma in the well). After the on-chip assay, no readable ink bar-chart was formed on the device for the six parallel channels (the distance is ~ 0). These results demonstrated that there is no hemolysis during the filtration or very minimal hemolysis that doesn't bring significant influence on the assay because of the efficient washing of the washing buffer, which washes away the minimal leaked enzymes/proteins (such as hemoglobin) before they contact with H_2O_2.

G. Collection of clinical samples. This project for drugs of abuse detection was approved by the Institutional Review Board (IRB) at Houston Methodist Hospital (Houston, TX). Twenty-eight de-identified urine samples and twelve de-identified serum samples were obtained from the clinical laboratory and Associated Regional and University Pathologists, Inc. (ARUP laboratories, Salt Lake City, Utah) after clinical testing has been completed. Blood samples from drug-free volunteers were drawn in the clinical laboratory of Houston Methodist Hospital. Finger-prick blood samples from the drug-free volunteers were obtained by using contact-activated lancets (BD, Catalogue # 366594).

References

1. Huang, M.H. et al. Designed nanostructured Pt film for electrocatalytic activities by underpotential deposition combined chemical replacement techniques. *J Phys Chem B* **109**, 15264-15271 (2005).
2. Song, Y., Xia, X., Wu, X., Wang, P. & Qin, L. Integration of platinum nanoparticles with a volumetric bar-chart chip for biomarker assays. *Angew Chem Int Ed Engl* **53**, 12451-12455 (2014).
3. Lin, H.C., Wang, I.L., Lin, H.P., Chang, T.C. & Lin, Y.C. Enhancement of an immunoassay using platinum nanoparticles and an optical detection. *Sensor Actuat B-Chem* **154**, 185-190 (2011).
4. Hakkinen, H. The gold-sulfur interface at the nanoscale. *Nat Chem* **4**, 443-455 (2012).

