Development of a nano-bioplatform for SARS-CoV-2 specific antigens detection

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2022 IEEE International Conference on Design & Test of Integrated Micro & Nano-Systems (DTS) | 978-1-6654-9784-8/22/$31.00 ©2022 IEEE | DOI: 10.1109/DTS55284.2022.9809866

***Abstract*— Lateral Flow Immunoassay (LFIA) has been employed for the development of rapid, low-cost, and relatively simple devices for Covid-19 diagnosis. The proposed approach reports the use of the antibody labeled by Gold nanoparticles for the simultaneous detection of Nucleocapsid protein and Spike protein. A comparison with the results obtained by conventional analytical methods (PCR) has been considered in a significant number of nasopharyngeal swabs; 48 and 26 samples from positive and negative individuals respectively. This research provides the basis for the development of a more efficient antigenic test for SARS-CoV-2 detection as a low-cost and quick pre-screening tool.**

Keywords—LFIA, Gold nanoparticles, SARS-CoV-2 detection

I. Introduction

The discovery of the novel coronavirus (SARS-CoV-2) in Wuhan, China, in December 2019, initiated the COVID-19 global pandemic, placing not just the healthcare system, but also the global economy, in danger [1], [2]. The graph (Fig.1) presents total Cases (worldwide) including deaths and recovered or discharged patients. The 2019 coronavirus has been responsible for more than 515,000,000 infection cases and 6,272,000 deaths worldwide. Rapid detection, according to public health experts, is critical for controlling the virus's spread. The nucleotide-based test (qRT-PCR) of viral RNA, collected from the upper respiratory tract of suspicious patients, is the standard method for diagnosing COVID-19 [3]. Although this technique is sensitive and specific, it is constrained by high cost and long analysis time limitations. Therefore, diagnostic alternatives are essential in terms of simplicity of implementation, rapidity of results, and reasonable cost. To this aim, several detection devices based on lateral flow immunochromatographic tests (LFIA) have been developed and marketed [4].

Most FDA-approved commercial antigen kits target the nucleocapsid protein (N) of SARS-CoV-2. However, recent alerts from the Food and Drug Administration and the Centers for Disease Control and Prevention have raised concerns about the tendency of these devices to display false positive results while performing antigenic testing for quick detection of COVID-19 [5]. Therefore, a new alternative antigenic test based on the spike protein, including specific antibodies, directed towards distinct epitopes of this protein has been proposed [6].

In this study, we aimed to evaluate the potential use of the spike protein as a target for SARS-CoV-2 detection [4], [7]. Moreover, we propose that combining the two antigens will improve the results' validity. In this context, we have developed an additional assay, simultaneously targeting the nucleocapsid protein and the spike protein. This research portrays the initial optimization attempts of the development of a nano-bio platform for the diagnosis of SARS-CoV-2.

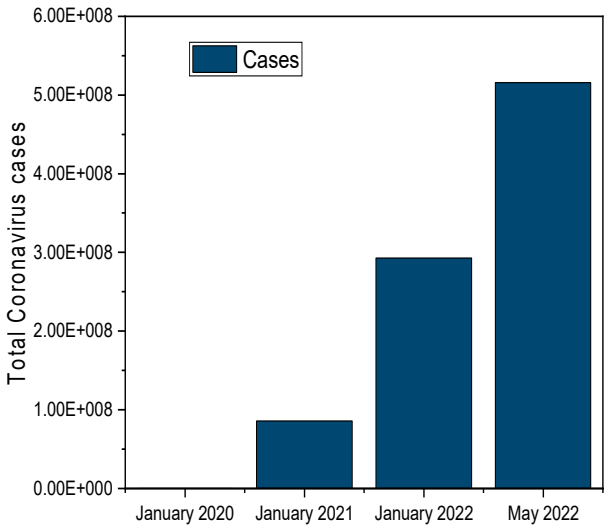

Fig. 1. Total Cases (worldwide). Source: Worldometer

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II. Experimental

Preparation of anti-N–AuNPs / anti-S–AuNPs conjugates:

AuNPs were prepared via an aqueous synthesis that was formalized in 1951 by Turkevich [8]. The pH of the colloidal gold solution was adjusted to ~8. 100 µl of borate buffer pH 8 and the volume of anti-N antibody / anti-S antibody (1 µg/mL) needed to obtain stable colloids (as defined by the flocculation stress test [9]) were added to 1 mL of pH adjusted AuNPs (optical density, OD=1) and incubated at 37°C for 30 min. 100 µl of 5% w/v casein was added, the mixture was again incubated for 10 min. After centrifugation at 12,000 rcf / 15 min, a clear supernatant of unbound antibodies was removed and dark red sediment of AuNps-Ab conjugates was re-suspended in 0.5% w/v casein and centrifuged. The recovered pellet was re-suspended in a storage borate buffer (0.5% w/v casein, 2 % w/v sucrose, 2.5 % v/v Tween 20 and 0.2 % v/v NaN_3) and centrifuged. Finally, the resulting AuNps-Ab conjugates were collected and stored at 4°C for further use [10, p. 20].

