Supporting Information

for

Integrated Smartphone-App-Chip System for On-Site Parts-Per-Billion-Level Colorimetric Quantitation of Aflatoxins

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Additional experimental results and data analysis including dimension of the plastic microchip, HPLC data, comparison of experimental procedures of SPAC/HPLC/ELISA methods, Bland-Altman plots showing the SPAC/HPLC and SPAC/ELISA agreements, and the cost calculation for the SPAC tests (9 pages).

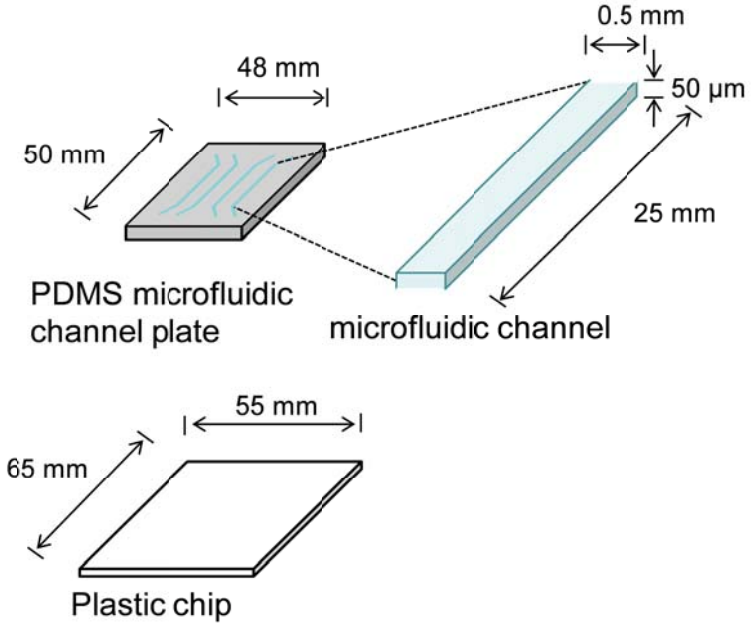

Figure S1. Physical dimensions of the PDMS microfluidic channel plate and PC plastic chip used for the SPAC assay.

