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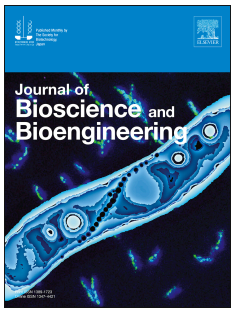

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Development of Nucleic Acid Based Lateral Flow Assays for SARS-CoV-2 Detection

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Short title: Molecular detection of 2019-nCoV_ N3

ABSTRACT

SARS-CoV-2 is still threat for humanity and its detection is crucial. Although real time reverse transcriptase polymerase chain reaction is the most reliable method for detection of N protein genes, alternative methods for molecular detection is still needed. Thus, lateral flow assay models for 2019-nCoV_ N3 were developed for molecular detection. Briefly, gold nanoparticles were used as label and three sandwich models (1A, 1B, 1.2) were designed. Prob concentrations on gold nanoparticles, types of sandwich model and membrane, limit of detection of target gene and buffer efficiency were studied. Model 1B has shown the best results with M170 membrane. Lower limit of detection was achieved by model 1.2 as 5pM. All parameters have significant role for molecular detection of SARS-CoV-2 by lateral flow assays, and these results will be useful for nucleic acid based lateral flow assays for viral detection or multiple detection of mutated forms in various detection systems.

Keywords

rapid test, SARS-CoV-2, sandwich assay, 2019-nCoV_N, detection

INTRODUCTION

Wuhan Viral Pneumonia seen in the late 2019 was called as SARS-CoV-2 and COVID-19 caused by SARS-CoV-2 was named as disease by World Health Organization (WHO). SARS-CoV-2 is an RNA virus belonging to the β and it has at least four structural proteins including Spike (S) protein, envelope (E) protein, membrane (M) protein and nucleocapsid (N) protein. S protein is widely used for diagnosis because of antigenicity and commonly chosen as target for neutralizing antibodies. However, N protein become attractive for molecular diagnosis of COVID-19 as highly protected protein sequences and high immunogenicity (1). Additionally, N protein is abundantly expressed protein during the infection and causes to protective immune response for SARS CoV and COVID-19 (2). The sequence similarity of protein coding region of COVID-19 was found as 89.74%, 48.59% and 35.62% with SARS CoV, MERS-CoV and HCoV-OC43, respectively (3) and 96% with bat coronavirus in the whole genom level (1). Comprehensive domain structure of N proteins among the four coronaviruses (SARS-CoV-2, SARS-CoV, MERS-CoV, and HCoV-OC43) and complete genome of SARS-CoV-2 were reported in the literature respectively (3). To say that the characteristics of the surface electrostatic potential of N terminal domain of SARS-CoV-2 N protein is different even if it is similar to the other coronaviruses.

N region of SARS-CoV-2 was determined as target sequence for SARS-CoV-2 specific gene. WHO proposed a few primer sets for N gene and reported that 2019-nCoV_ N3 (USA) and NIID_2019-nCOV_N (Japan) primers are the most sensitive for real time reverse transcription polymerase chain reaction (rRT-PCR) respectively (4). Therefore, among the specific regions N gene regions are widely accepted for diagnosis as the high similarity between SARS-CoV-2 and SARS-CoV causes to mistake in molecular diagnosis. SARS-CoV-2 RNA may be obtained from bronchoalveolar lavage, nasal/pharingeal swab (53.6% - 73.3%) (5), salivary/sputum (74.4%-88.9%) (6), feces/urine, blood samples and anal/oral swabs (7-9). Additionally, it is

known that virus may be alive at suitable environmental conditions after leaving from human body and join to waste water. Thus, SARS-CoV-2 and newly developed coronaviruses will always threaten the public health since the development of antiviral drugs or therapeutics takes long time. In this reason, early molecular viral detection is crucial to get under control the epidemy/pandemia.

