Optimising Lateral Flow Assays for diagnostic applications

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In the current pandemic Lateral flow assays (LFAs) are used for broad screening of the public, as a fast, but less sensitive supplement to PCR. The standard LFA platform offers simple handling and reading, and a very fast response time. The major drawback of the platform is, however, the sensitivity [1], which has been shown to vary greatly, i.e. for SARS-CoV-2 diagnostics from 0-98% [2]. Tremendous efforts have been put towards enhancing the sensitivity. In our work, we have investigated several aspects of sensitivity enhancement of LFAs, including orienting the capturing biomolecule, signal enhancement using larger nanoparticles, inducing geometric changes to manipulate the flow rate, as well as a digital readout. The platform itself is typically nitrocellulose produced with a proprietary surface, which greatly challenges the ability to transfer a chemical optimisation step between different manufacturers. Instead, physical changes in the geometry, providing an overall change in flow rate, can be universally implemented. A locally reduced flow rate promotes biomolecule interactions, even at lower antibody-antigen affinities. By conducting a set of simple experiments, some key parameters describing flow through the membrane can be found, so that the flow can be numerically described. Afterwards these parameters, incl. porosity, permeability and capillary pressure, can be used to conduct simulations for finding novel geometrical designs ensuring larger time for antibody-antigen interaction.In addition, the binding kinetics between analyte (virus, bacteria, protein etc.) and antigen are greatly affected by the amount of available binding sites, both on the analyte and the immobilised biomolecule. By implementing a protein able to correctly orient the capturing biomolecule, a 5-fold sensitivity enhancement is observed (figure 1.a). We also investigated the effect of the label used for signal generation. We show that the label particle size along with antibody concentration is an important parameter to consider. Finally by digitalizing the output, i.e. automatically reading the color in controlled light conditions, we can further enhance the sensitivity relative to the naked eye.

REFERENCES

[1] J. D. Bishop, H. v. Hsieh, D. J. Gasperino, and B. H. Weigl, *Lab on a Chip*, vol. 19, no. 15 (2019) pp. 2486–2499
[2] J. Dinnes *et al.*, *Cochrane Database of Systematic Reviews*, vol. 2020, no. 8 (2020)

FIGURES

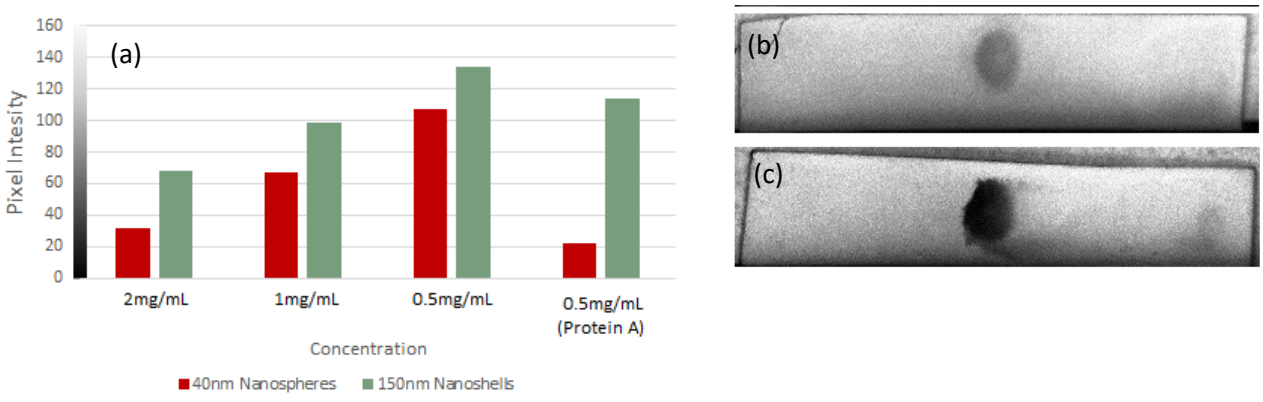

Figure 1: (a) Sensitivity enhancement by using 150 nm nanoshells or antibody orientation by using protein A. The intensity scale shows that the lower the pixel intensity the higher the color signal. (b) LFA image of 150 nm nanoshells with 0.5 mg/ml of capture antibody oriented by protein A. (c) LFA image of 40 nm nanoparticles with 0.5 mg/ml of capture antibody oriented by protein A figure (Arial 10)