Electronic Supplementary Information (ESI)

Colorimetric immuno-microarray for the quantitation and direct visualization of illicit drugs in body fluids

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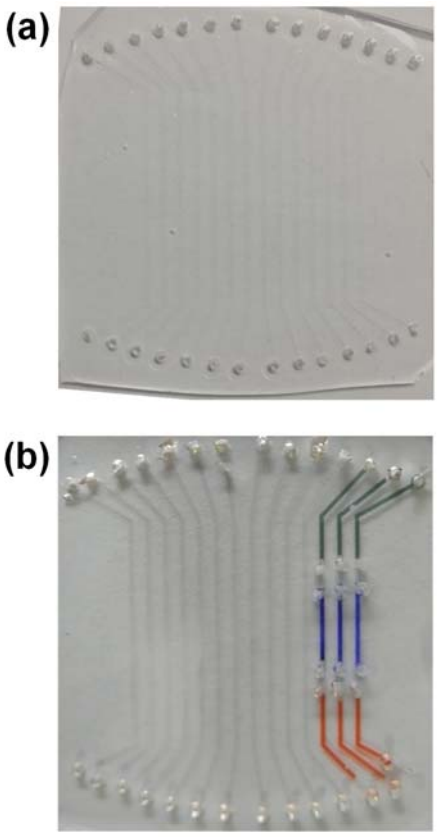
Additional experimental results and data analysis including the design of PDMS channel plates for the preparation of immuno-microarrays, the optimization of reaction conditions for the indirect competitive immunoassays for illicit drugs, the detection in serum, and the selectivity/ stability test of the immuno-microarray chips (PDF, 11 pages).



Scheme S1. Photos of (a) the PDMS microchannel plate with 14 channels for the immobilization of BSA-drug conjugates, the delivery of competitive reaction samples and silver staining regents, and (b) a specially designed PDMS plate with 11 channels for individual testing, and 9 sectional channels ("divided" from three channels on the right side) used for the delivery of mixed samples and different staining reagents; ink solutions in different colors were injected to these channels to highlight the design. See the main text for the assay preparation details.