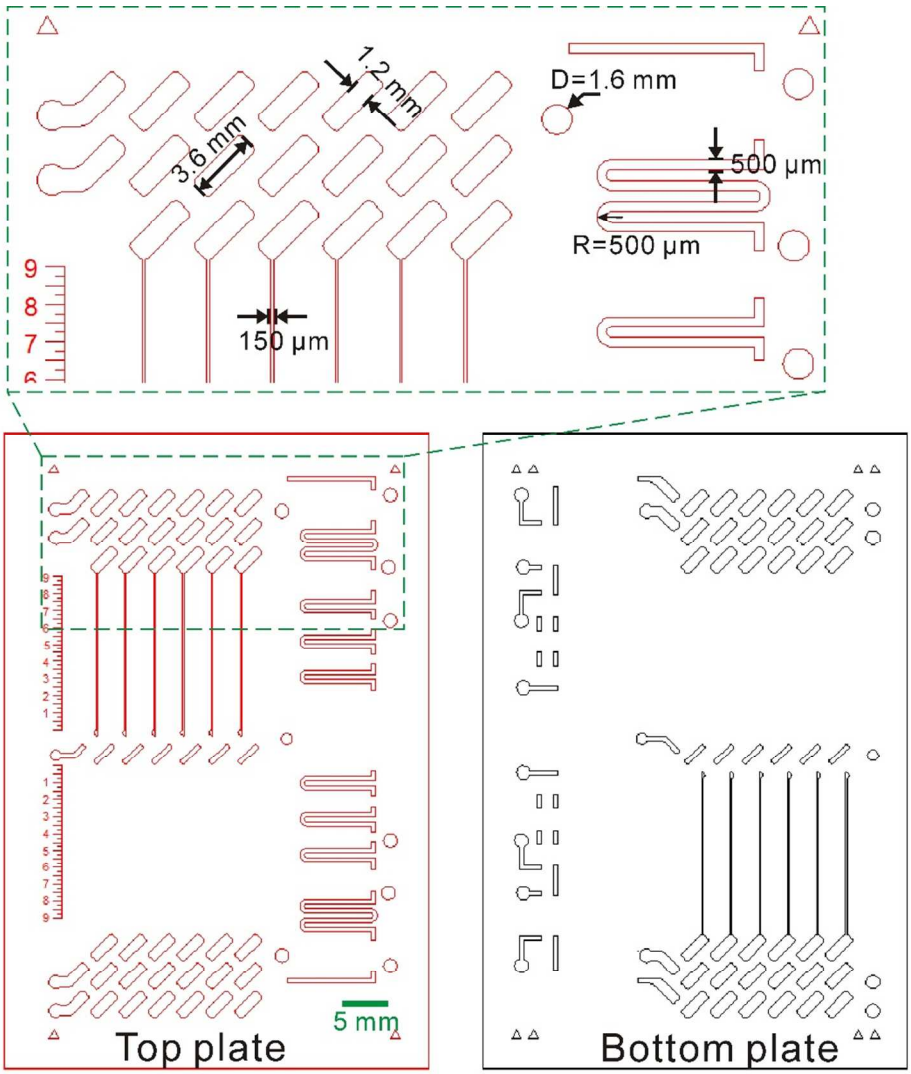

Figure S1 Design of the integrated CV-Chip. The top plate and bottom plate are shown in red and black, respectively. The enlarged view showed the size details for the wells and channels.

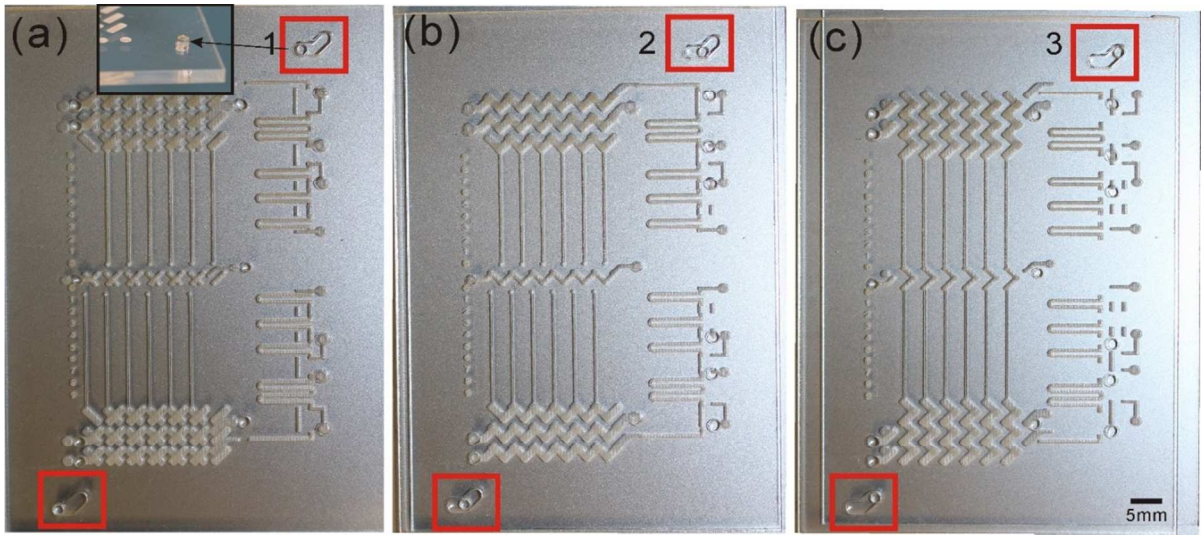

Figure S2 Plastic-based 6-plexed integrated CV-Chip with tracks. (a-c) The three status of the device during the operation. Red rectangles highlight the track designs. Track was fabricated on the top plate. 1, 2 and 3 indicate the positions of the cylinder on the bottom plate (the inset in a) in the three status. This plastic-based device was fabricated by using laser cuter/engraving system (Universal Laser Systems, Arizona).

