SUPPLEMENTARY DATA

A Lab-in-a-briefcase for rapid prostate specific antigen (PSA) screening from whole blood

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Supplementary Methods

PSA Immunoassay Optimization in the MCF

The effect of CapAb concentrations (10, 20 and 40 µg/ml) and DetAb concentrations (0, 0.5, 1 and 2 µg/ml) on colorimetric signal was tested by comparing absorbance values obtained in test strips incubated with 0 ng/ml (negative control), 1.5 ng/ml (lower end) and 30 ng/ml (upper end) recombinant protein diluted in the assay buffer. Once the optimum CapAb and DetAb concentrations were established, the effect of DetAb concentration and DetAb incubation times (0, 2.5, 5, 10 minutes) was fully tested using 4.0 ng/ml of recombinant protein. A third set of experiments was performed which aimed optimising the conditions for the enzyme complex, High Sensitivity Streptavidin-HRP (0, 1, 2 and 4 µg/ml). All core sandwich PSA immunoassay steps were optimised in respect to maximum signal-to-noise ratio.

A chromogenic OPD enzymatic substrate was used, which consisted in 1 mg/ml OPD and 1 mg/ml H_2O_2 (enhanced recipe) or 4 mg/ml OPD and 1 mg/ml H_2O_2 (fully optimised recipe); note the manufacturer recommended 0.4 mg/ml OPD and 0.4 mg/ml of H_2O_2 for ELISA in MTPs. Horseradish peroxidase is an enzyme with a large turnover, therefore we have found that higher concentrations of OPD and/or H_2O_2 results in one order of magnitude increases in absorbance in the microcapillaries, where the diffusion of a small molecule such as OPD and DPA across the whole diameter of the capillary can happen in few seconds.

PSA Immunoassay in the Microtiter Plate (MTP)

Table S1. Experimental conditions used for sandwich ELISA detection of PSA in a 96 well MTP

Step	Concentration	Incubation Time	Volume (per well)	T (ºC)
CapAb	1 µg/ml	overnight	100 µl	4
Washing	-	-	4*100 µl	20
Blocking (BSA)	1% (w/v)	2 hours	300 µl	20
Washing	-	-	4*100 µl	20
PSA standards	0.9-60 ng/ml	120 minutes	100 µl	20
Washing	-	-	4*100 µl	20
detAb	0.2 µg/ml	120 minutes	100 µl	20
Washing	-	-	4*100 µl	20
Enzyme (Extravidin)	1 µg/ml	20 minutes	100 µl	20
Washing	-	-	4*100 µl	20
Substrate (OPD)	0.4 mg/ml	30 minutes	100 µl	20

Supplementary Results

PSA Immunoassay Optimization in the MCF

A concentration of 20 µg/ml of capAb and 2 µg/ml of detAb resulted in the highest absorbance signal for the assay as shown in Figure S4, however it also resulted in higher background signals (strip with 0 ng/ml of recombinant protein) with incremental CapAb concentrations for all range of DetAb concentrations tested. With 40 µg/ml of CapAb the concentration of DetAb significantly affected the background signal. This happened because the detection antibody is polyclonal, so it can bind directly to the capture antibody or to other proteins on the fluoropolymer surface significantly increasing the background signal. The best signal-to-noise ratio was therefore obtained with 40 µg/ml of CapAb and 0.5 µg/ml of DetAb. This was also valid for 1.5 ng/ml and 30 ng/ml PSA (lower and upper limits, respectively, of PSA studied range), based on 5 minutes substrate incubation time. The large

surface-area-to-volume ratio in the 200 μm MCF allows coating the plastic fluoropolymer surface with a concentration of CapAb more than 10x higher than on a 96-well plate. This can potentially improve the sensitivity of the assay in the MCF, as the kinetics of antigen-antibody binding is favoured by high surface density of CapAb molecules. This is only possible until a given surface coverage, above which the antibodies binding capacity decreases for reason well established in literature.[1]

