Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A microfluidic immunoassay platform for the detection of free prostate specific antigen: a systematic and quantitative approach

Narayanan Madaboosi[a #], Ruben R. G. Soares[a,c #], Virginia Chu[a] and João Pedro Conde[a,b] *

[a] INESC-MN – Microsystems and Nanotechnologies, Lisbon, Portugal.

[b] Department of Bioengineering, Instituto Superior Técnico, Lisbon, Portugal

[c] iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

[#] Equal Contribution

* Corresponding author email: joao.conde@tecnico.ulisboa.pt

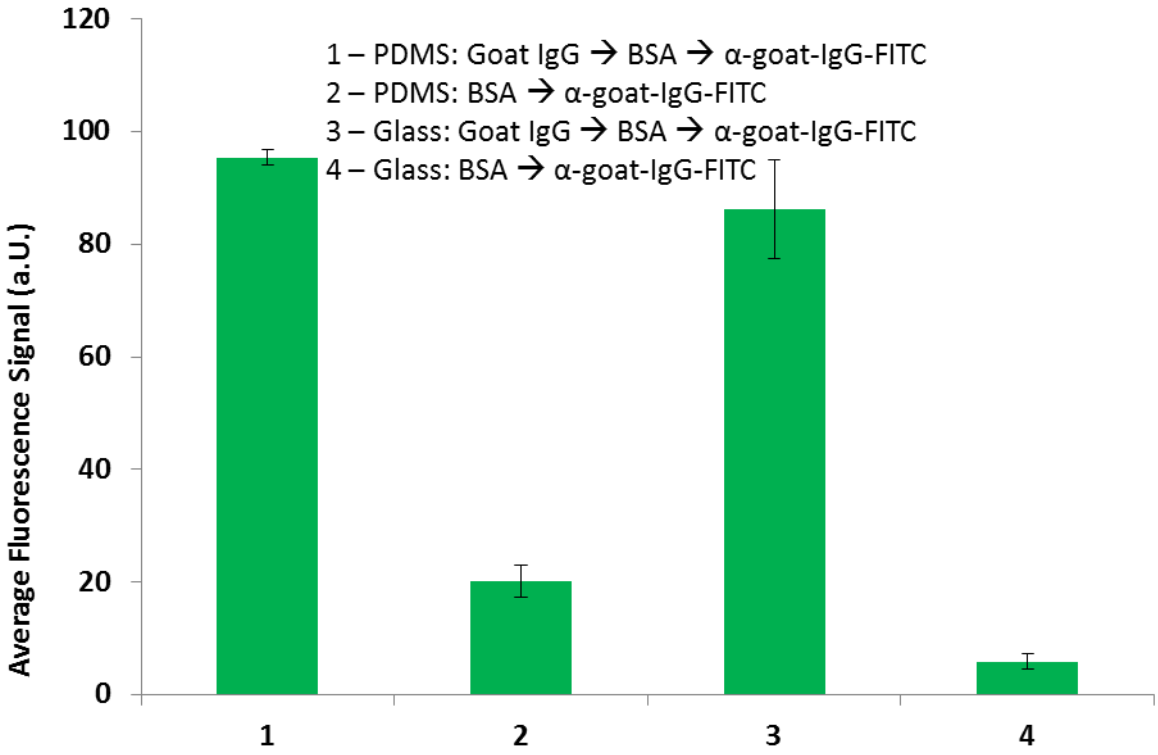

Fig. S1: Comparison between PDMS and glass substrates for surface coverage, when a primary Ab is adsorbed on their surface and detected by a secondary FITC-labelled anti-Ab. 1 & 3 refer to the experiments, and 2 & 4 refer to the controls for signal from the secondary Ab as well as the blocking, using BSA. Both the primary and the secondary Abs have been used at a concentration of 100 µg/mL. 4% BSA was used for blocking. 2 & 4 show a drastic reduction in the signal compared to 1 & 3, thus indicating the efficiency of blocking, also showing that the non-specific signal can be reduced by blocking.