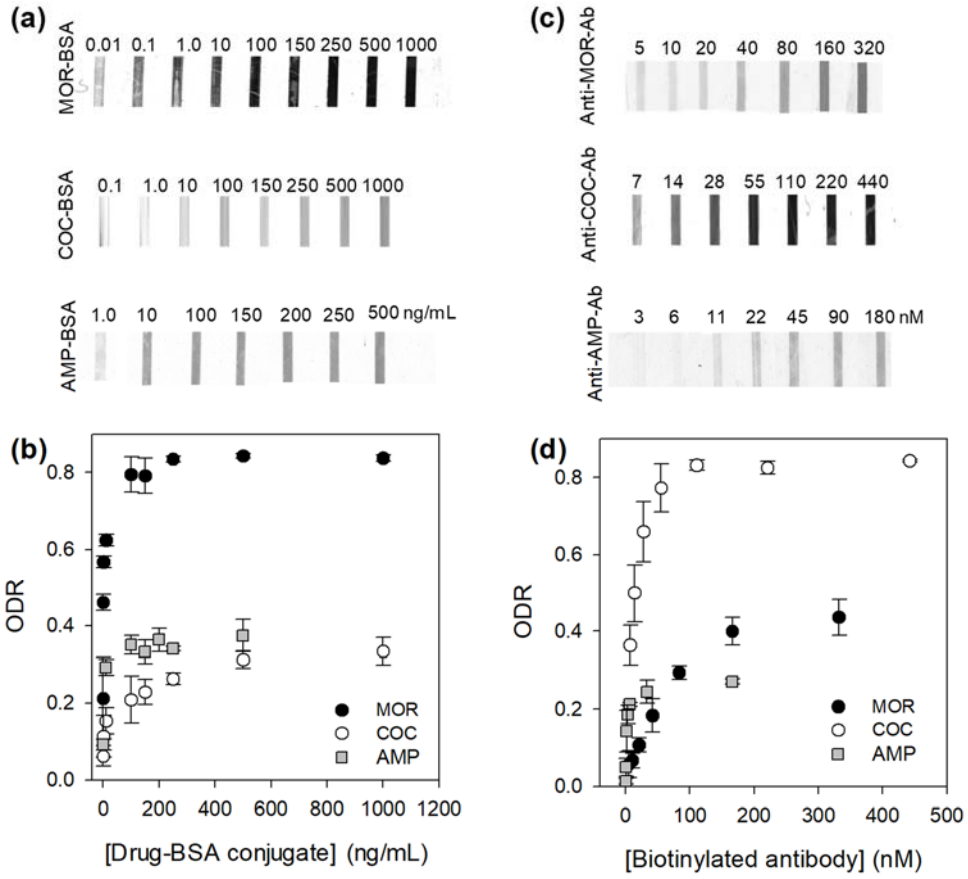


Fig. S1. Optimization of reaction conditions for the indirect competitive assays for MOR, COC, and AMP. (a) Photo of the line arrays of immobilizing different concentrations of BSA-drug conjugates (upon adding corresponding antibody at the same concentration, followed by introducing streptavidin-nanogold® conjugates and silver staining solution). (b) The dependence of ODR on the concentration of the Drug-BSA conjugates used for the probe immobilization (data extracted from (a)). (c) Photo of the line arrays of adding different concentration of biotinylated antibodies in the sample (with the same concentration of immobilized BSA-drug conjugates). (d) The dependence of ODR as a function of the concentration of biotinylated antibodies concentration used for the competitive reaction (data extracted from (c)). The ODR responses start to saturate when the concentration of the BSA-drug conjugates is higher than 100 ng/mL and that of the labeled antibodies is beyond 100 nM, suggesting that such values are concentrations for preparing the indirect competitive assays for these drugs.

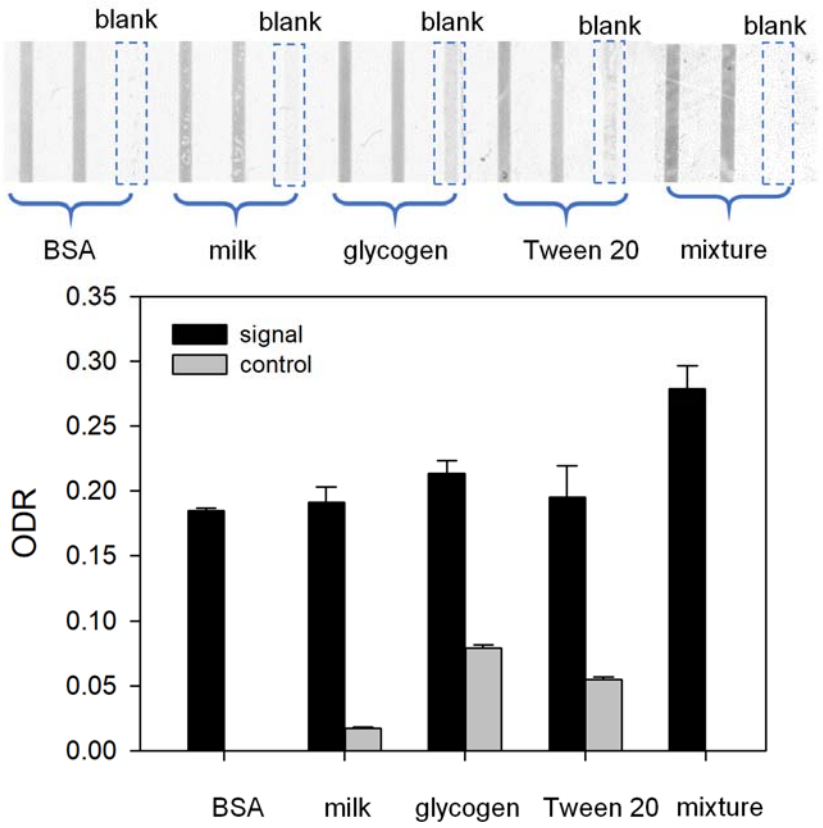


Fig. S2. Optimization of the blocking buffer composition (3% BSA, 6% milk, 1% glycogen, 0.1% Tween 20, mixture: 1% BSA, 0.5% glycogen, 0.1% Tween-20) used in the preparation of the indirect competitive assay for illicit drugs. The top inset shows the optical images of the assay when different blocking buffer was used. Before adding the sample (containing the drug and anti-drug antibody), the chip (immunized with BSA-drug conjugates) must be passivated to prevent the nonspecific binding of the antibodies and/or target drugs. It is apparent that a mixture of three popularly used passivation reagents (BSA, Glycogen, and Tween-20) produces the best assay results.