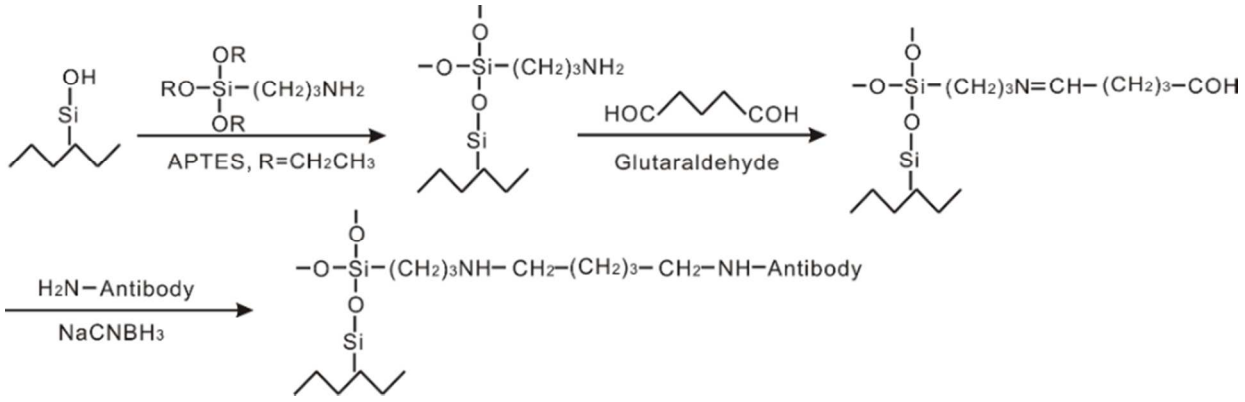

Figure S3 Procedures for the glass modification antibody coating.