Serology is a standart method for viral detection and based on the testing of antibody response coming from the immune system and antigen presence. However, it cannot be used for early detection since it is based on the measurement of antibody after infection, and could not be efficient for patients who are in risk groups. For instance, antibodies against to COVID-19 are developed in early stage (4-10 days for IgM) and late stage (11-24 days for IgM-IgG). Besides, cross antibody reactions may give a false positive result and producing of polyclonal antibodies may change from batch to batch. Nanoparticle based viral diagnosis (10) is another way and it was used for the detection of SARS specific sequence (11). However, there is lack of nano-based diagnosis systems for SARS-CoV-2 sequences even if antigen based detections are reported (12-17). rRT-PCR is the most reliable method for molecular detection of SARS-CoV-2 in the world and the first quantitative rRT-PCR was designed after the definition of virus by WHO in January 2020 (https://www.who.int/emergencies/diseases/novel-coronavirus). Although these assays are reliable, complex and expensive test protocols, need of educated personnel and diagnosis laboratories, taking time for sending the samples into reference labs are disadvantages. Similarly, conventional PCR needs to agarose gel loading and high copy number of target genes. For this reasons, rapid and naked eye molecular detection of SARS-CoV-2 is always be needed. In this regard, lateral flow assays (LFAs) or point of care tests could be an alternative to the molecular detection of SARS-CoV-2 as a rapid, cheap and simple way without advanced devices in a short time.

LFAs are portable, ready to use immunochromatographic diagnostic assays developed by antibodies, enzymes or nucleic acids (18) for various fields. They can also be helpful for epidemy/pandemia by sensitive detection of nucleic acids (19) and could make 8 times sensitive and rapid detection compared to the electrophoresis (20). While a number of LFAs were developed for COVID-19 they are mostly based on the antibody (IgG/IgM) detection of patients (21) and there is lack of nucleic acid detection of SARS-CoV-2 by LFAs. LFAs can be used with amplification systems producing any RNA (22). Although LFA for molecular detection of SARS-CoV-2 is reported in the literature it is based on CRISPR Cas12a dependent nucleic acid detection (23) and needs the complex experimental steps. Similarly, Broughton et al., developed the LFAs for SARS-CoV-2 using the RNA extracts related with respiratory swab. It is based on the CRISPR–Cas12 for the detection of E and N gene (24). However, extra labelling with Fluorescein amidites and sensitive steps including enzymatic restriction are needed, and assay was only developed for one region of N gene announced by US Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-detection-instructions.html) which are accepted regions for rRT-PCR. LFAs are encouraged to be developed for nucleic acid detection of SARS-CoV-2 based on PCR. Therefore, nucleic acid based LFAs are always become crucial and they can be adopted the new sequences occurred by mutations in the virus.

In this research, molecular detection of RNA region (2019-nCoV_N3) specific to SARS-CoV-2 by gold nanoparticles (AuNPs) based LFAs in 5-7 minutes was aimed. Test principle is based on the hybridization of oligonucleotides without complex enzymatic reactions and naked eye analysis.

MATERIALS AND METHODS

The chemicals used were all analytical grades. $HAuCl_4$. $3H_2O$, trisodium citrate dihydrate were purchased from Alfa Aesar (Kandel, Germany). Ultra-low range DNA ladder were from Thermo Scientific. Tris-HCl was purchased from Applichem, KCl and NaCl were purchased from Merck, MgCl2, CaCl2, nuclease free water and SSC buffer were purchased from Multicell. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was purchased from Sigma Aldrich. Synthetic oligonucleotides were purchased from Integrated DNA Technologies (Coralville, USA). Ultrapure water was used for the preparation of all solutions during the study. The nitrocellulose membrane cards were purchased from Whatman, Germany. Absorbent pad, sample pad and conjugate pad were purchased from Millipore, USA. Scanning transmission electron microscop (STEM-TESCAN), HORIBA Dynamic Laser Particle Size/Zeta Potential Analyzer and EPOCH2 Plate Reader/Spectrophotometer were used for analysis of synthesized AuNPs and AuNPs/prob concentration. As a probe 1A model: GNP probe 1A: 5’ cca atg tga tct ttt ggt gta aaa aaa aaa-3 -SH’; test line for 1A: 5’bio aaa aaa a gca ttg tta gca gga ttg c 3’and control line for 1A: 5’ tac acc aaa aga tca cat tgg ttt 3’bio were used. As a probe 1B model: GNP probe 1B: 5’ ttt ggt gta ttc aag gct ccc aaa aaa aaa-3 -SH’; test line for 1B: 5’bio aaa aaa tg cgg gtg cca atg tga tct 3’ and control line for 1B: 5’ ggg agc ctt gaa tac acc aaa ttt 3’bio were used. As a probe 1.2 model: GNP probe 1.2: 5’ t gcc aat gtg atc ttt tgg tg aaa aaa aaa-3 -SH’; test line for 1.2: 5’bio aaa aaa gc agc att gtt agc agg att 3’ and control line for 1.2: 5’ ca cca aaa gat cac att ggc a ttt 3’bio were used. 72 base long target N gene region 1 (2019-nCoV_N3), 5’-ggg agc ctt gaa tac acc aaa aga tca cat tgg cac ccg caa tcc tgc taa caa tgc tgc aat cgt gct aca-3’, and 50 base long target N gene region 1.2 (2019-nCoV_N3), 5’ aat aca cca aaa gat cac att ggc acc cgc aat cct gct aac aat gct gc -3’, were experienced. 5’ggg agc ctt gaa tac acc aaa a 3’ and 5’tgt agc acg att gca gca ttg 3’ primers were used as forward and reverse, respectively for PCR reaction.

