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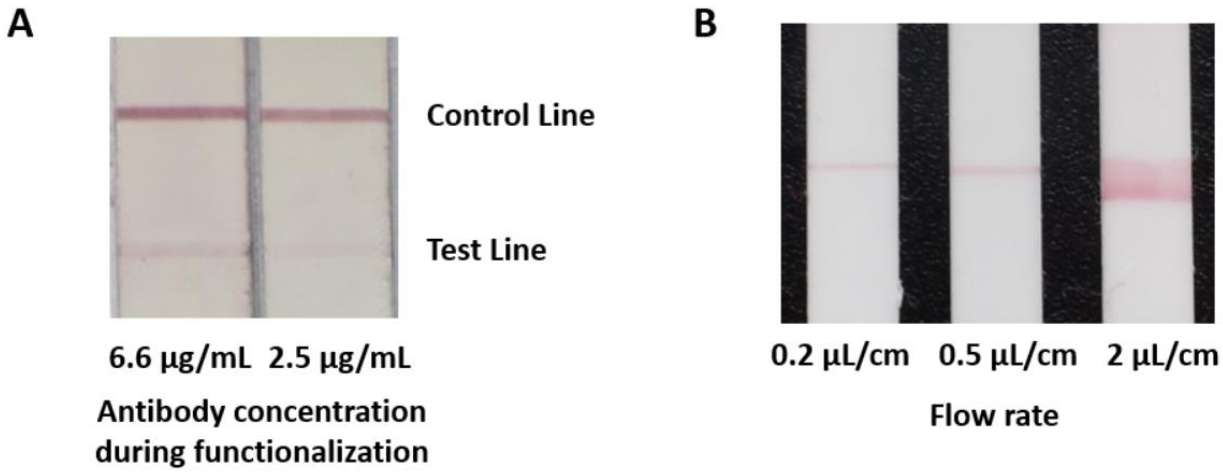
Supplementary information

Tutorial: design and fabrication of nanoparticle-based lateral-flow immunoassays

In the format provided by the authors and unedited



Supplementary Fig. 1

Examples of how AuNP conjugation and the striping of the test line can affect the LFA signal.
A, Using a concentration of 6.6 µg/ml (left) versus 2.5 µg/ml (right) of anti-human IgG during the AuNP functionalization (steps 10–16 in Box 2) produces different signal in LFAs challenged with a blank solution (all the other parameters in the LFA fabrication were fixed). When using 6.6 µg/ml, a higher non-specific signal (stronger red line) is produced compared to using 2.5 µg/ml. Thus, a careful optimization of the nanoparticle conjugation can lead to consistent improvements in the overall LFA performance. **B**, By fixing the dispense speed at 50 mm/s, the use of three different flow rates during the membrane striping produces lines with different widths. As expected, using a flow rate of 2 µl/cm generates a thicker line than those obtained using lower flow rates.

Supplementary Table 1 – Costs associated with a single LF strip

Material/Reagent	Cost in USD per 6 mm strip	Material cost, USD per [unit]	Quantity of material per strip
Laminated card	0.0080	0.013 [cm]	6 mm
Nitrocellulose membrane	0.0204	0.034 [cm]	6 mm
Conjugate pad	0.0044	0.007 [cm]	6 mm
Sample and Absorbent pad	0.0046	0.004 [cm]	2 x 6 mm
Antibodies for test and control lines	0.0450	75.00 [mg]	2 x 0.3 µL (1 µg/µL)
Gold nanoparticles	0.1700	340.00 [g homemade AuNPs]	0.46 mg $HAuCl_4$ 12.5 mg sodium citrate
Conjugate pad buffer	0.0031	0.35 [g salts mixture]	1 mg $Na_2[B_4O_5(OH)_4]\cdot 8H_2O$ 1 mg H_3BO_3 60 mg sucrose
Antibodies for conjugate	0.1416	140.00 [mg]	10 µL (0.1 µg/µL)
Sample pad buffer	0.0047	0.45 [g salts mixture]	1 mg NaH2PO4 1 mg Na2HPO4 0.5 µL Tween 20 25 mg BSA
Total	**0.4018**		

This estimation is based on the product prices provided by suppliers in 2019 and considering a 6 mm wide strip. To the final cost of a single LFA of 0.4018 $ must be added the cost for the dispenser (which can be around 20,000.00 $, but can be even lower for lab scale applications), the storing containers (aluminium pouch and desiccating agents) and the salary of personnel/facility (not estimable since it may vary significantly between countries).