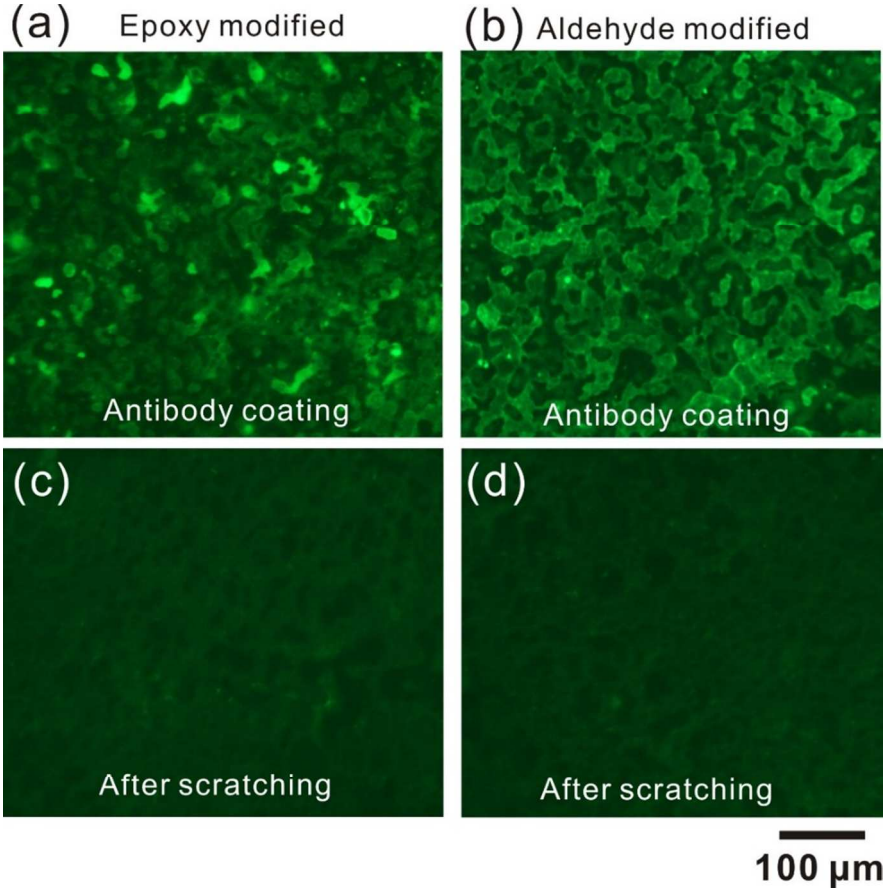

Figure S4 Comparison of the performance of epoxy- and aldehyde- modified glass surface for the coating of mouse anti-cocaine antibody. (a, b) Fluorescence of the FITC-conjugated, anti-mouse IgG secondary antibody on the epoxy (a) and aldehyde (b) modified surface. (c, d) The surface in a and b after scratched using a pipette tip.

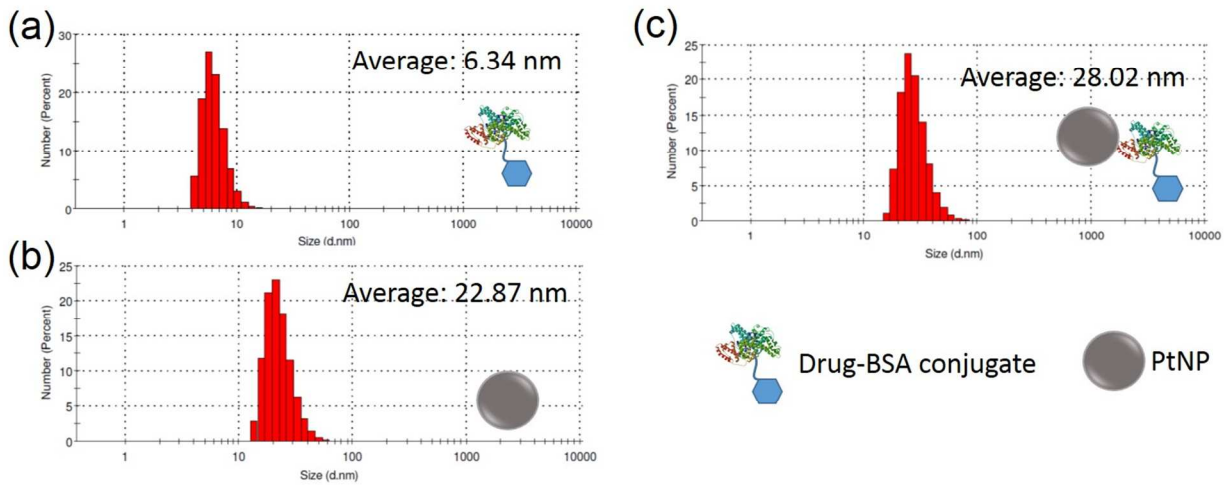

Figure S5 Average size of the cocaine-BSA (a), PtNP (b) and cocaine-BSA-PtNP conjugate (c), measured by dynamic light scattering (DLS). The results showed that the size of PtNP increased by 5.15 nm after the conjugation, which confirmed the success of the conjugation.

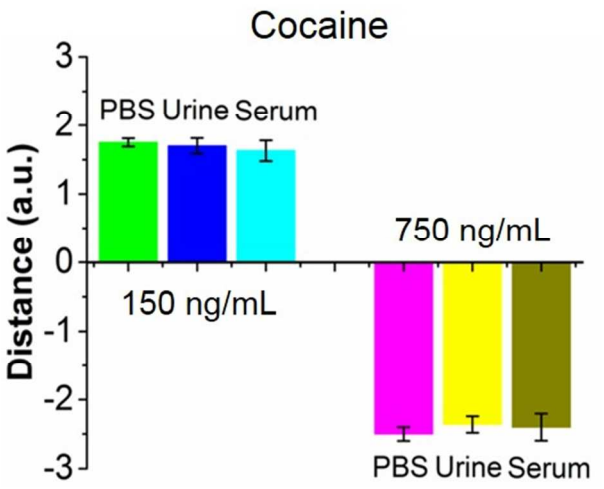

Figure S6 Test result of two samples (150 and 750 ng/mL) spiked in PBS, urine and serum on the integrated CV-Chip. This result demonstrated that the complex matrix provided little or no interference with the performance of the device.