Synthesis of Gold Nanoparticles and Conjugation with oligonucleotide probes

AuNPs were synthesized by reducing the $HAuCl_4$. $3H_2O$ with sodium citrate (25), in conical flask. All the glass materials used were cleaned with acid solution and rinsed with distilled water. Briefly, 100 mL of 1mM $HAuCl_4.3H_2O$ solution was boiled by stirring continuously. Then 1% sodium citrate was added and the color changed from black to red in 2-3 minutes. Boiling was continued about 10 minutes and colloidal solution was allowed to cool. Synthesized AuNPs were filtered by 0.45µm cellulose acetate and concentrated by centrifugation at four times (4X AuNPs) before the conjugation with oligonucleotides and stored at 4˚C. To make a conjugate with oligonucleotide probes, thiol modified probes were initially activated by TCEP for one hour at room temperature. In this purpose, three probe concentrations (2µM, 4µM, 8µM) were used for conjugation in order to see the effect of probe concentration on assay efficiency. Then solution was added into 1 ml of 4X AuNPs solution and incubated for overnight at room temperature. After that 0.01M phosphate buffer saline (PBS) was added as final concentration for salt aging and incubated for overnight. Then, the solution was centrifuged at 12000 rpm and pellet was resuspended in resuspension buffer (20mM sodium phosphate buffer containing 5% BSA, 0.25 % Tween 20 and sucrose). Conjugate was washed with the resuspension buffer as twice and stored at 4˚C after resuspending in the same buffer.

Preparation of LFAs

The components of the LFAs are sample pad, conjugate pad, nitrocellulose membrane and absorbent pad. The design of strip assay was manually performed according to our previous study (26). Two different cellulose membranes having different flow rates were used in this study (M170-M120). In short, sample pads were treated with two different buffers called as buffer 4 (0.05M Tris-HCI, 0.25% Triton X 100, 0.15M NaCI, pH 8.0) and buffer 5 (PBS, 0.1mM NaCI, 0.2 % Tween 20), separately and dried at 37˚C or room temperature. Conjugate

pads were soaked with AuNPs/Probe conjugate and dried at 37˚C for 1 hour. Buffer 14 (20mM Tris, 50mM NaCI, 5mM KCI, 5mM MgCI2, 2mM CaCI2, 0.1mM BSA, 1.7% Triton X 100, pH 8.0), PBS and saline-sodium citrate (SSC) was used as running buffer for optimizing the assay. Test and control lines are prepared by the principle of streptavidin-biotin interaction. Briefly, biotinylated oligonucleotides were conjugated to streptavidin and then immobilized on the cellulose membrane using micropipette, manually. For the assay development three sandwich models (1A, 1B, 1.2) were prepared for hybridization on LFA and experienced, separately. Two of them (1A, 1B) were for 72 base long 2019-nCoV_N3 and the last one (1.2) was for 50 base long which was obtained from shortening the 2019-nCoV_N3 region which is still specific for SARS-CoV-2.

Polimerase Chain Reaction for 2019-nCoV_N3

In order to see the application potential of developed LFAs for real samples, PCR was performed by plasmid DNA including N gene of SARS-CoV-2 and specific primers for 2019-nCoV_N3. After the reaction was completed, PCR product was run on agarose gel electrophoresis along with the Ultra-low DNA ladder at 90V for 1 hour to be sure that correct gene region was obtained before applying to the LFA. The PCR reaction was performed as 34 cycle for each tube and finally extended as 72°C for 4 minute. PCR products were heated for denaturation and then applied to the strip assays.

RESULTS AND DISCUSSION

Synthesis of Gold Nanoparticles and Conjugation with oligonucleotide probes

Synthesized AuNPs were analyzed by STEM, UV-Vis spectroscopy and Dynamic Laser Particle Size Analyzer. According to the analysis, homogenously distributed spherical colloidal AuNPs were measured as about 13 nm and λ_{max} was 521 nm as expected (Fig. S1). Additionally, the

measurement of STEM analysis showed that Value: l [nm], Obj. count: 85, Summation: 1071.01, Min. value: 9.44, Max. value: 20.26, Mean value: 12.60 and Std. dev. :2.21. The concentration of synthesized AuNPs was also calculated as 0.4nM according to the extinction coefficient of 13 nm at 521 nm wavelength (27).