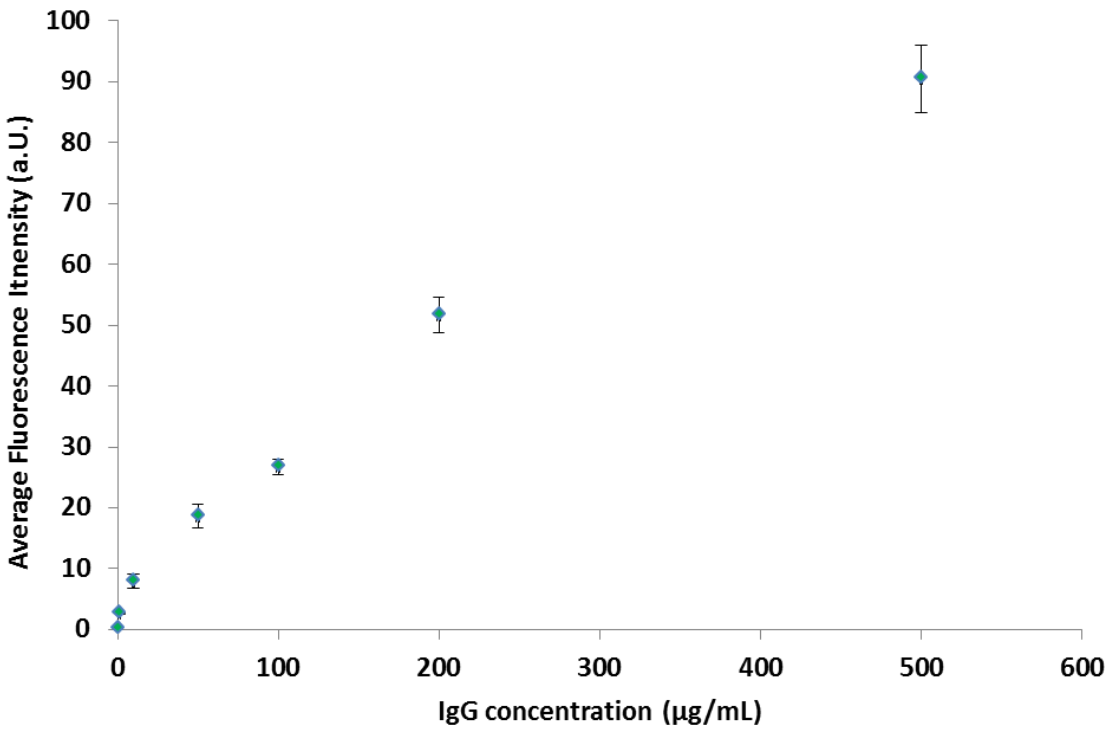

Fig. S2: Relation between the concentration of the immobilised Ab (α-mouse IgG labelled with FITC) and the absolute signal from the spot measured in microscope.