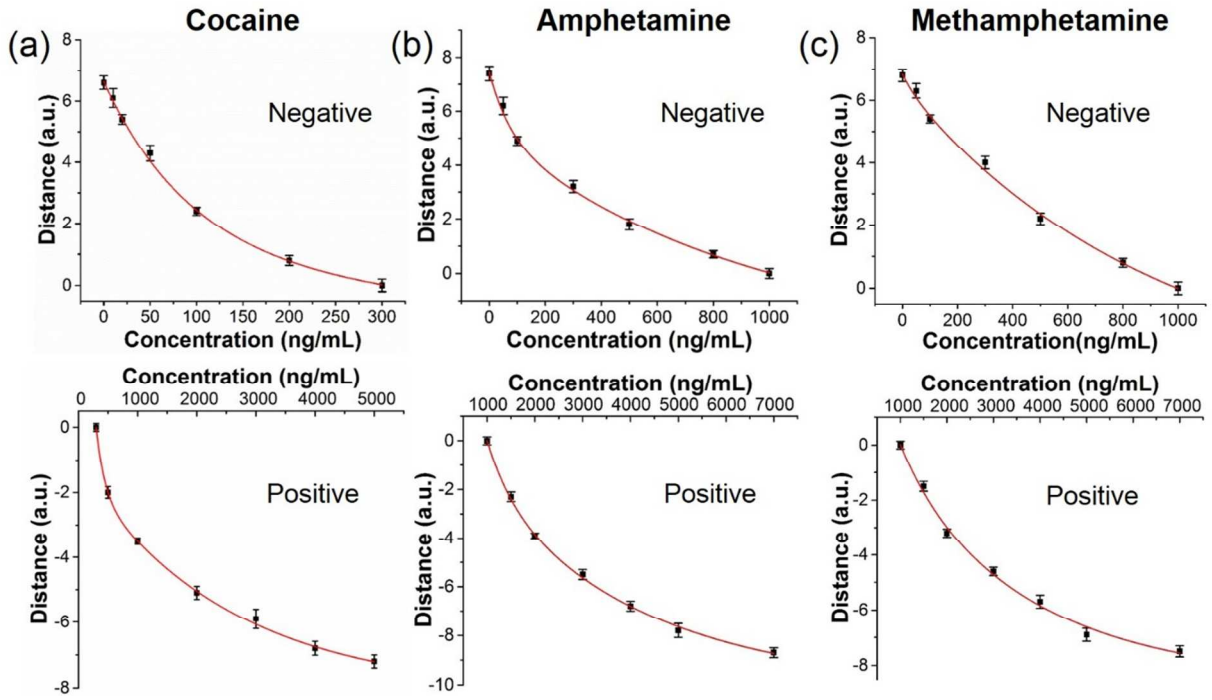

Figure S7 Calibration curves for cocaine (a), amphetamine (b) and methamphetamine (c) for negative and positive urine samples. Cutoff values used for cocaine, amphetamine and methamphetamine is 300, 1000 and 1000 ng/mL, respectively.