After the conjugation of AuNPs with thiol modified probes their max absorption peaks were shifted from 521 nm to 526 nm as expected (Fig. S2). Because coating of AuNPs by probes (4µM, 8µM) for three sandwich models caused the changing of surface charges of AuNPs and resulted with max absorption peak shift. While the concentration of probes on AuNPs was enough for 4µM and 8µM for three models, 2µM probe was not enough for sustaining the stability of AuNPs since it caused the aggregation of AuNPs (data not shown). Therefore, 4µM and 8µM coated AuNPs were used for further studies for three LFA models.

Preparation of LFA models

The components of LFAs were manually prepared and three sandwich models (1A, 1B, 1.2) (Fig. S3) were applied to assay, separately. These models were designed to make comparison between the models and find the best one for molecular recognition of SARS-CoV-2 by LFAs.

Agarose Gel Electrophoresis of 2019-nCoV_N3 PCR

PCR was performed by using plasmid DNA including N gene and primers specific for 2019-nCoV_N3 were used for amplification of 72bp target. This was made for verification of presenting the target gene in our sample and mimicking the real PCR samples coming from the patients, which will be applied to the LFAs for further studies. PCR products for 2019-nCoV_N3 (72 bp) was shown in Fig. S4.

Application of targets to the LFAs models

Here probe concentration on AuNPs, sandwich models, membrane types, limit of detection (LOD) of target gene and buffer efficiency for molecular detection of 2019-nCoV_N3 on designed LFAs were studied. LFAs were prepared by four membranes and target was used as synthetic oligonucleotide sequence of 2019-nCoV_N3. Initially, buffer optimizations were experienced. For this purpose, sample pads were soaked with two buffers, buffer 4 and buffer 5, and three different buffers, buffer 14, PBS and SSC were used as running buffer. In these conditions the control lines of LFAs should always be seen as red and in order to say that test is positive both the test and control line should be seen as red. Results verified that membrane type and designed models have significant differences when they are used with different buffers and temperatures (data not shown). For instance, model 1B has shown the best results with M170 membrane compared to the M120 membrane for using 4µM and 8µM probe concentrations at 37˚C drying (Fig. 1, B1-B10; D1-D4), while the model 1A has weak test lines with M170 and M120 membranes at the same temperature (Fig. 1, A1-A10; C1-C4). Although there are no significant differences between buffer 4 and 5 for assay results, SSC buffer was used for further studies as it has clear red color intensity on both the test and control lines and showed no nonspecific bindings on LFAs for all models using 4µM and 8µM probe concentrations. This finding is meaningful as SSC buffer has commonly positive effect on oligonucleotide hybridizations. To highlight, all developed strips have selectively detected the target and showed no nonspecific binding to the Mers CoV_N2, Mers CoV_N3 and RdRp/Orf1 sequences of SARS CoV_2, which are specific for Mers CoV and SARS-CoV-2, respectively. This means that designed LFA strips are suitable and usable for the molecular detection of SARS-CoV-2, reliably. Additionally, all strip assays worked truely since all the control lines are visible even if two of them are weak in positive assay (Fig. 1, A1,C1). This is probably caused by the weak interaction between the capture and detection reagent for prob 1A.

Application of PCR products to LFAs designed by three models

PCR products were applied to the developed LFAs using three models and two type of membranes. To make sure that for selective detection of target, random oligonucleotide sequences such as Se20_60 bio, Crn2SH and Mers CoV_N2, Mers CoV_N3 and RdRp/Orf1 of SARS-CoV-2, SSC running buffer were used as negative controls. Findings showed that all models recognized the target sequence, selectively without any nonspecific bindings of negative controls (Fig. 2). Although there is no significant differences between the strip assays, model 1B on both membranes might be considered as the best for the detection of 2019-nCoV_N3 in PCR sample by two probe concentrations (Fig. 2 strip 6, 8, 11, 16). Although the test line intensity on model 1.2 (Fig. 2, A1) and model 1A (Fig. 2, B14-B15) is weak compared to model 1B they could make selective detection without any nonspecific binding to the negative controls (Fig. 2, A2-A5, Fig. 1, A, C).

