## **Supplementary Information**

## Lab-in-a-syringe using gold nanoparticles for rapid immunosensing of protein biomarkers

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Set-up for the vertical flow immunoassay (VFIA) with syringe pumping



**Figure S1**: Photo of the set-up used for the VFIA. Conjugate and detection cartridges are serially connected to a standard syringe used for acquiring the sample and subsequently pumping it through the pads. The paper pads are hold/fixed inside just by pressure while screwing the threaded pieces of the cartridge.



Figure S2: Scheme of the different antibodies and AuNP/antibody conjugates used in

the LIS assays.

## Quantitative assays using detection/control pad

A design of the detection pad including an additional spot for the control of the VFIA was also developed. The wax ring defines in this case two holes of 4 mm in diameter where the anti-HIgG (detection; right) and the anti-goat (control; left) antibodies were immobilized. The obtained results for different concentrations of IgG are shown in figure S2. The intense red color in the control pad is ensuring that the assay is working properly.



**Figure S3**: Calibration curve and corresponding pictures of the control (left)/detection (right) pads.

## Procedure for measuring color intensity with Image J software

1) Install Image J (v. 1.47) on your computer;

2) Open the File menu of ImageJ;

4) Select the image that will be analyzed in Image J;

5) The color analysis requires a gray-scale image. The simplest method to convert to gray-scale is through *Image*>*Type*>*8-bit*;

6) Now you must configure ImageJ to measure the color intensity of fixed areas. For this, open the ANALYZE menu, and select SET MEASUREMENTS. In this window, select GRAY MEAN VALUE and AREA (everything else should be unchecked);

7) For choosing the area, select CYCLE SELECTIONS tool from the ImageJ toolbar;

8) Draw a circle around the first test point. In the ANALYZE menu, select MEASURE.

A new window should appear containing two numbers: one corresponds to the circle area and the other one to the circle intensity;

9) Place the circle with the same area upon the second test point of the figure. In the ANALYZE menu, select MEASURE. Other line should appear containing two numbers: one corresponds to the area (that should be equal to area of the first test point) and the other one corresponds to the second test point.

10) Repeat the procedure for all test points.