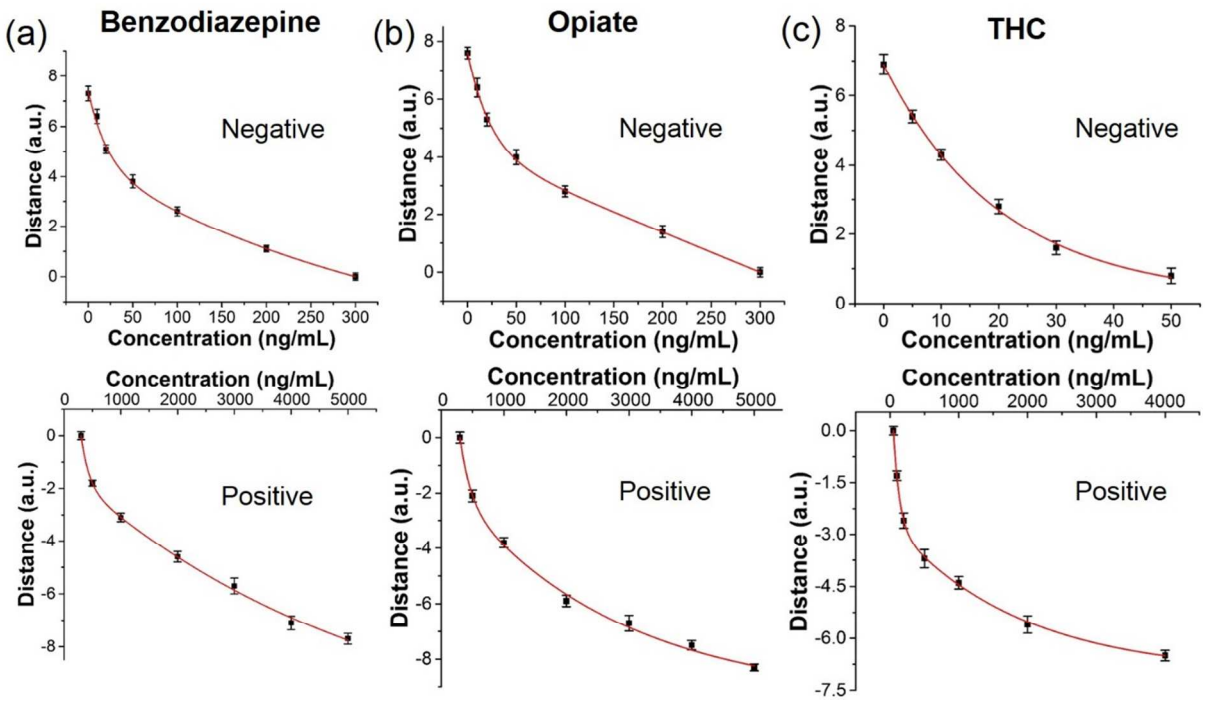

Figure S8 Calibration curves for benzodiazepine (a), opiate (b) and THC (c) for negative and positive urine samples. Cutoff values used for benzodiazepine, opiate and THC is 300, 300 and 50 ng/mL, respectively.

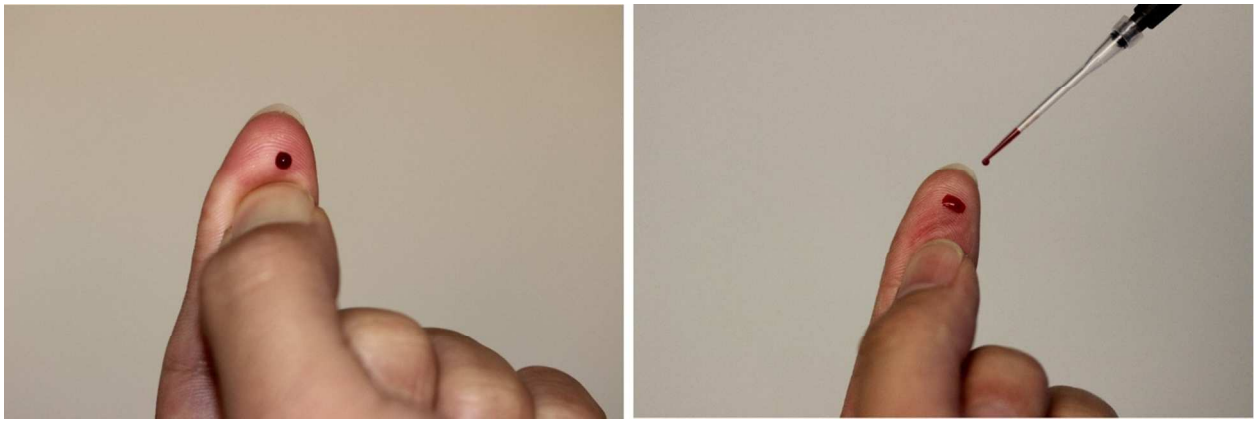

Figure S9 Blood collection from finger-prick. About 20 μL blood can be obtained in one finger-prick.