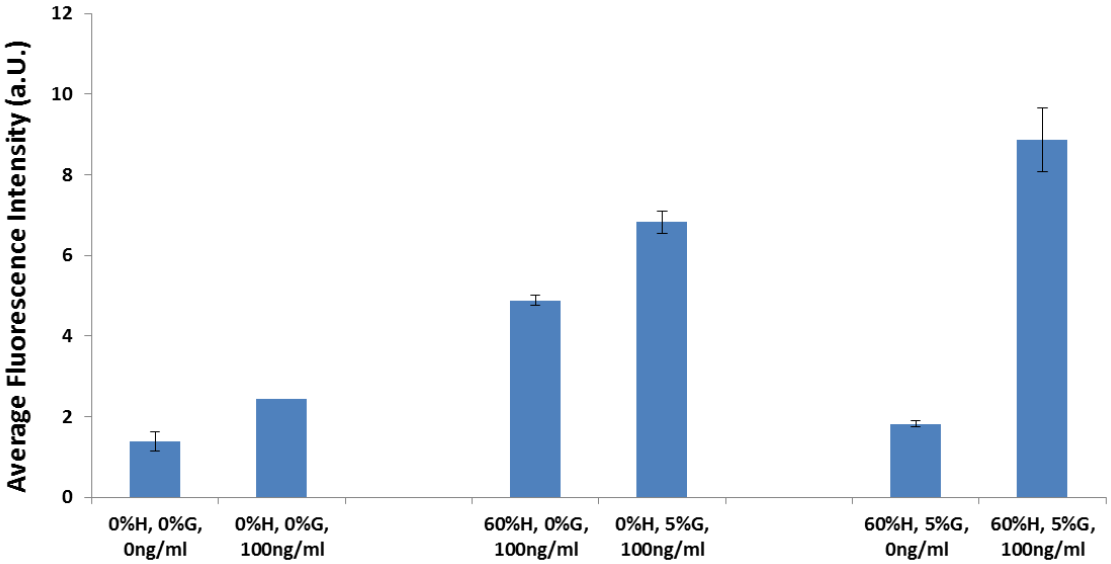

Fig. S3: Optimisation experiments showing the effect of humidity (H) and glycerol (G) for experiments performed with spotted α-PSA Ab (Ab10187). After sealing with corona, 100 ng/mL PSA was captured by the spotted Ab and detected using FITC labelled α-PSA Ab (Ab178776). 60% humidity and 5% (v/v) glycerol help in establishing a clear difference between the control (0 ng/mL PSA) and the sample (100 ng/mL PSA).

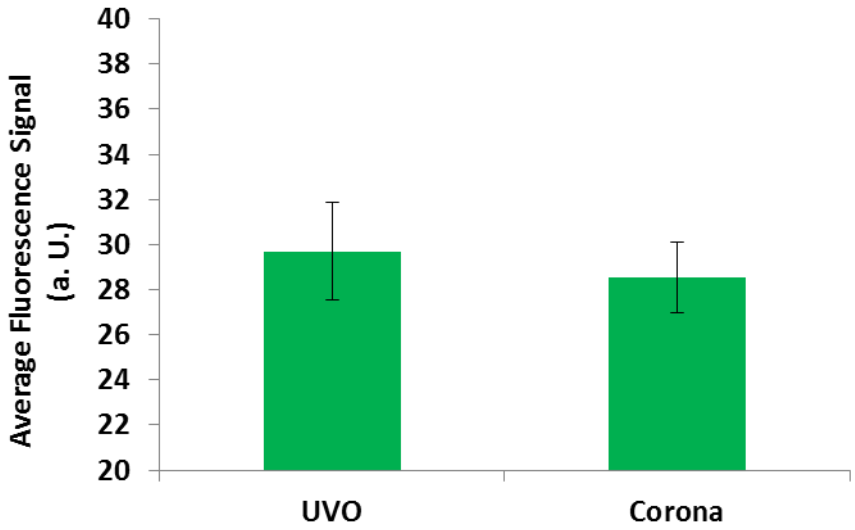

Fig. S4: Effect of sealing on signal. FITC-labelled α-goat Ab (50 µg/mL) was adsorbed by flowing in the microchannels, after sealing by either UVO or corona methods. Upon washing with PBS, the final signal is essentially the same independently of the channel sealing method.