Briefly, all these results mean that designed assay models could be good candidate for naked eye analysis of SARS-CoV-2 without needed advanced rRT-PCR devices and agarose gel electrophoresis. Additionally, as very low amount of PCR product, 5 µL, is loaded on developed LFAs it seems advantageous and sensitive compared to the traditional agarose gel electrophoresis. Because agarose gel electrophoresis needs to the high amount of PCR product and long time for analysis. To highlight, there is no significant difference between two probe concentrations (4µM-8µM) for detection of PCR products.

Limit of Detection of LFAs designed by three models

LOD experiments were performed by using synthetic target with developed LFAs by three sandwich models and both membrane types. It is clearly seen that model 1A works efficiently since the control lines of all strips are seen and results showed that 0.1µM target, 72 base long,

was sensitively recognized by model 1A using both membrane types (Fig. 3, B3 and D1, D3). Interestingly, there is significant difference in terms of the detection of this amount by M120 membrane. The line intensities of 0.5µM target (Fig. 3, C) are weak compared to the 0.1µM target (Fig. 3, D).

It might be said that the sensitivity of LFAs based on hybridization is significantly affected by the amount of target and membrane type. It means that there is an optimum concentration between the capture and detection oligonucleotides for effective hybridization and it is not directly related with high amount of target for this model designed with this membrane. However, the line intensities on M170 membrane were gradually become weak when the concentration of target was decreased for two probe concentrations (Fig. 3, A-B). While the LOD is 0.1µM target by using 8µM probe (Fig. 3, B3), it was 0.5µM using 4µM probe on this membrane. These findings suggested that probe concentrations on AuNPs has significant role for sensitive detection along with the membrane type. This can also be verified by comparing the strips developed by 4µM probes with M170 (Fig. 3, B1) and M120 membrane (Fig. 3, D1) for the same LOD.

LOD experiments were experienced by model 1B using both membranes and two probe concentrations (Fig. 4). According to the results 0.1µM target, 72 base long, was detected by M170 membrane using both probe concentrations (Fig. 4, B) while it was 0.005µM by M120 membrane using 8µM probe (Fig. 4, F). These results verified that membrane types and probe concentrations used in LFAs have significant role for sensitive detection. Here, used sandwich model, another important point for sensitive detection, seems better than model 1A since the all strips have clear line intensities and lower detection limit.

LOD was also experienced by model 1.2 using both membranes and two probe concentrations (Fig. 5). According to the results the minimum amount of target, 50 base long, was detected as 5pM by M170 membrane using 8µM probe (Fig. 5, C1) while it was 100pM by M120 membrane using 8µM probe concentration (Fig. 5, E3) without any nonspecific bindings. This amount is either lower than the reported nucleic acid based LFAs (28,29) or similar with the amount of SARS CoV_2 N protein detection (30). Thus, to make sensitive recognition 8µM probe could be used for this model. Lastly, model 1.2 allowed effective hybridization on both lines and this was resulted by clear line intensity and the lowest detection amount of target.

When compared to all models in terms of the LOD, the length of the target has crucial role for LFA efficiency. It could be inferred from these results 50 base long target sequence could be sensitively recognized compared to the 72 base long as the base length of target sequence become shorter, LOD was observed as lower. This may be caused by the high probability of the hairpin structures in long bases, which can interfere the hybridization on the assay. Therefore, sensitive detection is closely related with the length of target sequence and hybridization models between the target and capture reagents for LFA. Along with this, it should be highlighted that the main gene length, 72 base long, is also clearly detected by developed strip assay models and could be used for the detection of 2019-nCoV_N3.

Lastly, LFAs were also prepared by different times in order to see the stability of conjugates and LFAs efficiency. Therefore, assays were applied by 6 months awaited conjugates. It was found that conjugates are still stable and works efficiently in terms of the hybridization on the LFAs and both lines on the strips were observed, clearly (data not shown). Since the stability of the conjugates is also highly related with the red color of suspension they still have their original color (data not shown). Therefore, designed LFAs with these models have potential for

long shelf life if they are fabricated. Because the developed method is also consistent in terms of the reproducibility and there was no difference in batch to batch production or preparation of all LFA strips.

