Electronic Supplementary Information

Laser-printing of toner-based 96-microzone plates for immunoassays

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The electronic supplementary information (ESI) brings additional information about the contact angle measurements and scheme related to direct and indirect ELISA procedures. Complementary results found for the analytical performance and ELISA with reduced incubation time are also presented.

Experimental

Contact Angle Measurements

Contact angles of static drops placed at each zone were measured on images captured with an optical microscope equipped with a digital camera with 1.3 MP-resolution. Measurements were taken at room temperature using drops of a MB solution which were defined by volumes ranging from 10 to 50 μL. Images were analyzed in the software Corel Draw version 11.0 and all recorded contact angles were the average of three data points.

ELISA procedures

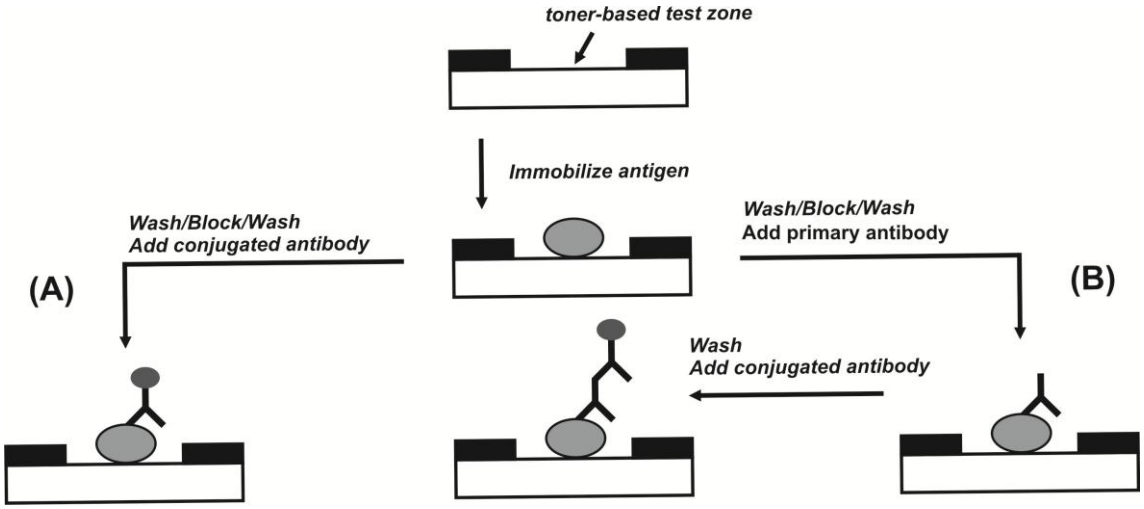

Figure S-1. Simplified scheme of the (A) direct and (B) indirect ELISA procedures performed on toner-based microzones.

Results

Analytical curve for the methylene blue solution

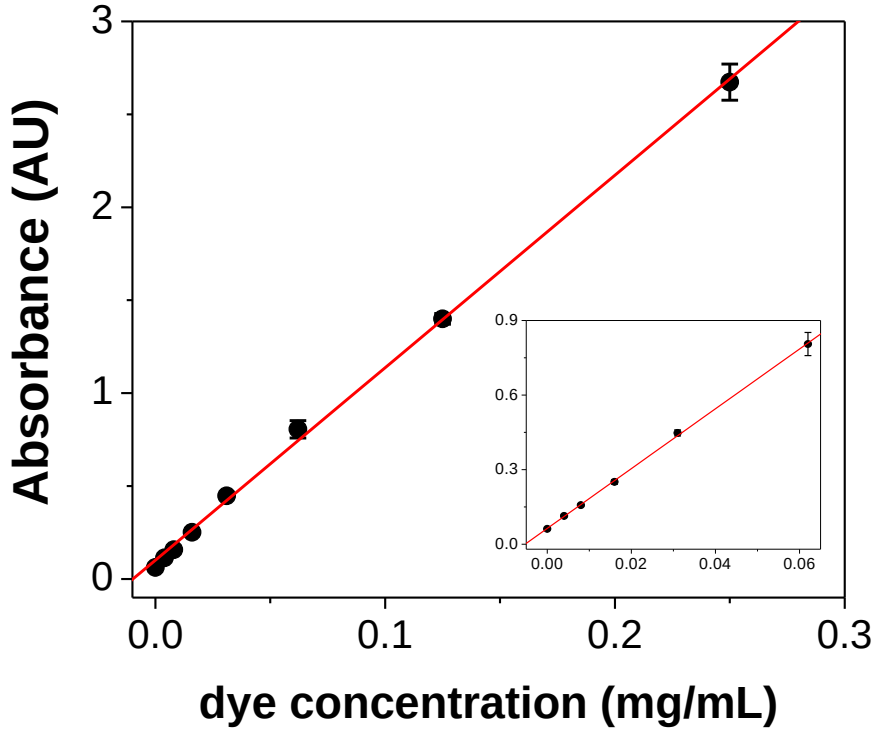

Figure S-2. Analytical curve for the methylene blue dye. The points plotted in this Figure are the mean values of twelve independent values recorded in different zones. The error bars mean the standard deviation for these data.