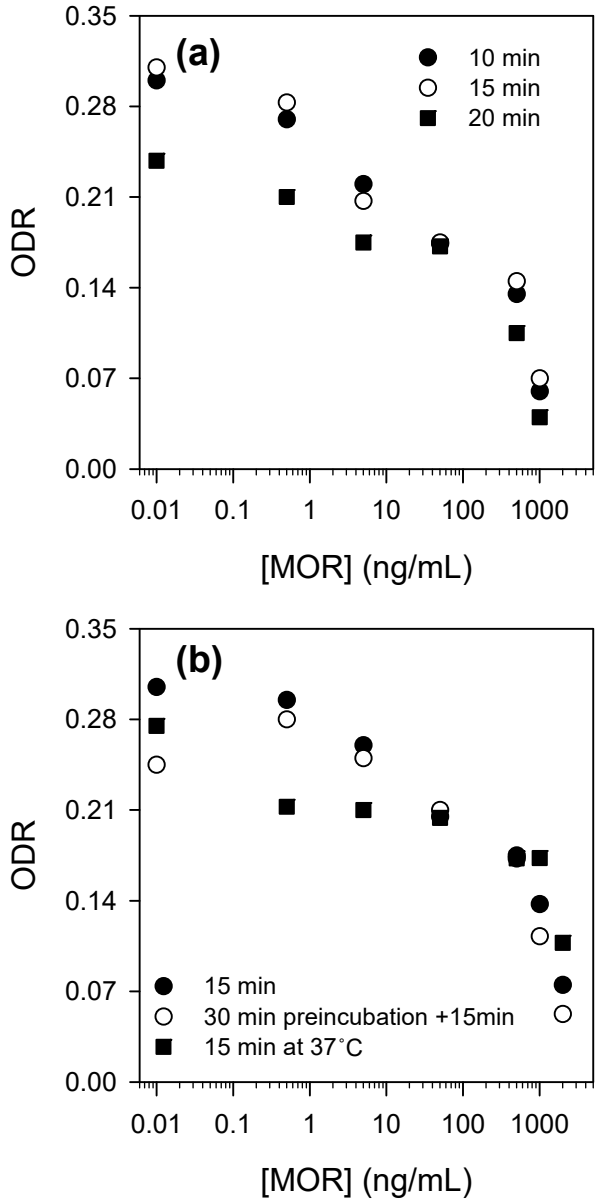


Fig. S3. Optimization of the competitive assay time (a) and temperature (b) for MOR. It is clear that 15-20-min time are adequate for the reaction (i.e., prolonged reaction does not improve the results). Also, evaluated temperature does not help the assay reaction either.