As a conclusion, the detection of SARS-CoV-2 by targeting the 2019-nCoV_N3 gene region was succeeded by designed LFAs models as a first study according to the best of our knowledge. Although the detection of virus by LFAs is commonly based on the immunoglobulins of patients or antigens these models are for the molecular detection of SARS-CoV-2 since it is the most reliable method in the world. To highlight that designed LFAs are also cost effective as they are naked eye analysis assays and need to conventional PCR products instead of the expensive devices and reagents for analysis such as rRT-PCR. We believe that findings will be valuable for various molecular detection methods for SARS-CoV-2 and its mutants. Because assay is based on the hybridization and can be rapidly designed for specific sequences of mutant viruses. Thus, the detection of either mutated or conserved regions could be possible by these type of assay models. Since the PCR products were recognized and parameters were optimized in designed LFAs they might also be a candidate for point of care diagnosis in terms of the molecular detection of SARS-CoV-2 for further fabrication. In this perspective, applying the developed LFAs to the real samples coming from the patients will be planned for the future work.

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Figures Legends

Figure 1. LFAs developed by M170 (A, B) and M120 (C, D) membrane using model 1A (A, C) and model 1B (B,D) at 37˚C drying. Strips A1-A5; B1-B5; C1-C2; D1-D2 were prepared by 4µM probe and strips A6-A10; B6-B10; C3-C4; D3-D4 were prepared by 8µM probe. target: 2019-nCoV_N3 (72 bp), SSC: running buffer. Arrows show the test and control lines.

Figure 2. Application of PCR products to LFAs developed by two types of membranes and three models. Strips 1-8 were prepared by 4µM and strips 9-16 were prepared by 8µM. Strips 1-15 were prepared by M170 and strip 16 was prepared by M120 membrane. Strips 1-5: model 1.2, Strips 6-13,16: model 1B, strips 14-15: model 1A. Non heated: Non heated PCR product. Se20_60 bio, Crn2SH, Mers CoV_N2, Mers CoV_N3 and RdRp/Orf1 and SSC are negative controls. Arrows show the test lines.

Figure 3. LOD of target by model 1A using M170 (A-B) and M120 (C-E). A) 0.5µM, B) 0.1µM, C) 0.5µM, D) 0.1µM, E) 0.005µM target. Strips 1-2 were prepared by 4µM probe and strips 3-4 were by 8µM. Strip E1 was prepared by 4µM and E2 was by 8µM, and then target was applied to both. All 1-3 strips were applied by target and strips 2-4 were by buffer as a negative control. Arrows show the test and control lines.

Figure 4. LOD of target by model 1B using M170 (A-C) and M120 (D-G). A) 0.5µM, B) 0.1µM, C) 100pM, D) 0.5µM, E) 0.1µM, F) 0.005µM, G) 100Pm target. Strips 1-2 were prepared by 4µM probe and strips 3-4 were by 8µM. Strips F1-F2 were prepared by 8µM. All 1-3 strips

were applied by target and strips 2-4 were by buffer as a negative control. Arrows show the test lines.

Figure 5. LOD of target by model 1.2 using M170 (A-C) and M120 (D-F). A) 0.005µM, B) 50pM, C) 5pM, D) 0.005µM, E) 100pM, F) 50pM target. Strips 1-2 were prepared by 4µM prob and strips 3-4 were by 8µM. Strips C1-C2 were prepared by 8µM. All 1-3 strips were applied by target and strips 2-4 were by buffer as a negative control. Arrows show the test lines.