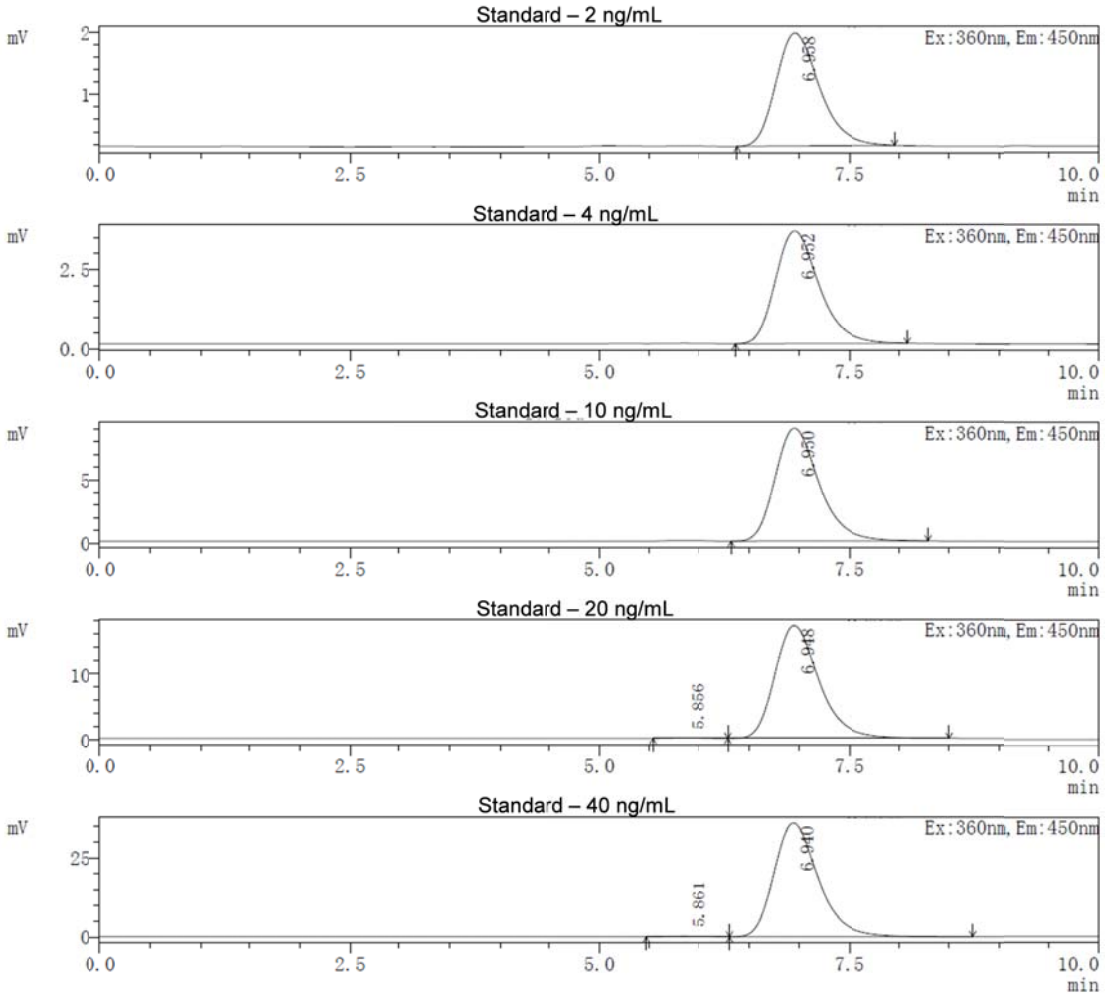

Figure S2. HPLC chromatograms of standard solutions of AFB1, prepared at 2, 4, 10, 20, and 40 ng/mL (equivalent to μg/kg, or ppb).