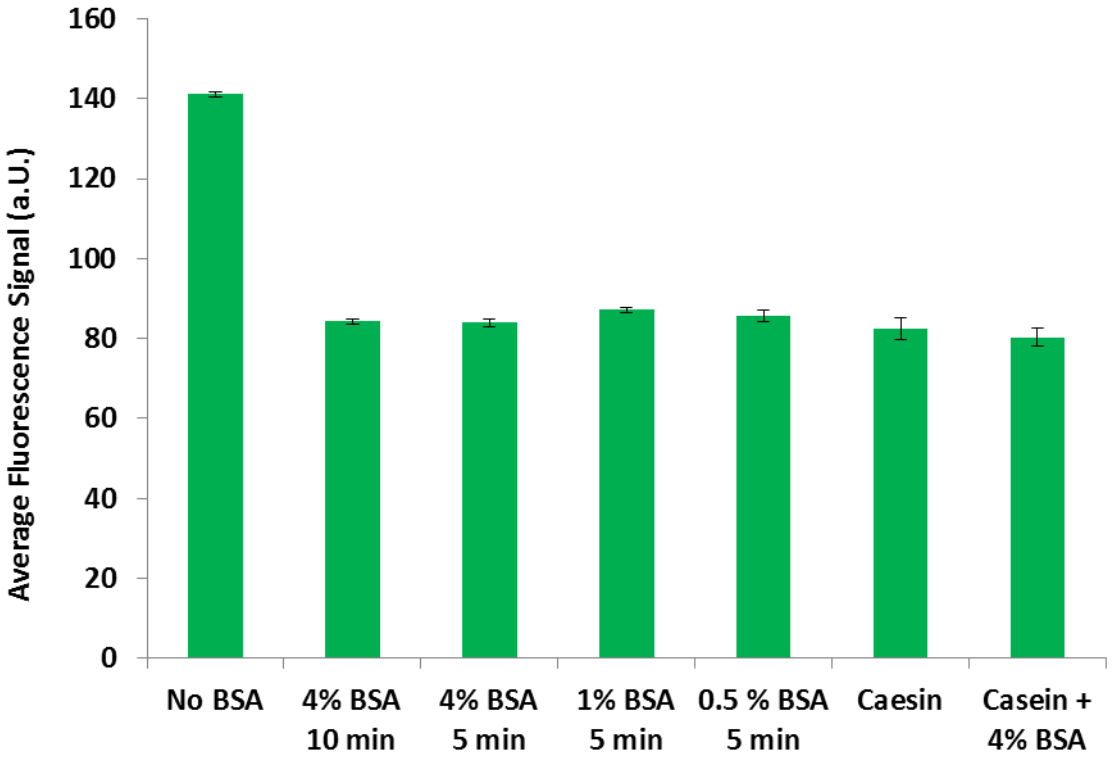

Fig. S5: Effect of blocking on final signal: Mouse IgGs were first adsorbed on the surface, followed by blocking using the indicated parameters in the figure and finally, FITC-labelled α-mouse IgGs were flowed inside the microchannel. With no blocking, the background signal resulting from the non-specific adsorption is very high, while blocking reduces the non-specific adsorption. All types of blocking, be it with BSA or casein or a mixture of both proteins, as well as be it for 5 or 10 min yield essentially the same specific signal.