Table S1 The abbreviation and cutoff value in urine and serum of the seven abused drugs. For the cut-off values in urine, we chose them based on those widely used in most drug screening kits (such as Alere Triage® TOX Drug Screen). The cutoff values in serum were chosen in reference of those used by ARUP laboratories. Unit, ng/mL.

Drug name	Cocaine	Amphe-tamine	Methamphe-tamine	Benzodiazepine	Opiate	Tetrahydro - cannabinol	Methadone
Abbreviation	COC	AMP	mAMP	BZO	OPI	THC	MTD
Cutoff value in urine	300	1000	1000	300	300	50	300
Cutoff value in serum	30	30	30	75	30	30	40

Table S2 Comparison of assay time on the previous CV-Chip and the integrated CV-Chip. The total on-site assay time of the integrated CV-Chip is significantly reduced due to the integrated channels and the improved sensitivity (e.g., surface modification and ELISA probe).

ELISA	Pre-prepared							On-site assay				
	Antibody coating	Washing	Blocking	Washing	Drug derivate binding	Washing	Assembling	Loading sample and Biomarker binding	Washing	silding	Reaction and Readout	Total on-site assay time
Previous CV-Chip	1h at RT	2 min	1h at RT	2 min	1h at RT	2 min	2 min	1.5 h	2 min	10 s	15 min at 37 °C	~107 min
Current integrated CV-Chip	1h at RT	2 min	1h at RT	2 min	1h at RT	2 min	2 min	5 min			5 min at RT	10 min

RT represents room temperature.

Table S3 LC-MS/MS data for cocaine in the 10 urine samples obtained from ARUP laboratories and the on-chip data. Unit for the data is ng/mL.

Sample	Cocaine	Age	Sex	On-chip result
1	200	73	F	342±19
2	292	27	M	375±49
3	365	23	F	331±23
4	>5000	50	F	5106±141
5	>5000	48	M	4890±214
6	2669	32	M	2512±18
7	1796	72	M	1630±123
8	270	34	F	390±35
9	2808	27	F	2608±261
10	4495	50	F	4600±162

Table S4 LC-MS/MS data for benzodiazepine/metabolites in the 10 urine samples obtained from ARUP laboratories and the on-chip results. Unit for the data is ng/mL.

Sample #	Diaz Diaz	Oxaz	Temaz	Nordiaz	Loraz	Alpraz	Clonaz	7ACLON	Midaz	Chlordiaz	Age	Sex	On-Chip result
11	<20	>4000	2688	851	<20	155	21	920	<20	<20	32	F	4890±189
12	<20	79	25	<20	<20	356	<5	<5	<20	<20	49	F	316±23
13	<20	863	934	923	<20	<5	<5	<5	66	<20	42	F	1580±112
14	<20	380	136	70	<20	<5	<5	<5	<20	<20	49	M	369±42
15	<20	<20	<20	<20	528	<5	<5	<5	<20	<20	35	F	480±71
16	<20	437	194	76	<20	<5	<5	<5	<20	<20	17	M	439±47
17	<20	300	595	327	<20	<5	<5	<5	<20	<20	46	F	632±56
18	<20	<20	320	<20	<20	48	<5	47	<20	<20	21	F	338±62
19	<20	318	126	106	<20	<5	<5	<5	<20	<20	45	M	413±51
20	33	>4000	>4000	2050	<20	94	<5	<5	<20	<20	51	F	4960±143

Diaz: Diazepam; Oxaz: Oxazepam; Temaz:Temazepam; Nordiaz:Nordiazepam; Loraz: Lorazepam; Alpraz :Alprazolam; Clonaz:Clonazepam; Chlordiaz:Chlordiazepoxide;7ACLON: 7-Amino Clonazepam; Midaz: Midazolam.