In a further set of experiments a MCF reel was coated with 40 μg/ml of CapAb varying the DetAb concentration and DetAb incubation time. Then, the full sandwich assay was completed using 4 ng/ml of recombinant protein. Figure S4B shows that for all DetAb concentrations studied the absorbance values increased until 1 μg/ml of DetAb. The highest absorbance signals were obtained for 5 minutes incubation time of DetAb, followed by 10 minutes and 2.5 minutes. The background signal increased with the increase in the concentration and incubation time of the DetAb, being 10 minutes incubation and 2 μg ml of DetAb the conditions resulting in higher background signals. Figure S4B shows that 1 μg/ml of DetAb incubated for 5 minutes resulted in the highest signal-to-noise ratio with 40 μg/ml of CapAb. The use of a detection monoclonal antibody would increase the sensitivity of the assay, however this issue can be easily overcome by varying the concentration and incubation time for the DetAb.

In respect to the enzymatic complex, the highest signal was obtained with 4 μg/ml of High Sensitivity Streptavidin-HRP with 5 minutes incubation (Figure S4C), however it is important to take in consideration that these conditions also led to the highest background signal. The presence of colorimetric signal at 0 μg/ml of enzyme showed enzymatic conversion of OPD in the absence of enzyme which might have been induced by the transmitted light in the flatbed scanner.

