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BIOSENSORS FOR PANDEMICS

AuNP-based lateral flow assay for detection of SARS-CoV-2 nucleoprotein: implicit design challenges in a new pandemic

Liming Hu [a], Enric Calucho [a], Celia Fuentes-Chust [a], Ruslan Álvarez-Diduk [a], Andrea Idili [a], Claudio Parolo [a], Arben Merkoçi [a,b]
a Nanobioelectronics & Biosensors Group, Institut Català de Nanociència i Nanotecnologia (ICN2), Campus UAB, 08193 Bellaterra, Barcelona, Spain
b Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain

ABSTRACT 2020 was marked by a SARS-CoV-2 outbreak that has claimed more than 2 million lives around the world [1], pushed healthcare systems to their limits, shaken the economy at all levels and greatly affected people's daily life. Roughly one year after the appearance of the first case of COVID-19, although there are vaccines available, the eradication of the pandemic is an enduring challenge due to the extremely rapid infectivity and the emergence of new strains of the virus. Therefore, there is the need of diagnostic devices dedicated to each situation: general screening, herd immunity, strain identification, etc.

Lateral flow assays make ideal point-of-care testing platforms due to their simplicity, low cost and user friendliness [2]. Moreover, paper can be easily functionalized with different kind of bioreceptors (e.g., antibodies, DNA, etc.). Herein, we present a AuNP-based lateral flow device for the detection of SARS-CoV-2 nucleoprotein, from the screening of antibodies to the preliminary testing of the device [3].

In the scenario of a new pandemic, the initial lack of bioreceptors for the development of diagnostic platforms creates the conditions for a race towards the launch of the first commercially available products. Therefore, the performance of these new bioreceptors is unbeknownst to researchers during the first stages of the pandemic. Here we will show and discuss the standardization procedures and previous validation of receptor as guidelines to be used for urgent design and application of LFA. ELISA tests were carried out to determine the most efficient set of antibodies for its later transfer to a paper-based format. However, changing the environment on which antibodies work doesn't always translate well, thus we take the opportunity to raise this issue in the context of a congress devoted to biosensors for pandemics.

Nucleoprotein LFA design

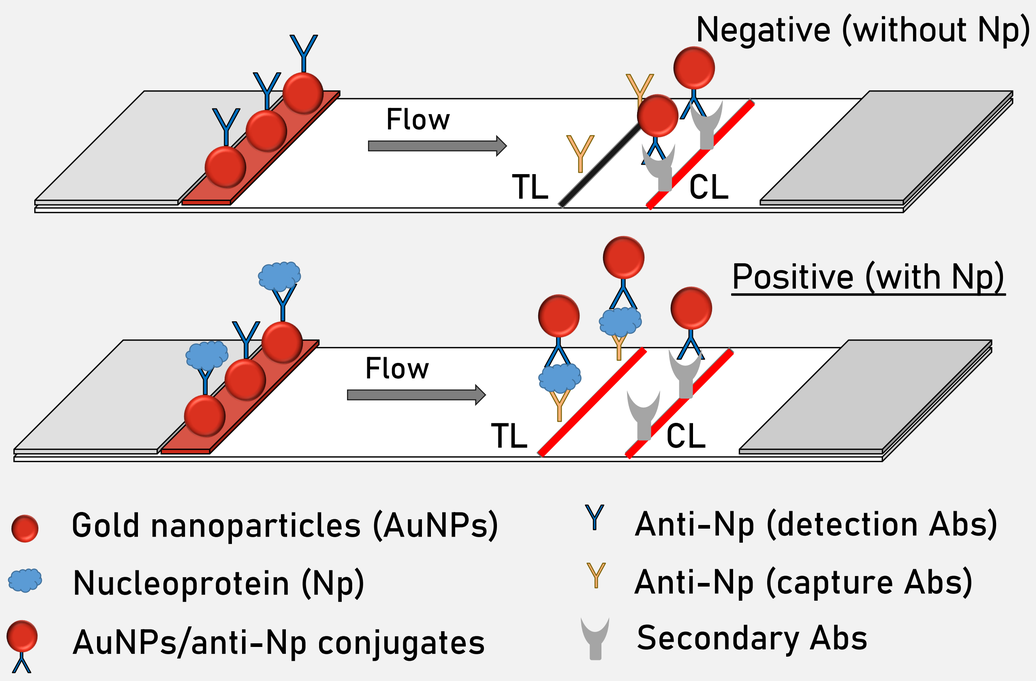

Figure 1. Scheme of LFA for detecting SARS-CoV-2 nucleoprotein.