Table S5. LC-MS/MS data for the 8 patient urine samples. For clarity of presentation, positive samples are shown in red fonts. Unit for the data is ng/mL.

Drugs			Samples							
Drug Class	**Items**	**Cut off**	21	22	23	24	25	26	27	28
Cocaine	Benzoylecgonine	50	16.5	6.2	352	Sat	380	Sat	365	1796
Amphetamine	Amphetamine	50	Sat*	No peak	No peak	0.102	No peak	0.02	0.17	No peak
Methaphetamine	Methamphetamine	50	Sat	No peak	No peak	Sat	<0	0.013	<0	0.042
Benzodiazepines	7-aminoclonazepam	10	4570	0.49	0.308	<0	0.003	0.149	<0	0.036
	Lorazepamglucuronide	40	0.594	<0	632	<0	0.45	<0	0.213	0.025
	Oxazepamglucuronide	50	<0	0.019	0.035	0.011	0.11	489	0.702	<0
	Clonazepam	10	284	0.327	0.138	0.03	4.1	2.1	0.526	<0
	Temazepamglucuronide	20	<0	<0	<0	<0	0.37	0.06	0.04	<0
Opiates	Codeine	20	59.1	<0	5.42	0.01	No peak	No peak	0.042	569
	Codeine-6-glucuronide	20	<0	3.69	0.77	1.52	0.29	<0	No peak	<0
	Hydrocodone	20	<0	325.9	<0	<0	<0	0.132	0.345	1.18
	Norhydrocodone	20	2130	207.3	0.39	324	0.06	0.13	<0	No peak
	Hydromorphone-3-glucuronide	20	3210	42.8	<0	2.56	0.37	0.06	<0	5.80
	Tramadol	50	<0	No peak	0.012	Sat	No peak	No peak	<0	<0
	O-Demethyl Tramadol	20	<0	No peak	No peak	1.97	<0	0.22	0.03	7.03
	Morphine	20	317	No peak	<0	No peak	0.19	<0	Sat	<0
	Morphine-3-glucuronide	20	No peak	<0	No peak	<0	0.64	0.006	0.438	<0
	Morphine-6-glucuronide	20	No peak	<0	No peak	<0	<0	No peak	0.08	0.09
Tetrahydro-cannabinol	THC-COOH glucuronide	50	369.2	306.5	100	<0	1.05	<0	0.72	1.034

Sat* means saturation.

Table S6 Data for methadone/metabolite in the 10 patient serum samples measured by LC-MS/MS from ARUP laboratories and the integrated CV-Chip device. Unit for the data is ng/mL.

Sample #	**Methadone (ng/mL)**	**Metabolite (ng/mL)**	**Age**	**Sex**	**On-Chip result**
29	963	153	28	F	912±58
30	524	76	28	F	490±21
31	271	25	38	F	285±12
32	520	124	22	F	585±45
33	298	41	36	F	272±23
34	911	84	29	F	985±66
35	1313	160	29	F	1454±89
36	434	43	54	M	412±17
37	675	75	54	M	625±35
38	1451	226	53	F	1381±101

Table S7. A comparison between the on-chip results and the LC-MS/MS method for all the tested samples.

		LC-MS/MS results	
		Positive	**Negative**
On chip results	**Positive**	True positive (N=47)	False positive (N=0)
	Negative	False negative (N=3)	True negative (N=28)

Movie S1 Time-lapse recording of bubbles induced by nitrogen gas generated from the reaction of luminol and H_2O_2, with HRP as the catalyst.

https://drive.google.com/open?id=0B6Ple8oWxMUvVTBxZDU3ZjZMTVU

Movie S2 Time-lapse recording of bubbles induced by oxygen gas generated from the decomposition of H_2O_2, with PtNPs as the catalyst.

https://drive.google.com/open?id=0B6Ple8oWxMUvWEZpVzhwMzFMMjg