Supplementary Figures

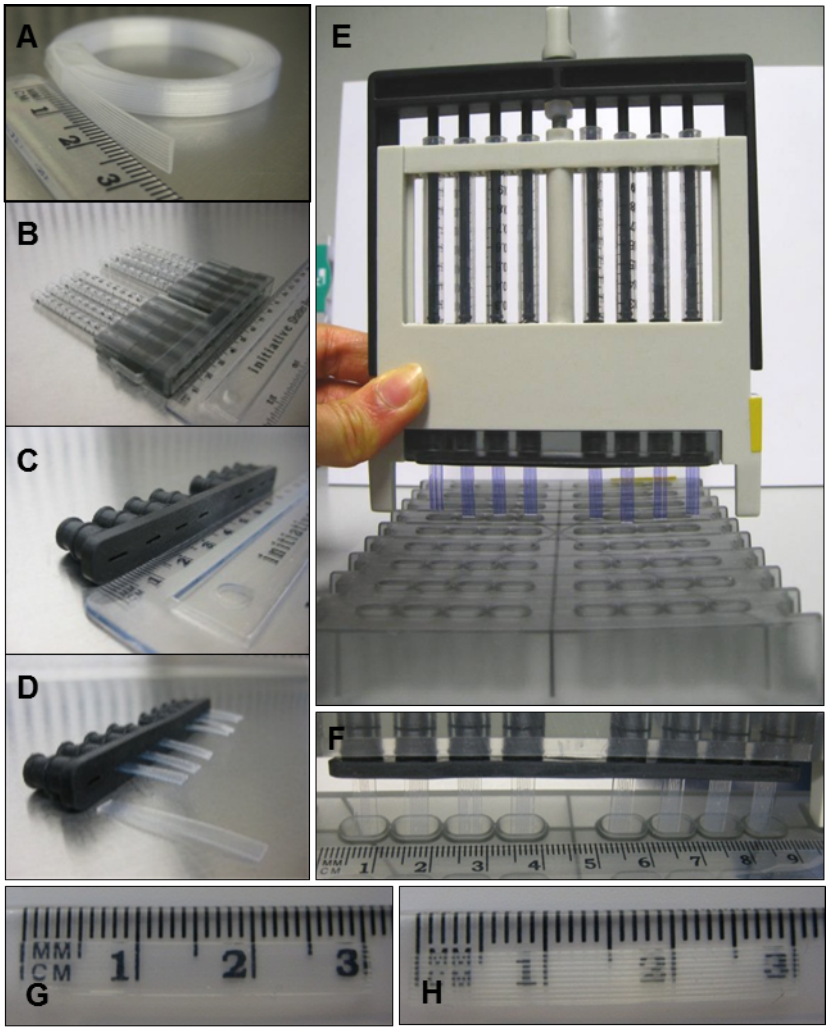

Figure S1. Overview of the "Lab-in-a-briefcase" components. **A** Close photograph of a melt-extruded fluoropolymer MCF reel with 10*200 µm i.d. embedded microcapillaries. **B** Cartridge incorporating an array of eight parallel 1 ml syringes used for fluid aspiration. **C** Close up at the rubber push-fit seal. **D** Push-fit of 30 mm long pre-coated fluoropolymer MCF strips in the rubber seal. **E** Fully assembled device, with a plastic frame used to hold syringes, a syringe plugholder with a central knob allowing fluid aspiration by rotation, a rubber seal with MCF test strips incorporated which interfaces with a sample tray with pre-loaded reagents. **F** Close up at the polyurethane microwell plate with 72 wells, organized in 8-wells arrows allowing all sandwich assays reagents to be pre-loaded. **G** Fluoropolymer MCF filled with PBS buffer, revealing excellent transparency. **H** Fluoropolymer MCF filled with air, revealing opaque film.