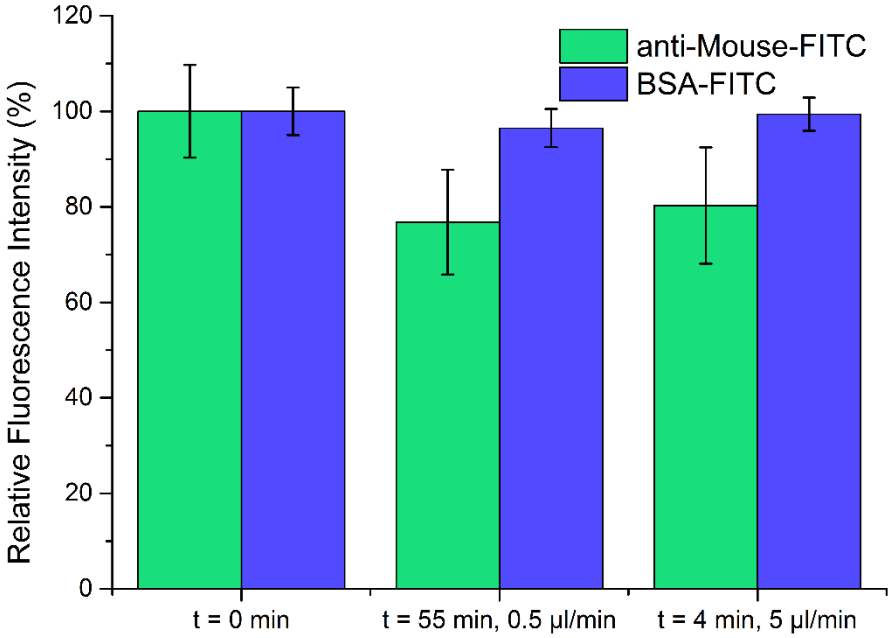

Fig. S6: Effect of incubation times and washing on the erosion of molecules adsorbed on the PDMS microchannel surface. For these experiments 50 µg/mL of BSA-FITC or 100 µg/mL of goat anti-mouse IgG-FITC were first adsorbed on the microchannels at 0.5 µL/min for 10 min, followed by a 10 mM PBS wash for 1 min at 5 µL/min. The incubation and washing steps were performed in series on the same microchannel. It can be seen that that while the 10 mM PBS incubation or washing steps do not provide a significant drop in fluorescence for the BSA, a drop in fluorescence of >20% down is observed for the adsorbed IgG molecules during a 55 min incubation step. The error bars represent the standard deviation from six measurements along the microchannels.

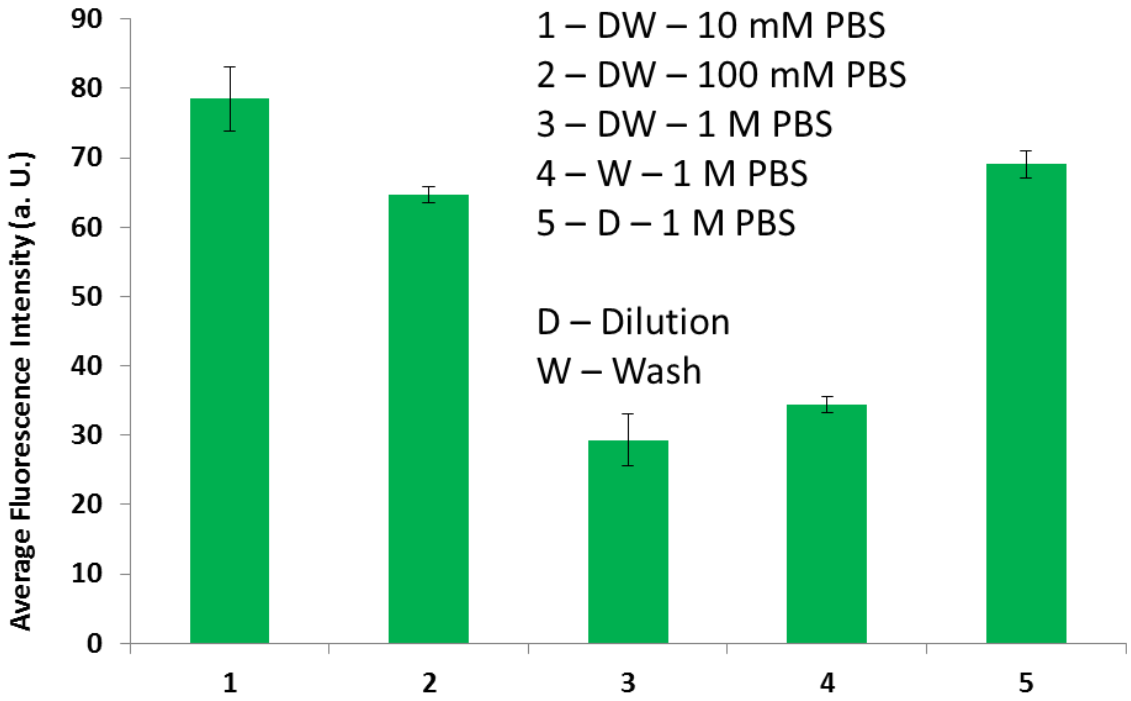

Fig. S7: Effect of salt. α-goat IgG-FITC adsorbed on the surface. 'D' refers to the dilution and 'W' to washing, and different concentrations of phosphate ions were used both for dilution as well as washing. DW – dilution and washing. PBS with 10 mM phosphate concentration, both for dilution of Abs as well as washing, yielded the maximum signal and was hence chosen for the experiments.