LFA fabrication and measurement

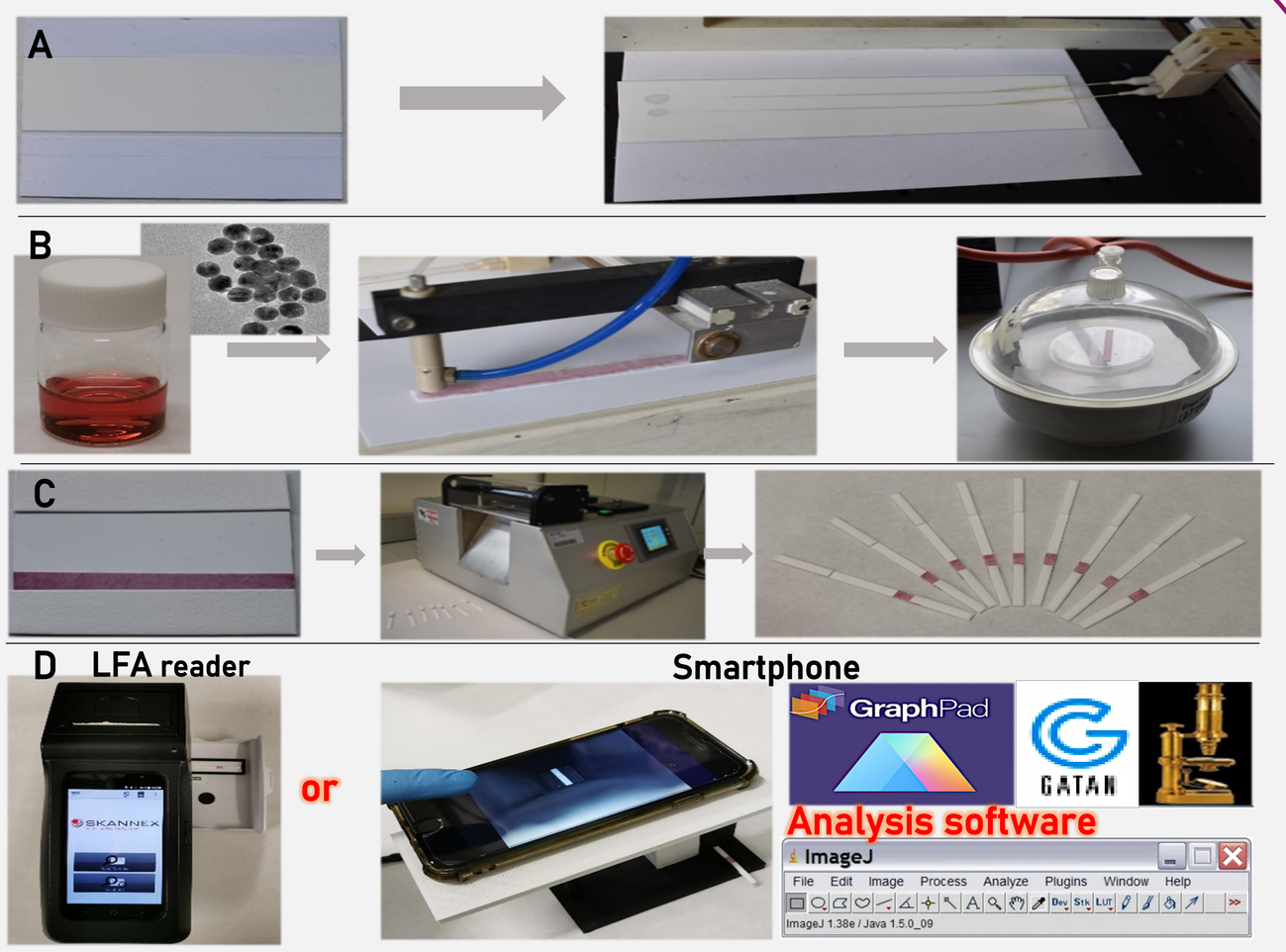

Figure 2. (A) antibodies are dispensed on nitrocellulose membrane to form the test line and control line; (B) Conjugation of AuNPs with antibodies are sprayed on glass fiber to form the conjugate pad; (C) Various pads are assembled and cut into lateral flow strips; (D) LFA reader or smartphone are used for detecting and analysis the lateral flow strips.

