Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Hydrogel Particles-on-chip (HyPoC): a fluorescence micro-sensor array for IgG immunoassay

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Spectrophotometric measurement of hIgG-atto647N

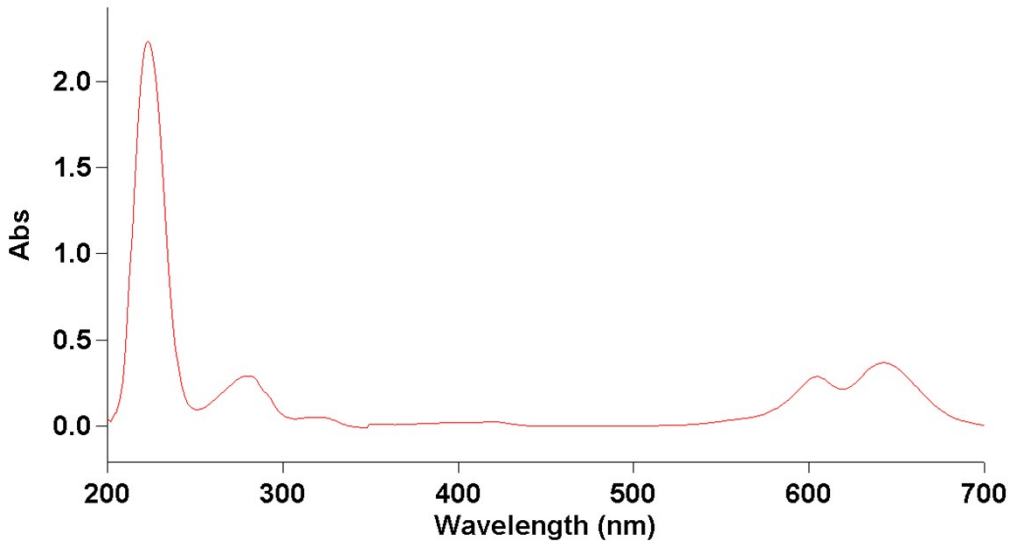

Figure S1: UV spectrum of hIgG-atto647N measured after dialysis at a concentration of 0.1 mg/mL in TRIS buffer.

Microfluidic device fabrication process

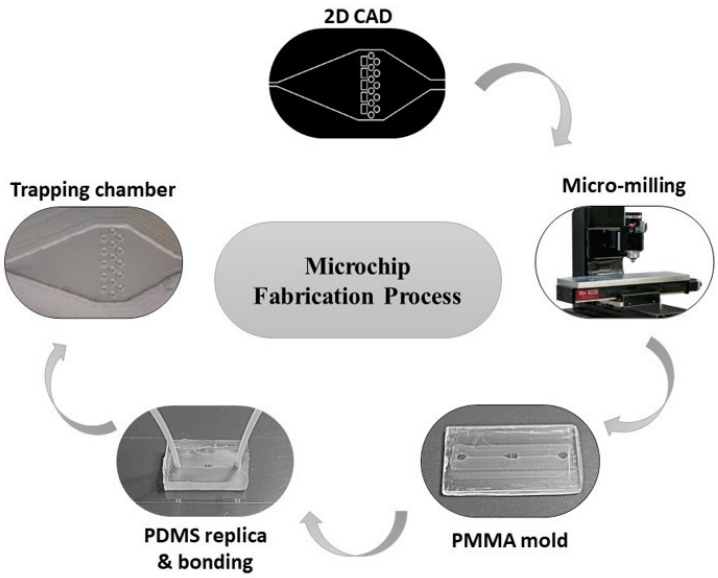

Figure S2: Schematic illustration of a microfluidic chip production process, which involves 4 phases: two-dimensional CAD drawing, micro-milling processing for the realization of the negative mold in PMMA, positive replication in hydrophilic PDMS of the device and finally its irreversible bonding on the slide through plasma and heat treatment.

LOD modulation with particles number

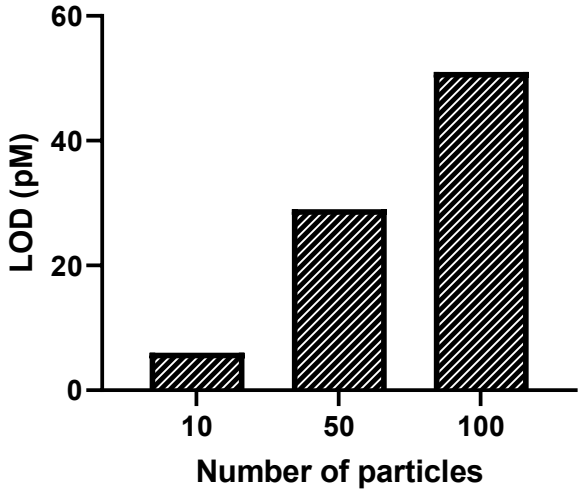

Figure S3: Bar graph showing the decrement of the limit of detection with the reduction of the number of particles used to perform the immunoassay.