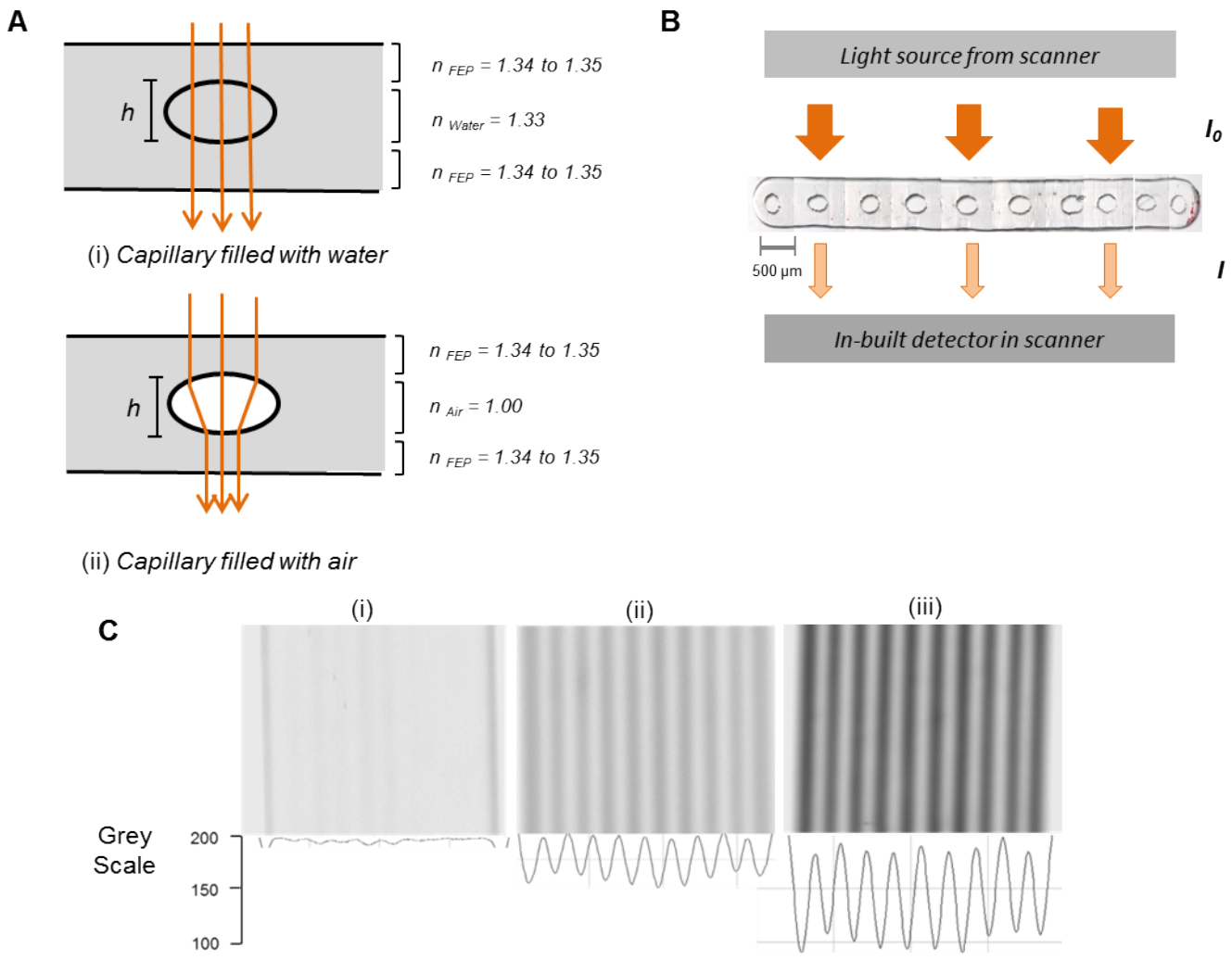

Figure S2. Signal quantitation in MCF ELISA. **A** Relevance of geometry and refractive index matching on singal-to-noise ratio. **B** Colorimetric signal quantitation in the fluoropolymer MCF using off-the-shelf flatbed scanner in transmittance mode. **C** Examples of blue channel image from scanned fluoropolymer MCF test strips following completion of a PSA sandwich assay using (i) 0 ng/ml, (ii) 15 ng/ml and (iii) 60 ng/ml of recombinant protein.