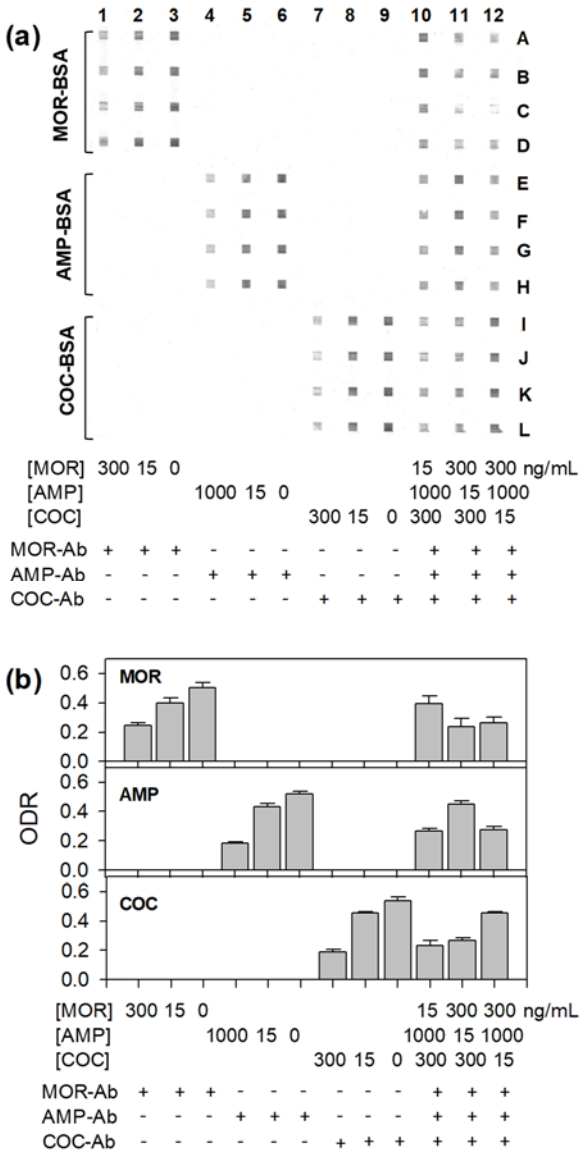


Fig. S4. Immuno-microarray for the quantitation of COC, AMP, and MOR in serum: (a) optical image showing the detection results with listed probe arrangement on the left and sample composition (as the bottom inset). (b) Histograms of ODR determined from the assay image shown in (a). For the assay tests, human serum was diluted 50 times using a 10 mM phosphate buffer (pH 7.4, 150 mM NaCl) and then spiked with 0, 15 and 300 ng/mL of MOR and COC, 0, 15 and 1000 ng/mL of AMP, respectively. The error bars show the standard deviation (SD) of the repetitive assays sites for the same concentration tested. It is clear that there are not any cross reactions observed in the individual sample testing zone of the array (shown in Fig. S4a), and near-idential ODR values were generated at the same concentration of drug presented in both the individual sample and mixed samples (shown in Fig. S4b).

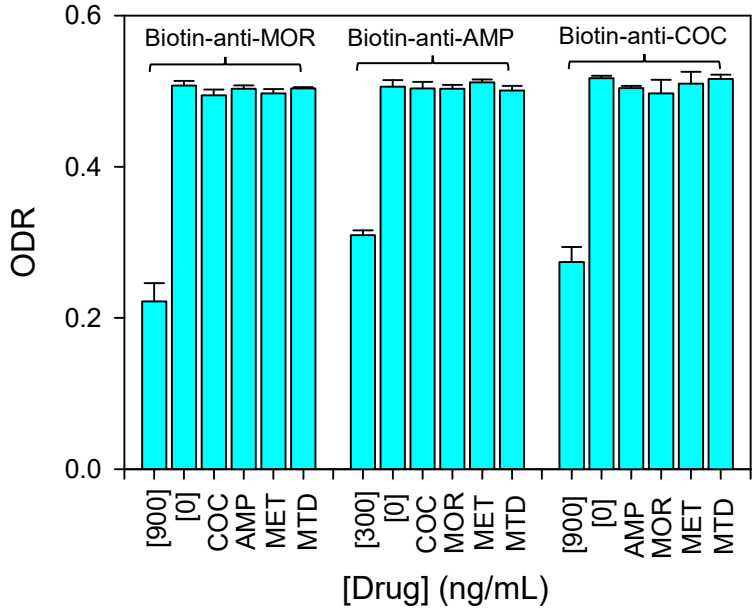


Fig. S5. Interference of among the three drugs (MOR, AMP, and COC) and others (methadonemm MET; methamphetamine, MTD) for running the indirect competitive immunoassay. Compared to the response of the positive control (0 ng/mL) and a high concentration of the tested drug, the ODR values of all other drugs were almost the same as the positive control, indicating no interreference among them.