The lateral flow immuno-strip assembly:

Lateral flow immunostrip assembly involves attaching five main components in an overlapping way. The reagent lines were deposited on the nitrocellulose membrane utilizing an automated dispenser (*BioDot automate µAirJet*™) in a uniform and reproducible manner (Fig.2).

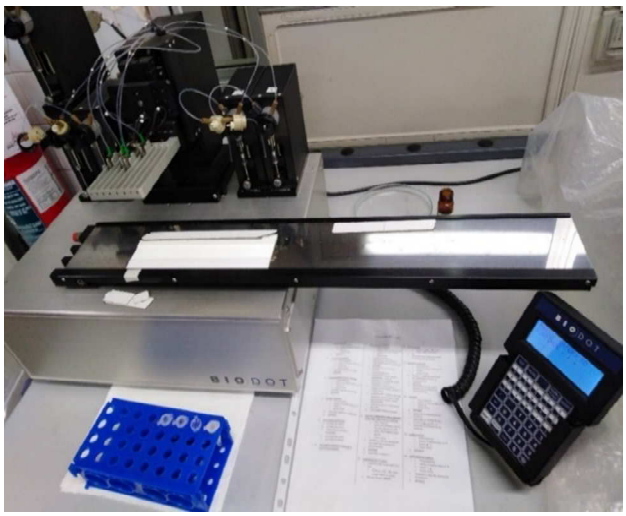

Fig. 2 . Automate µAirJet™ (BioDot)

III. Results and Discussion

- Characterization of antibody–AuNPs conjugates

Gold nanoparticles and the gold nanoparticle-antibody conjugates were characterized by Visible absorption. The Gold nanoparticles exhibited a plasmonic absorption band with maximum absorbance at the wavelength of 526 nm due to surface plasmon resonance [11]. The binding of the antibody to the AuNPs allowed indeed modifying the Vis spectra. In particular, λ max was increased for both antibodies by about 4 – 5 nm (526 nm for the AuNPs and 531 nm for the AuNPs-antiS / AuNPs-antiN), and this redshift was considered as the confirmation of the gold nanoparticle–antibody conjugates formation [12] (Fig.3).

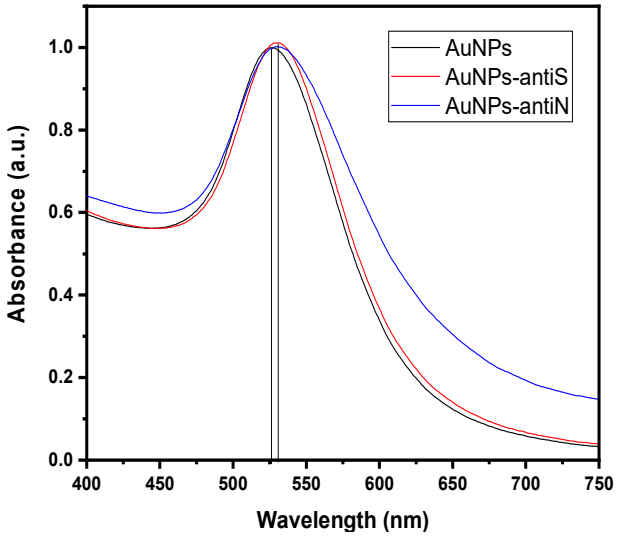

Fig. 3. Visible spectra of AuNPs, AuNPs-antibody-N conjugates, and AuNPs-antibody-S conjugates

- Detection of SARS-CoV-2 nucleocapsid and spike antigens

Recombinant spike protein and Nucleocapsid protein have been applied at varying doses to assess the sensitivity of the LFIA test designed. Intensity quantification of each test and background area on the strip was measured by QuantiScan software. The calibration curve for the Nucleocapsid antigen exhibited a linear range from 0 to 1 µg/ml and a visual limit of detection of 0.1 µg/ml (Fig.4). In the case of Spike protein, a calibration curve showed a linear range from 0 to 2.5 µg/ml and a visual limit of detection of 0.625 µg/ml. Above 2.5 µg/ml, saturation behavior has been observed (Fig.5).