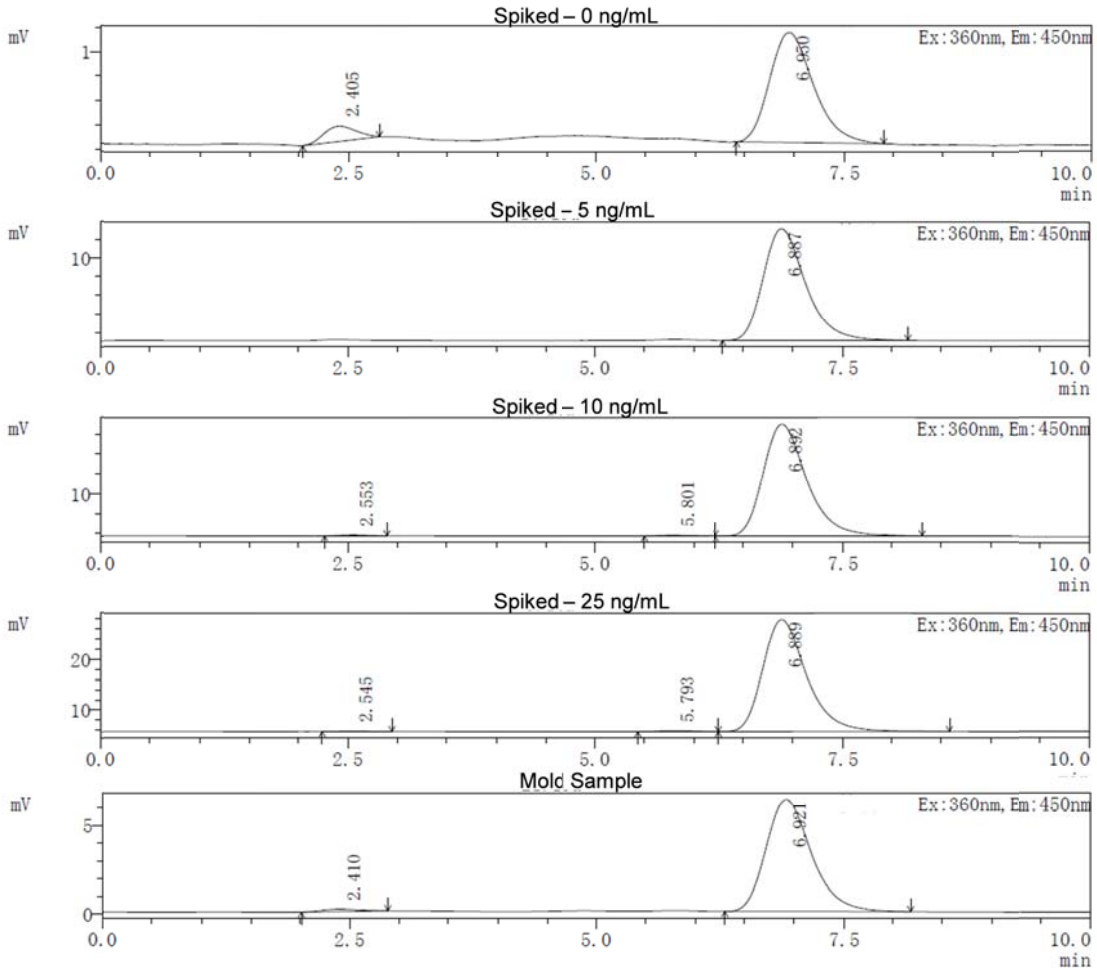

Figure S3. HPLC chromatograms of corn samples spiked with 0, 5, 10, 25 ng/mL AFB1 and a moldy corn sample.

Table S1. Summary of HPLC data for standard solutions of AFB1, corn samples spiked with AFB1 and moldy corn.

Sample (ng/mL)	Retention Time (min)	Peak Area	Concentration (ng/mL)
Std - 2	6.958	54458	2.189
Std - 4	6.952	106854	4.112
Std - 10	6.950	261920	10.071
Std - 20	6.948	521500	19.333
Std - 40	6.940	1092512	40.295
Spiked - 0	6.950	27352	1.193
Spiked - 5	6.887	179024	6.761
Spiked -10	6.892	344072	12.820
Spiked - 25	6.889	676046	25.007
Moldy corn	6.921	197856	7.453

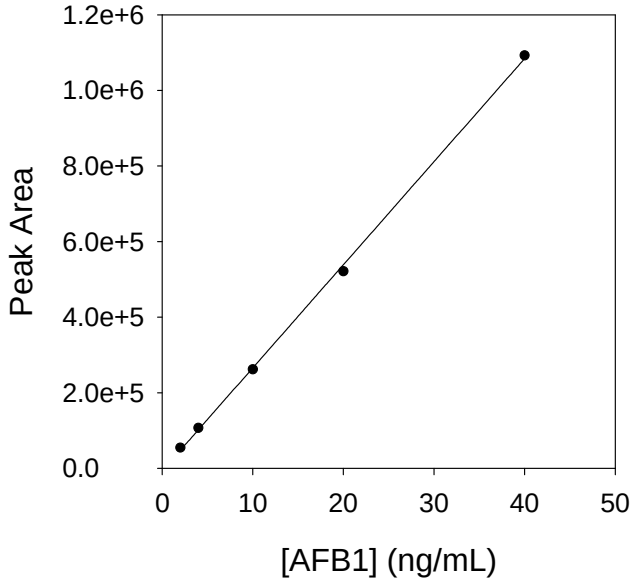

Figure S4. Calibration curve for AFB1 standard solutions (0-40 ng/mL) based on the HPLC data presented in Figure S2 and Table S1. The solid line shows the best fitting with an R^2 of 0.9994.

Table S2. Comparison of experimental procedures of the SPAC system with ELISA and HPLC methods.

Detection method		HPLC**	ELISA	SPAC
Sample preparation	Extraction	15 min	15 min	15 min
	Purification	30 min	n/a	n/a
	Derivatization	30 min	n/a	n/a
Sample detection	Chip preparation	n/a	8 h*	8 h*
	Chip blocking	n/a	3 h*	3 h*
	Sample loading/testing	30 min	30 min	30 min
	Signal enhancement	n/a	30 min	30 min
	Assay analysis	30 min	15 min	< 30 s

Note: n/a, not applicable; * these steps can be carried out prior to the tests (i.e., preloaded and blocked chips will be available for on-site testing. **HPLC does not involve the steps of chip preparation but the column needs to be preconditioned. It should be noted that the duration for each step varies significantly depending on the exact condition of the sample and instrumentation; here we just provide an estimation of the time needed for each method.