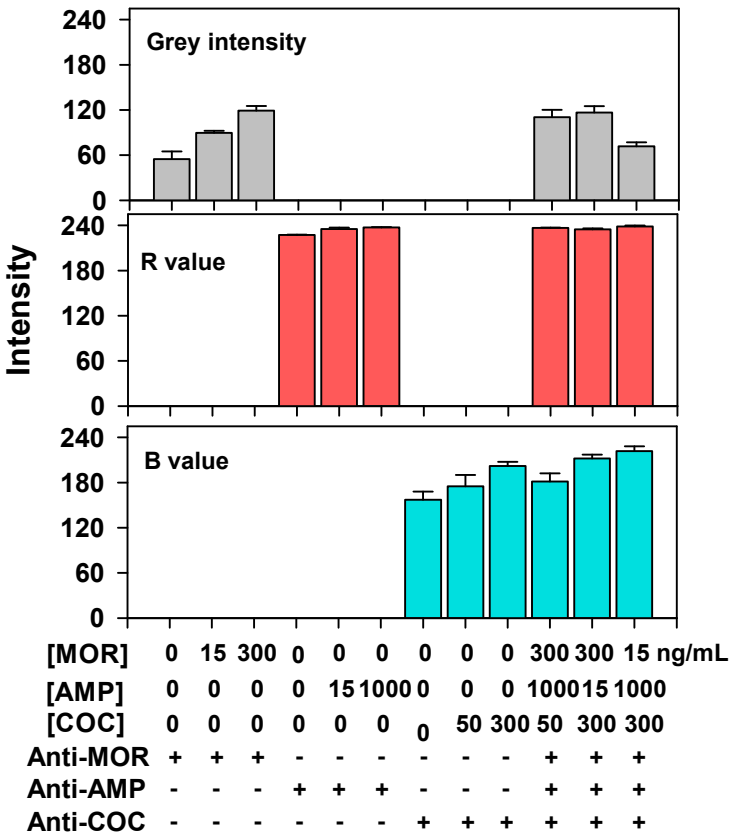


Fig. S6. Multi-color stained immuno-microarray for direct visualization of MOR, COC, and AMP. Histogram of color intensity determined from the different concentrations of drugs in each response zone. The bottom inset lists the amount of the drug and its corresponding antibody present in the sample. Compared to the determined R value (Firephos stained spots) and B value (TMB stained spots), the grey intensity showed the best correlation with the concentrations of drug presented in both individual and mixed samples. The R values provided relatively small changes, which limits it application for quantitation.

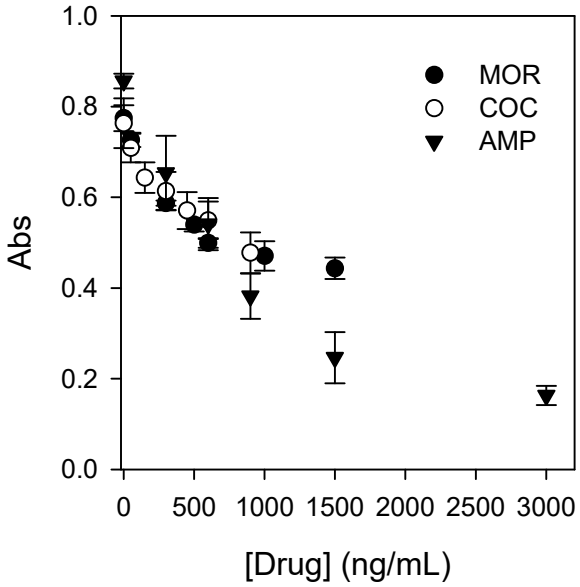


Fig. S7. ELISA validation of the drug detection via running indirect competitive immunoassays for MOR, COC, and AMP, respectively.