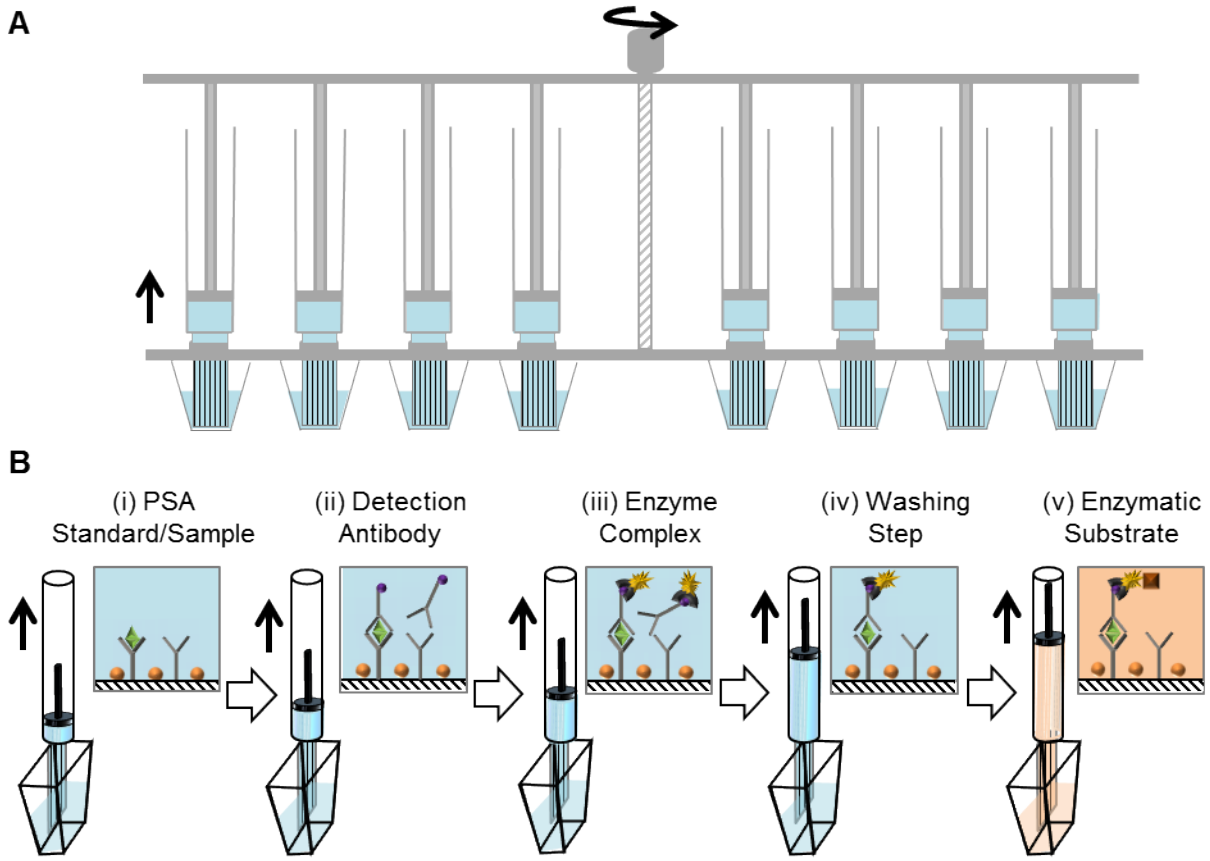

Figure S3. Sequential, semi-automatic fluid aspiration in the Multi Syringe Device (MSA). **A** With every full rotation of the central knob the syringes aspirate simultaneously 13 µl of reagents/samples from the wells through the fluoropolymer MCF strip; with 3 full knob rotations 8 MCF test strips can be filled with 40 µl of ELISA simultaneously. **B** Solution are sequently aspirated, with no need for intermediate washing steps; in step (i) PSA standard/sample is aspirated, and the MSA left to incubate on the well allowing the PSA to bind the capture antibody immobilised in the pre-coated strips; the MSA is then moved to next row of wells containing step (ii) pre-loaded detection antibody and the procedure repeated through steps (iii) to (v) for full sandwich assay completion.

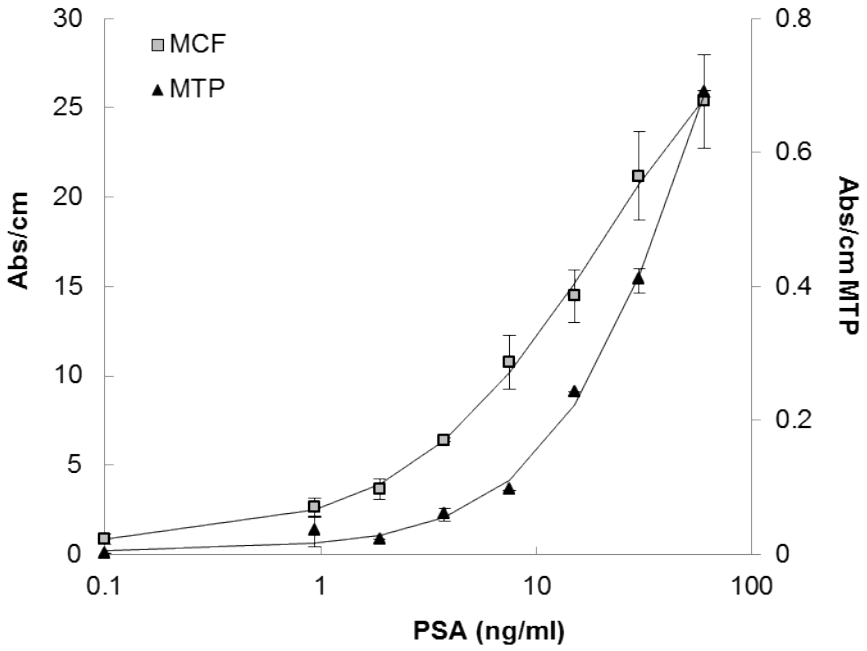

Figure S4. Comparison of MCF vs MTP PSA colorimetric immunoassay; MTP PSA assay was performed using conditions suggested by manufacturer and summarised in Table S1; MCF PSA assay was performed in the optimised conditions (Figure 4A and Figure S5).