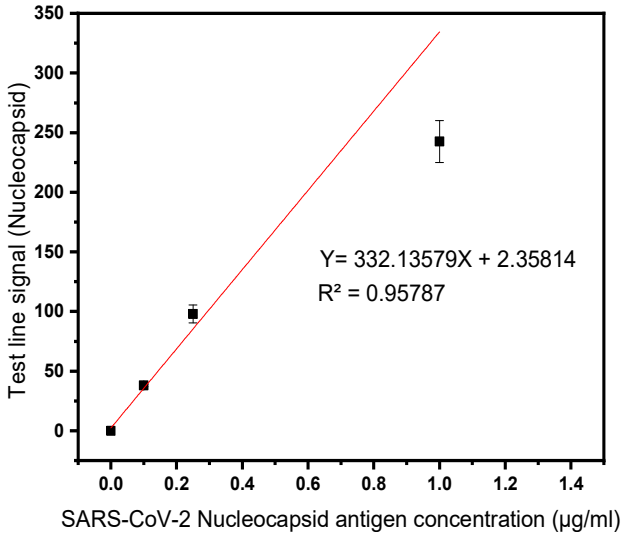

Fig. 4. Calibration curve of the Nucleocapsid antigen at different concentrations 1, 0.25, and 0.1 µg/ml

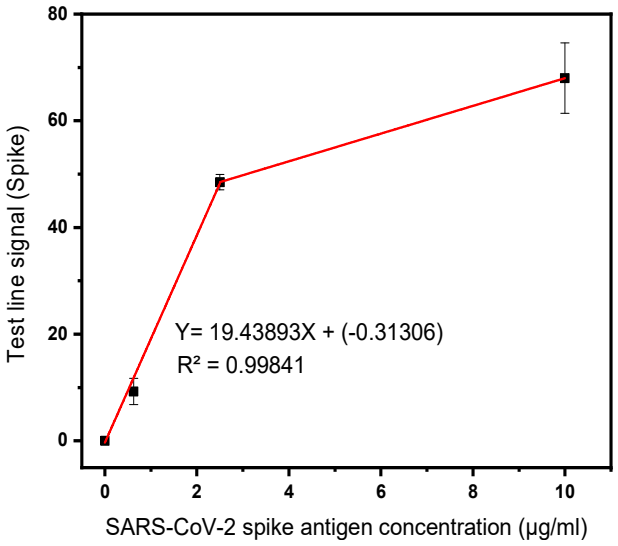

Fig. 5. Calibration curve using recombinant Spike protein at different concentrations 10, 2.5, and 0.625 µg/ml.

- DETECTION OF SARS-COV-2 NUCLEOCAPSID AND SPIKE ANTIGENS

The assay's sensitivity and specificity for detecting Nucleocapsid and Spike protein were investigated in a large number of nasopharyngeal swabs from PCR positive and negative individuals. 48 and 26 nasopharyngeal swabs from positive and negative individuals, respectively have been tested. For safety reasons, it is mandatory to inactivate the virus before using the sample.

The CT (cycle threshold) values of the positive test samples were evaluated by RT-PCR test against the signal of the 2 test lines (N and S proteins) of the antigenic assay developed. Indeed, the AuNP-modified antibody/antigen tests for inactivated real swab specimens were performed by signal measurement at the test line using a scanner and "ImageJ" software. Once the target analyte is captured by the antibody labeled with AuNPs, the labeled antibody/analyte complex will migrate to the nitrocellulose membrane where there is the test line and the control line. In fact, if the molecule of interest is present and is already bound to the antibody-nanoparticle conjugate, it also binds to the antibody on the test line, producing a positive signal with a red color intensity due to the AuNPs.

We could assume that strongly positive samples (low CT value, such as 25; 21) are generally weakly positive in the antigenic assay for S protein (a maximum S test line signal of 16) but strongly positive for N protein (a maximum N test line signal of 60) (Fig.6). In comparison to the nucleocapsid test, which was more sensitive (83,3%) but less specific (69,2%), the spike assay had higher specificity (80,8%) but a lower sensitivity (39,6%). Improved sensitivity for the N protein compared to S protein has been observed, which might be explained by several factors; (i) the inactivation step results in extracting the N protein from the inner part of the virus and thus facilitating its detection, however, this treatment could also damage the spike protein (ii) the nasopharyngeal swab may not be suitable for analysis of S protein. Table 1 shows a comparison of our results with the other studies reported in the literature.