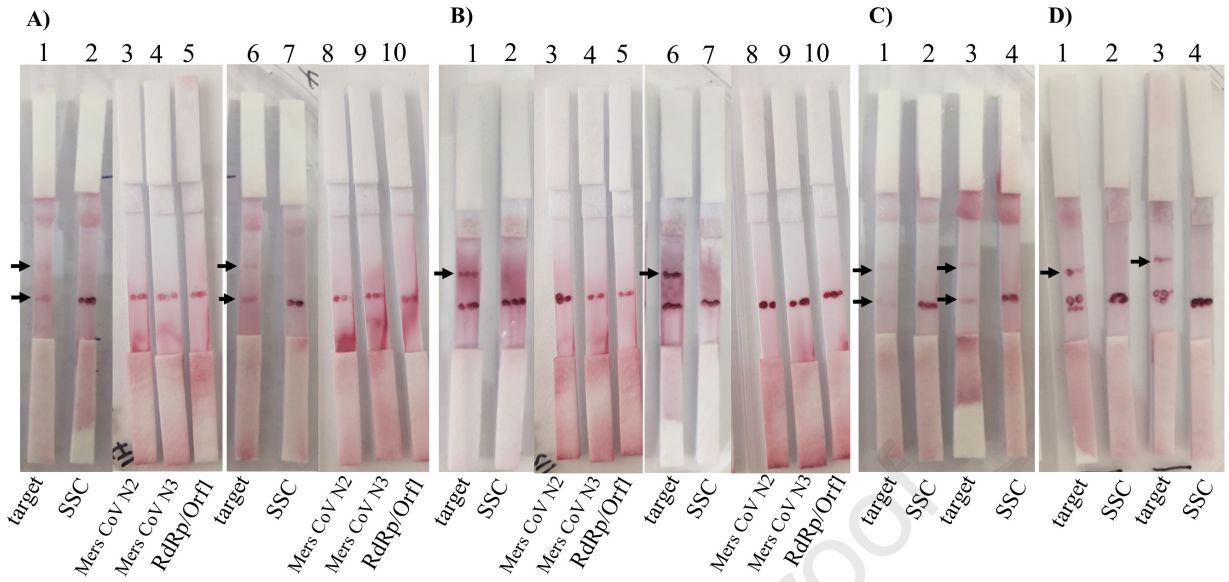
Supplementary Figures

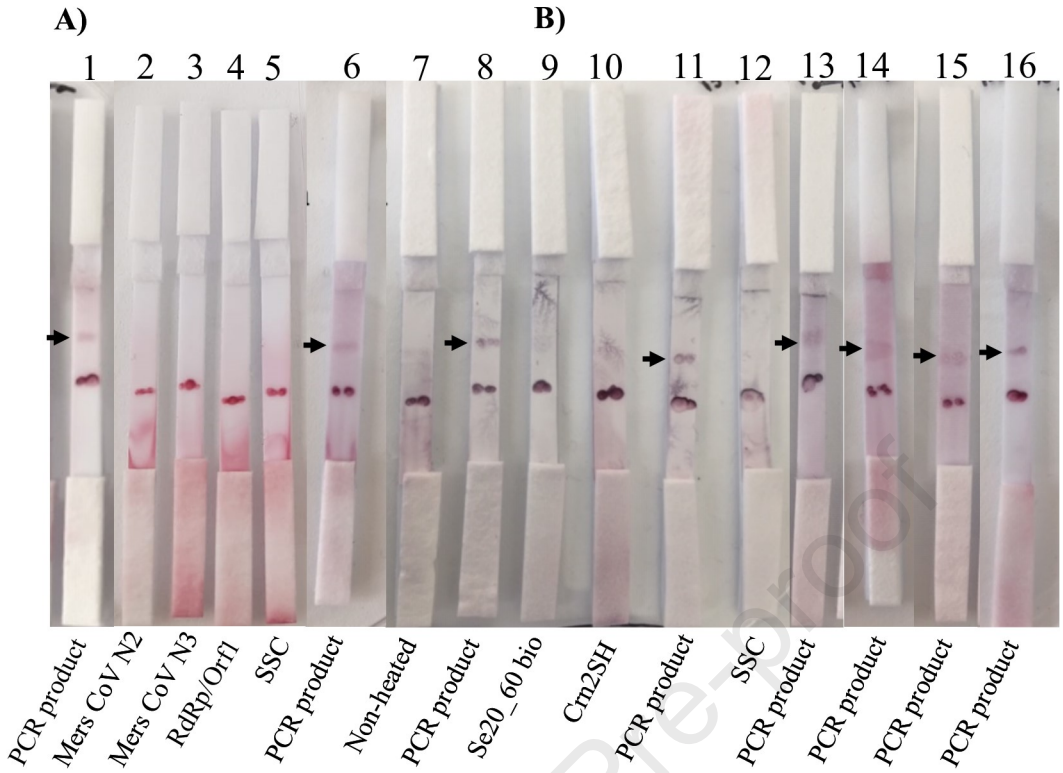
Fig. S1. UV-Vis Spectroscopy (A), Dynamic Laser Particle Size Analyzer (B) and STEM analysis (C) of synthesized AuNPs