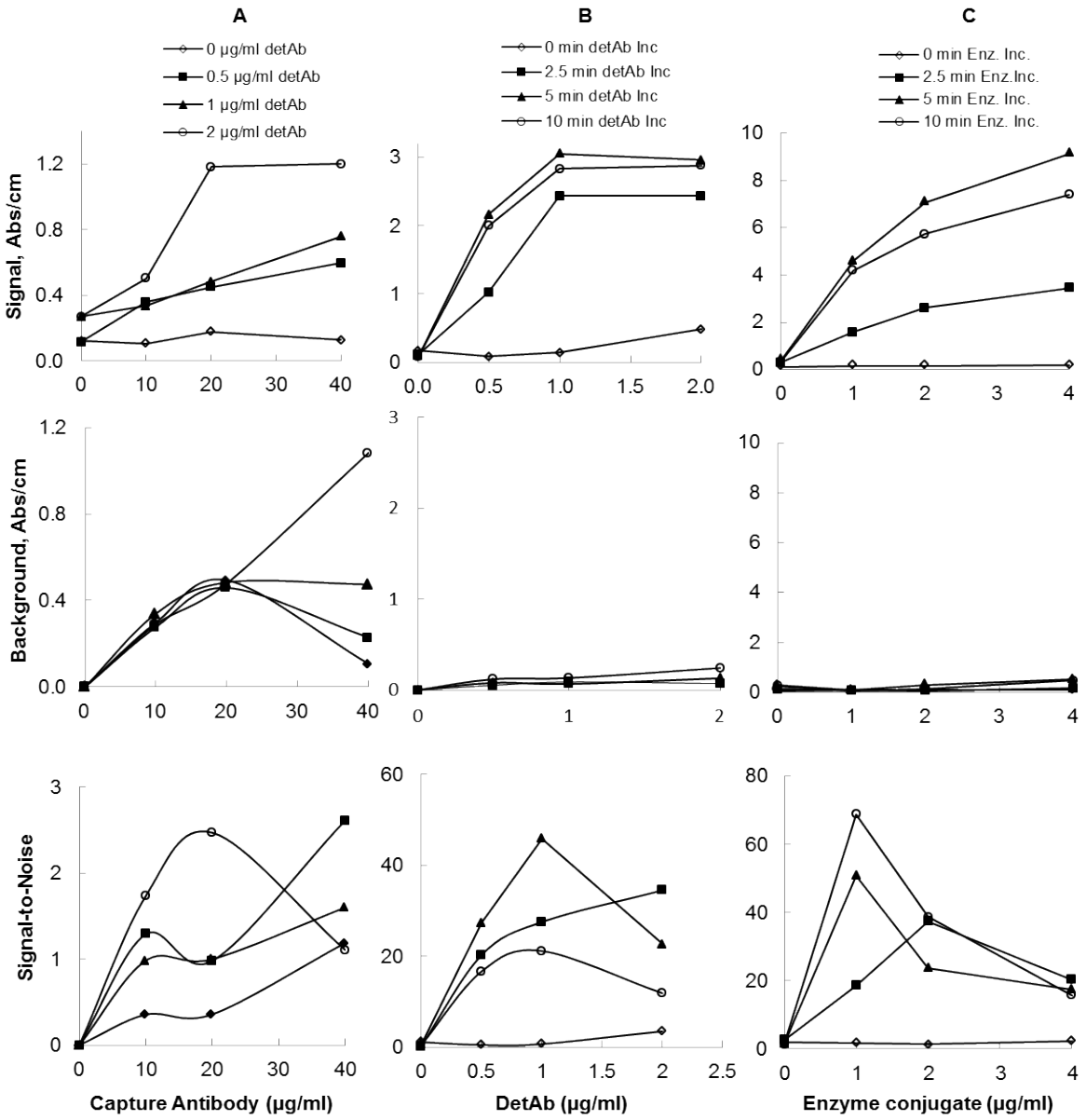

Figure S5. Optimisation of PSA sandwich assay conditions in the fluoropolymer MCF platform using the MSA. **A** Signal, background and signal-to-noise ratio of MCF PSA assay with varying concentartions of capAb and detAb concentration, using 1.5 ng/ml of recombinant protein and 4 µg/ml of Extravidin Peroxidase. **B** Signal, background and signal-to-noise ratio of MCF PSA assay for varying concentrations and inbuation times for detAb (capAb 40 µg/ml; 4 ng/ml recombinant protein; 4 µg/ml Extravidin Peroxidase. **C** Signal, background and signal-to-noise ratio of PSA assay with varying Enzyme concentration and Enzyme incubation times (40 µg/ml of capAb; 3.75 ng/ml of PSA concentration; 1 µg/ml of detAb concentration; High sensitivity streptavidin-HRP).

REFERENCES

1. M. E. Wiseman and C. W. Frank, *Langmuir : the ACS Journal of Surfaces and Colloids*, 2012, **28**, 1765–74.