Data generated according to the Hill model

As described in the experimental section, ELISA procedure has been carried out in both plastic and printed microplates using aliquots of 50 and 10 μL, respectively. The comparative data extracted from the Hill model are depicted in Table S-1.

Table S-1. Presentation of the ELISA results achieved on conventional and printed microplates.

Devices	A_{max} (AU)	[L_{50}] (in µg/mL)	n	LD (µg/mL)
Conventional microplates (50 µL of sample)	3.2 ± 0.1	0.45 ± 0.03	1.3 ± 0.1	0.05
Printed microplates (10 µL of sample)	2.4 ± 0.1	2.40 ± 0.26	1.4 ± 0.1	0.2

ELISA procedure with reduced incubation time

Once proved the capability of performing direct ELISA on toner-based zones, we also investigated the use of shorter time to experimental protocol. Basically, the coating time (immobilization) was reduced from overnight to 2 hours. In addition, steps related to the blocking and the complexing with the conjugated antibody were reduced to 15 min each. Figure S2 shows the results comparing the ELISA data obtained with conventional and reduced time. For each curve, we chose just six concentrations of IgG which provide a linear relationship using 5 µL of sample. ELISA was performed on printed microzones defined with 7 mm diameter and toner barrier with 1 mm wide. As it can be seen in Figure S-3, the sensitivity is clearly reduced. The equations of the curves obtained with conventional and reduced time were y = 0.00268 + 0.00730 x, and y = -0.00562 + 0.00238 x, respectively. Furthermore, other noticeable difference is attributed to the standard deviation (SD) values. For conventional procedure, the SD values were higher than those obtained with shorter time. The main reason associated to this parameter is the evaporation rate of the sample. For longer times, this effect is more pronounced.

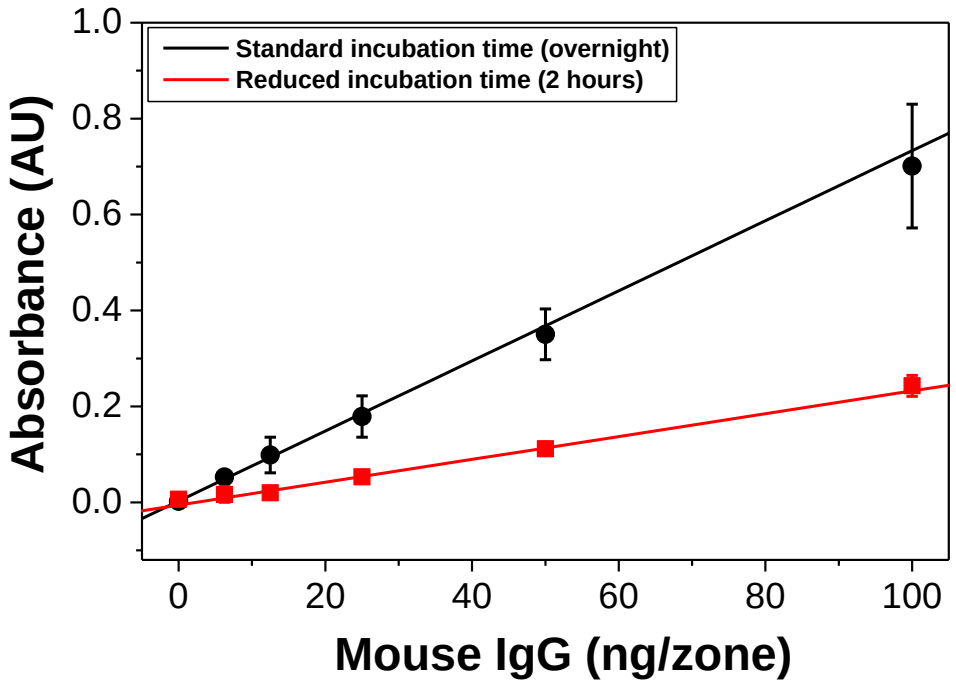

Figure S-3. Comparison of the ELISA results using standard and reduced incubation time. Sample volume = 5 μL. Other details: see Figure 4 in the main text**.**

Device-to-device repeatability

The plate-to-plate repeatability was investigated for three different devices, previously prepared for bioassays according to an indirect ELISA procedure described in the main text. The results found for this comparison are shown in Table S-2. It was found that the response for blank, control and a real sample of cell culture supernatant exhibited RSD values of 2.2%, 10.6 %, and 6.8%, respectively.

Table S-2. Data found for the plate-to-plate repeatability.

Devices	B (10^{-3} AU)	Control (10^{-3} AU)	Sample (10^{-3} AU)
Microplate 1	44	540	566
Microplate 2	46	466	525
Microplate 3	46	442	494
Average ± 1sd	45 ± 1	482 ± 50	528 ± 36