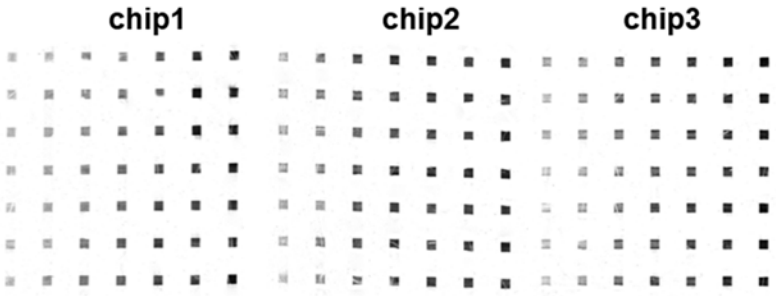


Fig. S8. Reproducibility of immuno-microarray chips for the detection of MOR (the concentration ranging from 0 to 900 ng/mL) shown on three independently prepared chips. The details of ODR values and their relative standard deviation (SRD) among three independently prepared immuno-microarrays (7×7) for the detection of MOR are displayed in the following table. The RSD is from 6.0% to 11.6% for chip 1, 4.1% to 14.4% for chip 2, and 6.5% to 13.8% for chip 3, showing the consistency among these chips.

ng/mL	ODR± SD (7 points of one chip); RSD			ODR± SD (3 chips); RSD
	chip 1	chip 2	chip 3	
0	0.84±0.050; 6.0	0.81±0.044; 5.4	0.87±0.065; 7.5	0.84±0.030; 3.6
1	0.75±0.087; 11.6	0.73±0.030; 4.1	0.76±0.064; 8.4	0.75±0.012; 1.6
5	0.67±0.063; 9.4	0.68±0.06; 8.8	0.69±0.045; 6.5	0.68±0.008; 1.2
50	0.54±0.062; 11.4	0.68±0.056; 8.2	0.63±0.046; 7.3	0.62±0.068; 11.0
300	0.46±0.044; 9.6	0.50±0.065; 13.0	0.49±0.066; 13.5	0.48±0.021; 4.4
600	0.34±0.037; 10.1	0.41±0.059; 14.4	0.34±0.047; 13.8	0.36±0.041; 11.4
900	0.23±0.032; 13.9	0.28±0.038; 13.6	0.24±0.029; 12.1	0.25±0.022; 8.8

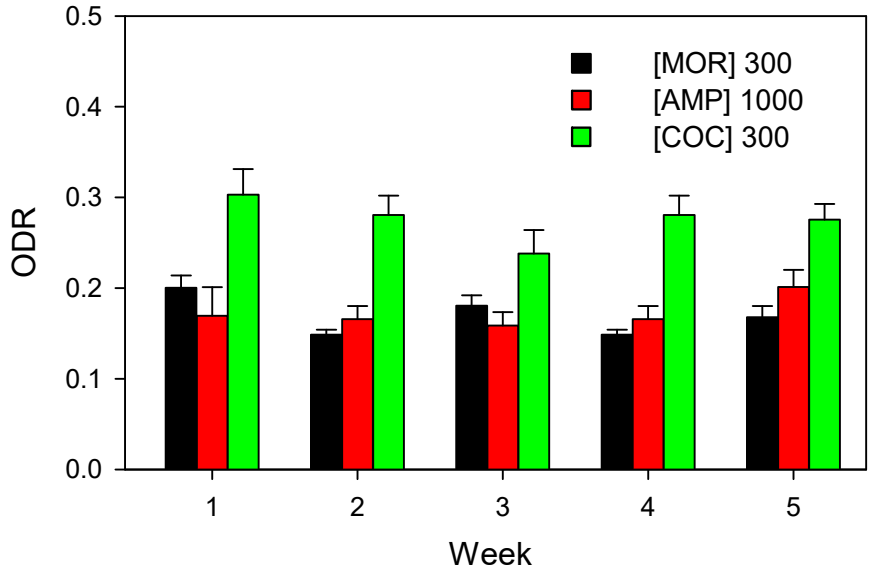


Fig. S9. Stability test of the immuno-microarrays. The chips with pre-immobilized probe coating conjugates (BSA-MOR, BSA-AMP, BSA-AMP) were either freshly prepared (control) or stored in a refrigerator (4 °C) for a period of time (from one week to five week). In all cases, the stability was evaluated by detection of MOR (300 ng/mL), AMP (1000 ng/mL), and COC (300 ng/mL). Compared with the ODR intensity performed on the freshly chips, the one tested on the "aged" chips displayed no substantial variations, indicating that the precoated chips are stable for over 5 weeks.