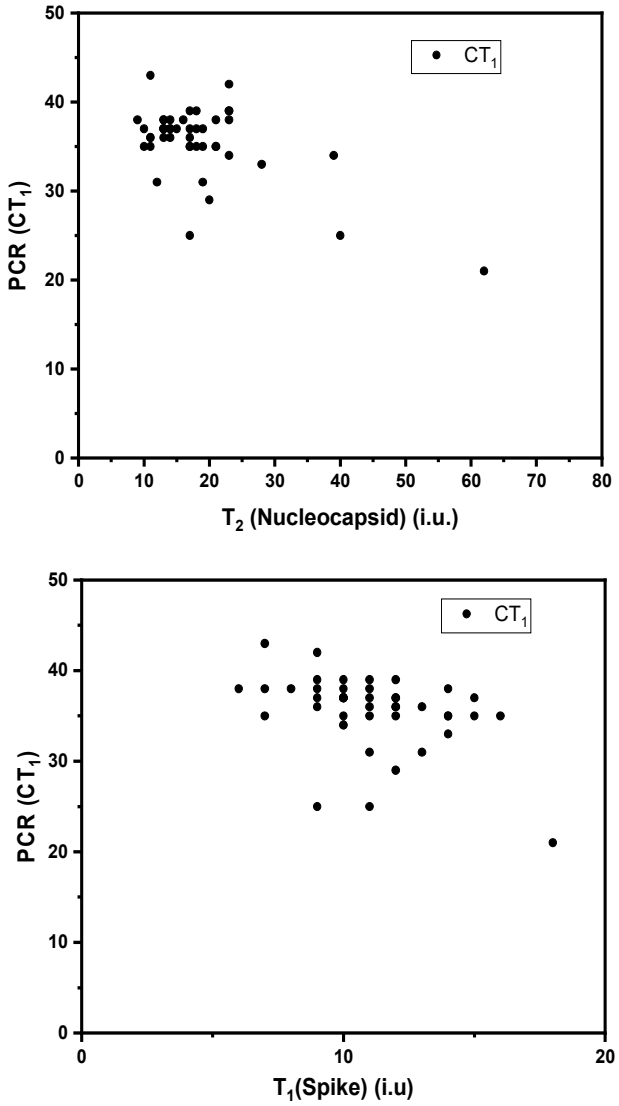

Fig. 6. CT values of the samples tested positive by PCR test Vs the signal of the 2 test lines (N and S proteins) of the antigenic test developed

TABLE I. COMPARISON OF OUR RESULTS WITH REPORTS IN THE LITERATURE

Target	Technique	Sensitivity	Specificity	Ref
S protein	LFA	26.5%	58.1%	[14]
N protein	ELISA	85%	87%	[4]
S protein	ELISA	99%	66%	[4]
N protein	**LFA**	**83,3 %**	**69,2 %**	**Our work**
S protein	**LFA**	**39,6 %**	**80,8 %**	**Our work**

In our study, the N test revealed good Sensitivity (83.3%) and moderate Specificity (69.2%) and in the case of the S test, moderate Sensitivity (39.6%) and good Specificity (80.8%)

were demonstrated. The manufacturer reported a Sensitivity of 98.1% and a Specificity of 99.8% when performed in symptomatic subjects, while when tested in asymptomatic subjects, the Sensitivity was lowered to 66.0 %.

Anyhow, the results showed a lower sensitivity as compared to ECDC and WHO recommended Sensitivity of effective rapid antigen detection. This observed moderate performance could be due to a possible high proportion of asymptomatic subjects in our population since we did not collect data on symptoms. Another possible explanation could be related to different test times, carried out in the early or late phase of the infection [13].

IV. Conclusion

A strategy based on the immobilization of gold nanoparticles (AuNPs) and appropriate antibodies for the selective and sensitive detection of antigens was studied. In this regard, an LFIA for the simultaneous detection of N and S protein was developed. The response of the developed device has been evaluated in nasopharyngeal swabs. The spike test line displayed high specificity (80,8 %) accompanied with a lower sensitivity (39,6 %), compared to the nucleocapsid test line which was more sensitive (83,3 %) while less specific (69,2 %). This research lays the path for the optimization of manufacturing parameters for a reliable fast antigenic test of SARS-CoV-2.

Acknowledgment

The authors gratefully acknowledge the Tunisian MHESR for supporting this work and the University of Sousse for the “Bourse d’ alternance” fellowship awarded to Mrs. Asma El Golli.

We also thank Dr. C Guiotto A.O. Ordine Mauriziano, Ospedale Umberto I di Torino, Turin, TO, Italy

Prof. F. Fagioli Department of Public Health and Paediatrics, Regina Margherita Children’s Hospital, University of Turin, Turin, TO, Italy.

Dr. M. Denina Department of Public Health and Paediatrics, Infectious Diseases Unit, Regina Margherita Children’s Hospital, Turin, TO, Italy for collecting saliva and nasopharyngeal samples and for the RT-PCR analysis.

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