Incubation time optimization

In order to optimize the hIgG antibody incubation time, different aliquots of conjugated microparticles (0.1 pmol/part anti-hIgG-FC, Sigma Aldrich) were incubated with a fixed concentration of hIgG-atto647N (500pM). The binding reaction was stopped by washing the microparticles at different times (0, 0.5, 1, 2, 3.5, 4.5 and 6 hours) to study its kinetics. After several washes to remove unbound hIgG-atto647N, images at confocal microscope (CLSM Leica SP5, Objective 10x DRY, scan speed of 400 Hz, excitation wavelength 633, emission wavelength 648-710nm) were acquired and analysed using ImageJ software to evaluate the residual fluorescence intensity within the microparticles. The error is represented as standard deviation over about 50 microparticles for each time point.

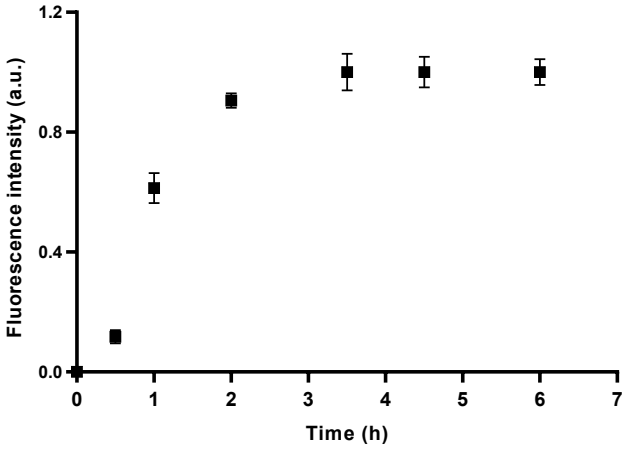

Figure S4: Experimental data of fluorescent hIgG binding kinetics.

In-gel immunoassay on 5 particles

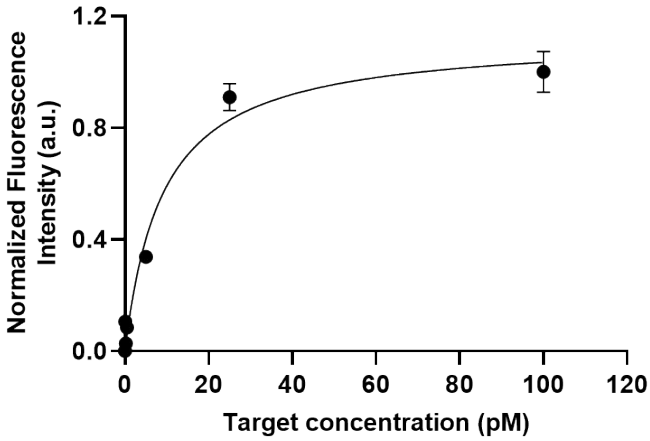

Figure S5: Data points and fit (non-linear regression based on Langmuir Isotherm Model R^2=0.97) of the mean calibration curve for hIgG detection obtained with three independent experiment performed on five functionalized hydrogel microparticles each.

Hypoc Device: loading

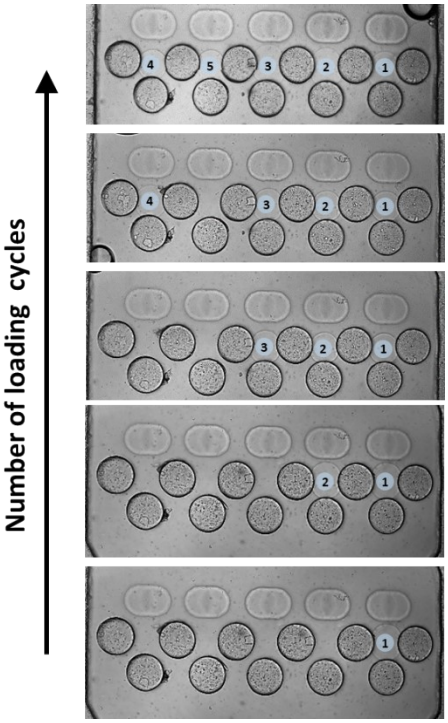

Figure S6: Optical images of microparticles trapped inside the device for each loading cycle, corresponding to an injection of 100µL of a diluted particles suspension (10 particles/ml). For each loading cycle, on average, a single particle is trapped into the device. Particles were numbered to improve their visibility.