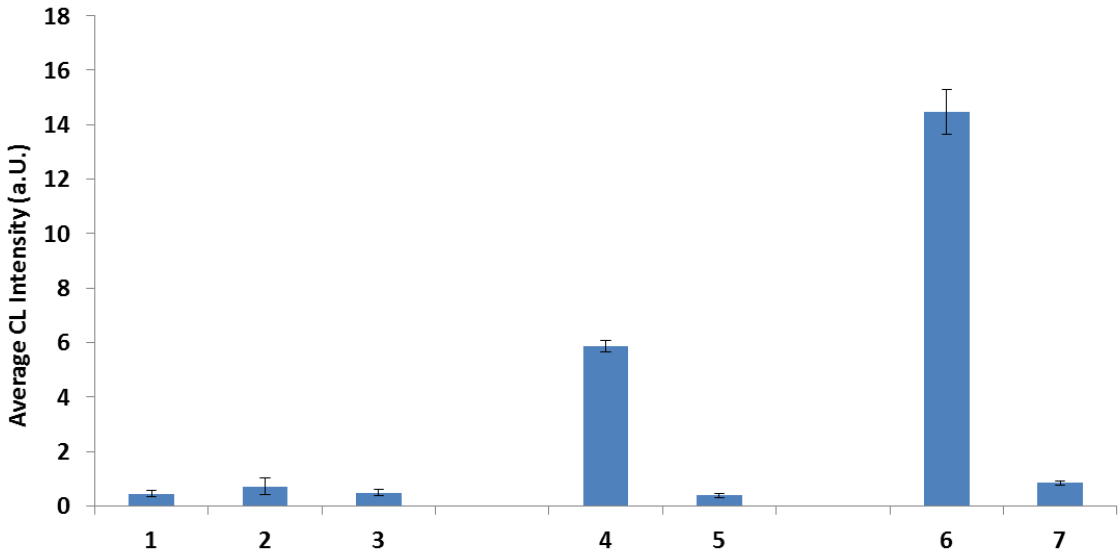

Fig. S8: Specificity check for fPSA assays. (1) 1 ng/mL IL6 captured using fPSA probe Ab (Ab10187) and detected using α-IL6-HRP Ab. (2) Surface blocked with 4% BSA and α-IL6-HRP Ab for checking the effective blocking to avoid non-specific adsorption. (3) 1 ng/mL IL6 captured using emtPSA probe Ab (Ab10189) and detected using α-IL6-HRP Ab. (4) 1 ng/mL IL6 captured and detected using IL6-specific Abs, with blocking step. (5) 1 ng/mL fPSA captured and detected using IL6-specific Abs, with blocking step. (6) Capture Ab for IL6 adsorbed on the surface, followed by detector IL6 Ab, without any intermediary blocking step. (7) Capture Ab for IL6 adsorbed on the surface, followed by detector IL6 Ab, with an intermediary blocking step.

Table S1: Control experiments for optimizing parameters related to flow of luminol for chemiluminescent mode of detection

Parameter	Condition	Chemiluminescent Signal (a.U.)
Speed of Luminol	a) 5 µL/min b) 15 µL/min	16 +/- 0.7 16.2 +/- 1
Batch of Luminol	a) Batch 1 b) Batch 2	20.5 +/- 2.5 29.2 +/- 0.9
Effect of External Light	a) With light b) Without light	25.3 +/- 1.2 29.2 +/- 0.9
Exposure Time	a) 10s b) 20s	29.2 +/- 0.9 46.7 +/- 1.6
Type of Luminol	a) Pico Luminol b) Femto Luminol	14.6 +/- 1.3 83.2 +/- 0.5