

## Supplementary information

Simple paper architecture modifications lead to enhanced  
sensitivity in nanoparticle based lateral flow immunoassay

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### **Preparation of gold nanoparticles (AuNPs)**

All glassware used in this preparation was thoroughly cleaned in aqua regia overnight and rinsed with double distilled H<sub>2</sub>O and reflux was used for all the procedure which was done as follows: a 50 mL aqueous solution of 0.01% HAuCl<sub>4</sub> was heated to boiling and vigorously stirred in a 250 mL round-bottom flask; 5 mL of 40 mM sodium citrate were added quickly to this solution. Boiling was continued for an additional 10 min. The solution was cooled to room temperature with a continuous stirring for another 15 min. The colloids were stored in dark bottles at 4° C.

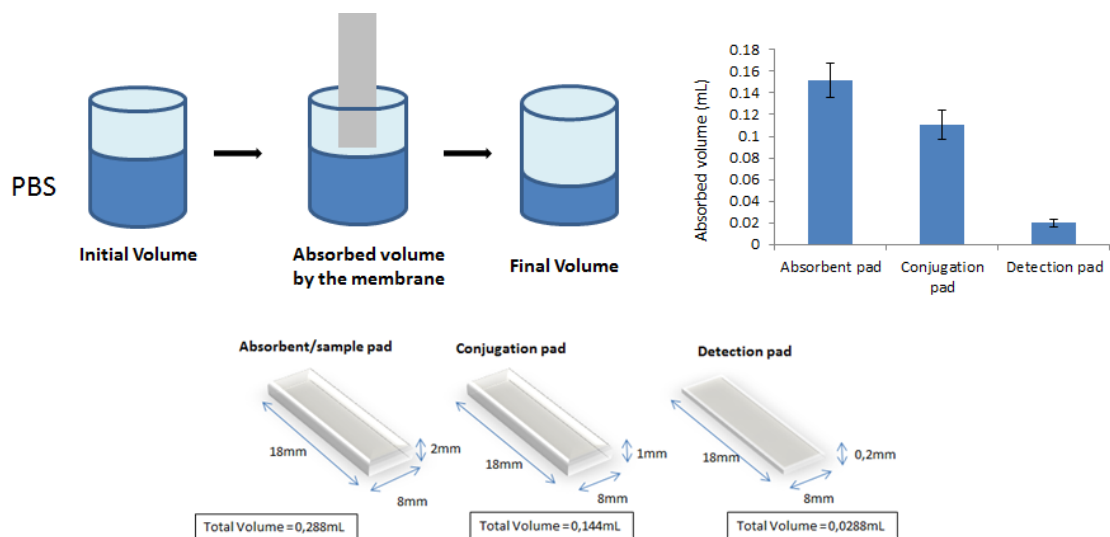
### **AuNPs modification with antibodies**

The AuNPs were then conjugated with the antibody  $\alpha$ HIgG  $\gamma$  chain specific. Briefly, 1.5 ml of AuNPs solution was adjusted to pH 9 with 10mM borate buffer pH 9.2. Then, without stopping the stirring, 100  $\mu$ L of the antibody solution (100  $\mu$ g/mL) were added drop by drop and the resulting solution was incubated for 20 min at 650 rpm. Then 100  $\mu$ L of 1 mg/mL BSA aqueous solution were added drop by drop and the stirring was continued for other 20 min at 650 rpm. Finally the solution was centrifuged at 14000 rpm for 20 min. The supernatant was removed and the pellet of AuNPs was re-suspended in 300  $\mu$ L of 2 mM borate buffer pH 7.4 containing 10% of sucrose.

### **Empirical calculation of pad constants**

The porosity and permeability constants of sample, conjugation and detection pads were calculated measuring the change in a fixed volume of PBS (PBS density = 1,97g/mL) after dipping the different pads as shown in figure S1. The sizes of the pads are also shown in figure S1. The porosities of the absorbent, conjugation and detection pad were respectively: 0.47, 0.23, 0.31; whereas the permeability were:  $7.5 \times 10^{-6}$ , 1.85

$\times 10^{-6}$ ,  $4.91 \times 10^{-7} \text{ m}^2$ , calculated considering the transversal area of the membrane and its porosity.



**Figure S1:** On the left: scheme of the experiment used to calculate the porosity and permeability of the membranes. On the right: results obtained for the different membranes. On the bottom: sizes and total volumes of the membranes used.