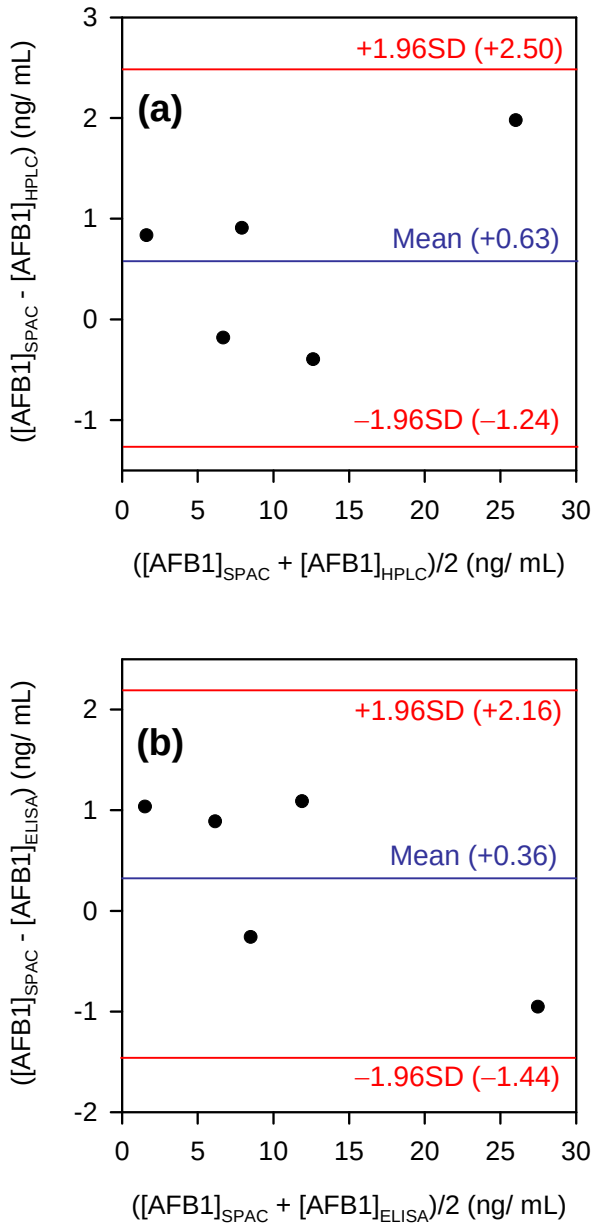

Figure S5. Bland-Altman plots showing the agreements between SPAC and HPLC (a), and between SPAC and ELISA (b), respectively. The data are based on the results presented in Figure 7(a) in the main text. For the detailed procedure to construct these plots, see Bland J. M.; Altman, D. G. Statistical methods for assessing agreement between two methods of clinical measurement, *Lancet* **1986**, *327*, 307–310.

Table S3. Cost estimation of the SPAC tests.*

Chip substrate and reagent		**Optical attachment**	
PC plate	$0.10	LED light source	$0.25
AFB1(standard)	$0.10	LGP diffuser reflector	$1.25
Anti-AFB1 antibody	$1.00	3D printing materials	$1.00
AFB1-BSA (coating antigen)	$1.30	Batteries	$0.40
Nanogold-streptavidin conjugates	$0.30		
Biotin labeling kit-NH2	$0.50		
Other reagents (Buffers, NHS, EDC, etc.)	$1.90		
Total	$5.20	Total:	$2.90

Note: The cost estimation listed above is in US dollars and based on multiple chips to be prepared and tested with one purchase of reagents. Taking AFB1 as an example, 5 mg AFB1-BSA was priced at ~$ 800, and can be used for making 600 chips. Therefore, the cost of AFB1-BSA for one chip is ~$1.30. For the PC substrate it was purchased at ~$70 for a large panel (2.6 m^2), from which about 700 chips can be made. Therefore, each of the PC substrates only costs ~$0.10. * It should be pointed out that due to variations in the price of reagents and materials purchased from different suppliers, it is impossible to calculate the exact expense for each chip but to provide a general idea of the cost effectiveness of the SPAC tests.