Nucleoprotein LFA results

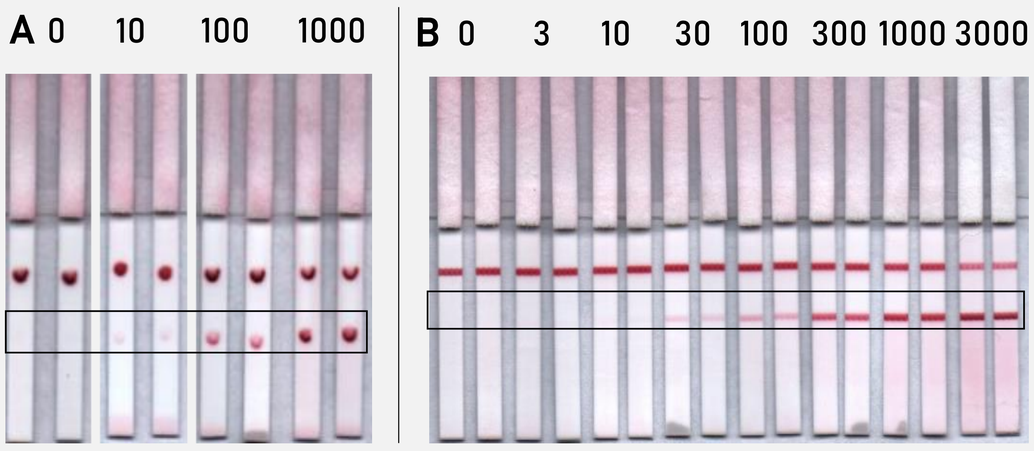

Figure 4. (A) Dot test for rapid evaluation of antibody performance; (B) LFA for detecting gradient concentration of Np (ng/mL) in PBS. Naked eye LOD: 10 ng/mL.

Nucleoprotein LFA development: antibodies selection

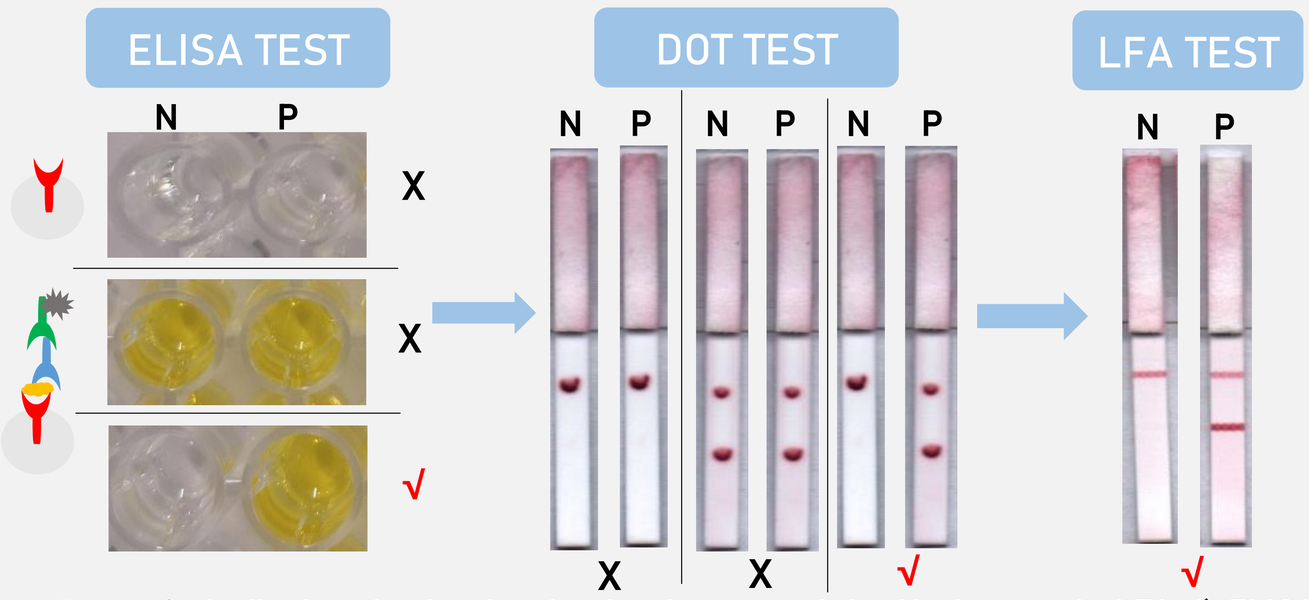

Figure 3. Antibody selection for the development of the Nucleoprotein LFA. 1) ELISA test for preliminary screening from a large number of antibodies; 2) Dot test for performance screening on nitrocellulose membrane of antibodies; 3) Antibodies with satisfactory performance on both ELISA and Dot tests are then used for developing a complete SARS-CoV-2 nucleoprotein LFA.

Note: N-negative, P-positive; 'X' means negative results and '√' indicates positive results

CONCLUSIONS Selection of high-quality antibodies is the most critical step to establish a specific and sensitive LFA. Compared with LFA, ELISA's various reaction conditions (long time, strict temperature control, shaking, free antibodies, and high enzyme sensitivity) are more likely to lead to immune reaction. Therefore, antibodies with good performance on ELISA tests are not necessarily fit for LFA, and antibodies with poor ELISA results are not tested on LFA. To develop a proper LFA diagnostic product as soon as possible in the context of a pandemic, high-throughput and rapid ELISA testing must be used to select antibodies with good sensitivity and specificity from a large number of commercial antibodies. Then dot test is used as a secondary screening, which is less time- and reagent-consuming than a full-fledged LFA, while still having strong conformity between them. Here, we formally adopted this process to develop a SARS-CoV-2 nucleoprotein LFA.

CONTACT PERSON

Arben Merkoçi
arben.merkoci@icn2.cat

NANO Bioelectronics & Biosensors Group
ICN2 Institut Català de Nanociència i Nanotecnologia
ICREA
BIST Barcelona Institute of Science and Technology
CSIC

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