Washes on chip optimization

The device was first loaded with five functionalized microparticles (conjugated with 0.1 pmol/part of anti-hIgG-FC) and then a solution containing a high concentration of fluorescent target hIgG (1 nM) was flushed with a syringe inside the chip. After, the fluorescence emission from the five microparticles was registered and measured, corresponding to point “zero” shown in figure S7. Moreover, the fluorescence signal from the channel surface was also considered for evaluating background noise and subtracted from the intensities recorded within particles. After the target perfusion (1 minute), the hydrogel microparticles were washed with a buffer solution (45µL at a time) for three times. At each washing step, the intensity of the signal decreases, stabilizing already after the second wash. The recorded signal is indicative of an efficient recognition and capture of the target by the particles in a single perfusion step.

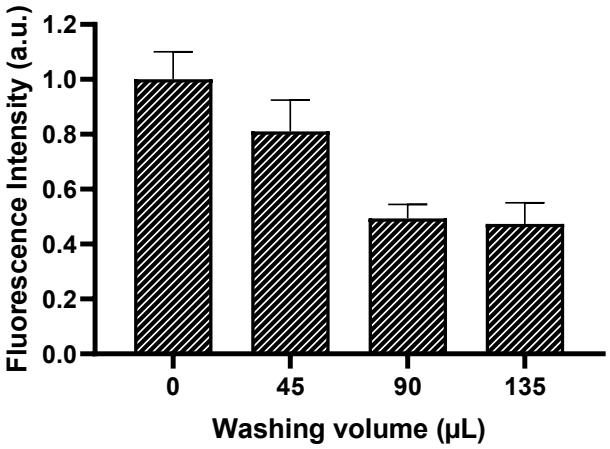

Figure S7: Bar-graph showing the average fluorescence intensity inside the trapped microparticles subtracted from the signal emitted by the channel (background). The washing volume 0 indicates the moment in which the labelled hIgG is injected into the device. The fluorescence signal reflects the target binding, which is efficiently recognized and captured by the particles.

HyPoC Immunoassay

Table S1: Normalized fluorescence intensity values for hIgG detection with HyPoC. Each value represents mean and standard deviation over the five functionalized microparticles loaded in every single device.

hIgG (nM)	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 4**
0	0 ± 0.036	0 ± 0.055	0 ± 0.035	0 ± 0.045
0.1	0.265 ± 0.092	0.259 ± 0.076	0.279 ± 0.067	0.282 ± 0.072
0.15	0.323 ± 0.089	0.345 ± 0.080	0.352 ± 0.070	0.361 ± 0.076
0.2	0.460 ± 0.091	0.465 ± 0.078	0.510 ± 0.077	0.529 ± 0.083
0.3	0.589 ± 0.071	0.564 ± 0.067	0.645 ± 0.056	0.639 ± 0.061
1	0.705 ± 0.086	0.701 ± 0.074	0.694 ± 0.084	0.722 ± 0.091
2	0.799 ± 0.078	0.820 ± 0.062	0.854 ± 0.075	0.872 ± 0.081
3	0.904 ± 0.083	0.906 ± 0.092	0.917 ± 0.101	0.975 ± 0.101
4	0.953 ± 0.075	0.944 ± 0.085	0.970 ± 0.105	0.985 ± 0.102
5	1 ± 0.0931	1 ± 0.096	1 ± 0.103	1 ± 0.101

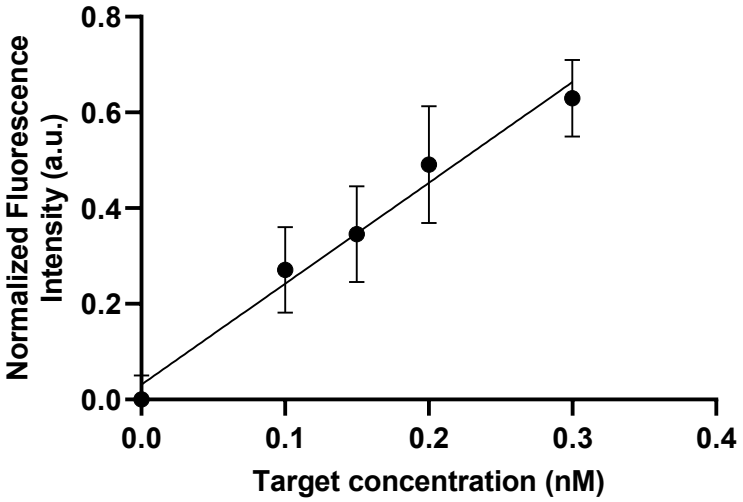

Figure S8: Data points, standard deviation and linear fit (R^2=0.98) of the linear fraction of the mean calibration curve for detection of hIgG with HyPoC.