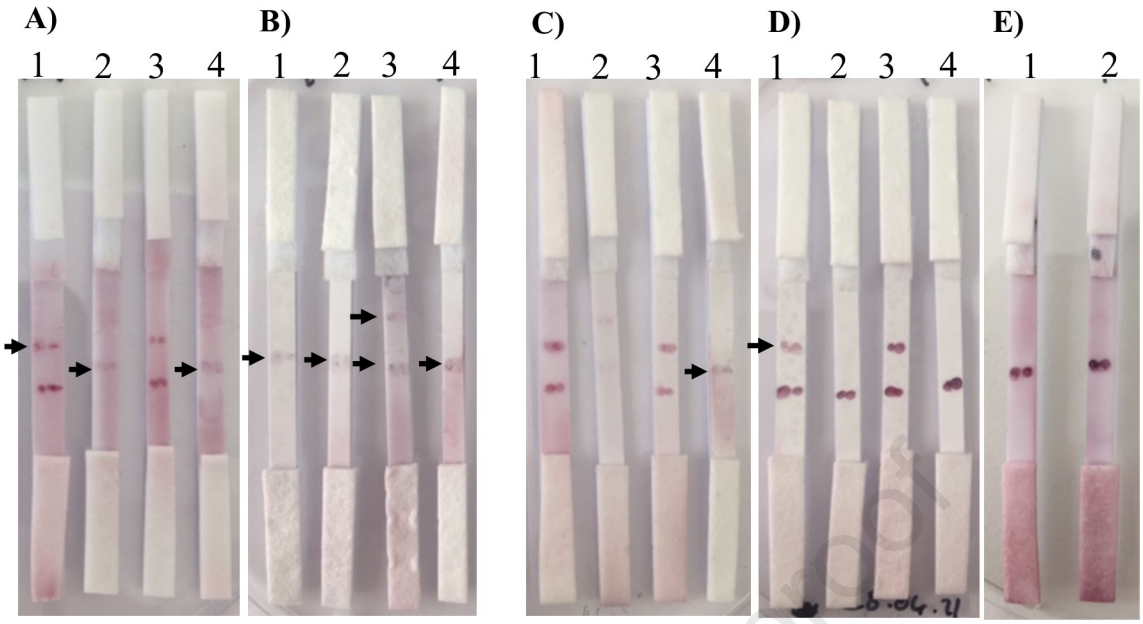
Fig. S2. The UV-Vis spectra of AuNPs after the surface modifications with probes. A) Prob 1A prepared by 4µM probe B) Prob 1A prepared by 8µM probe C) Prob 1B prepared by 4µM probe D) Prob 1B prepared by 8µM probe E) Prob 1.2 prepared by 4µM probe F) Prob 1.2 prepared by 8µM probe

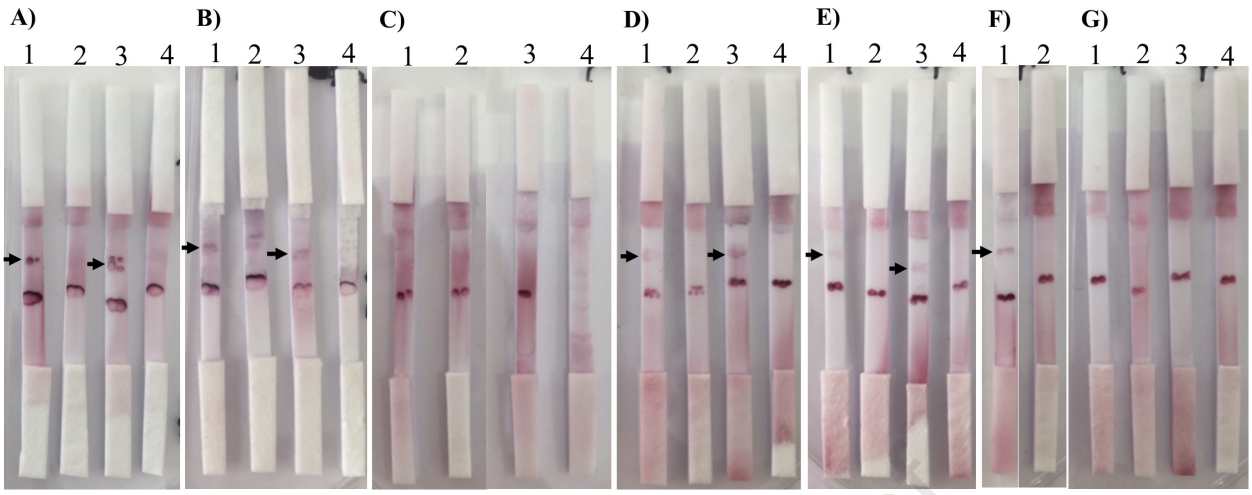
Fig. S3. LFA sandwich models for the detection of SARS-CoV-2 N gene region. 1A and 1B is designed for 72 base long N gene (2019-nCoV_N3) and 1.2 is for 50 base long N gene (2019-nCoV_N3). COVID-19 N gene: 2019-nCoV_N3

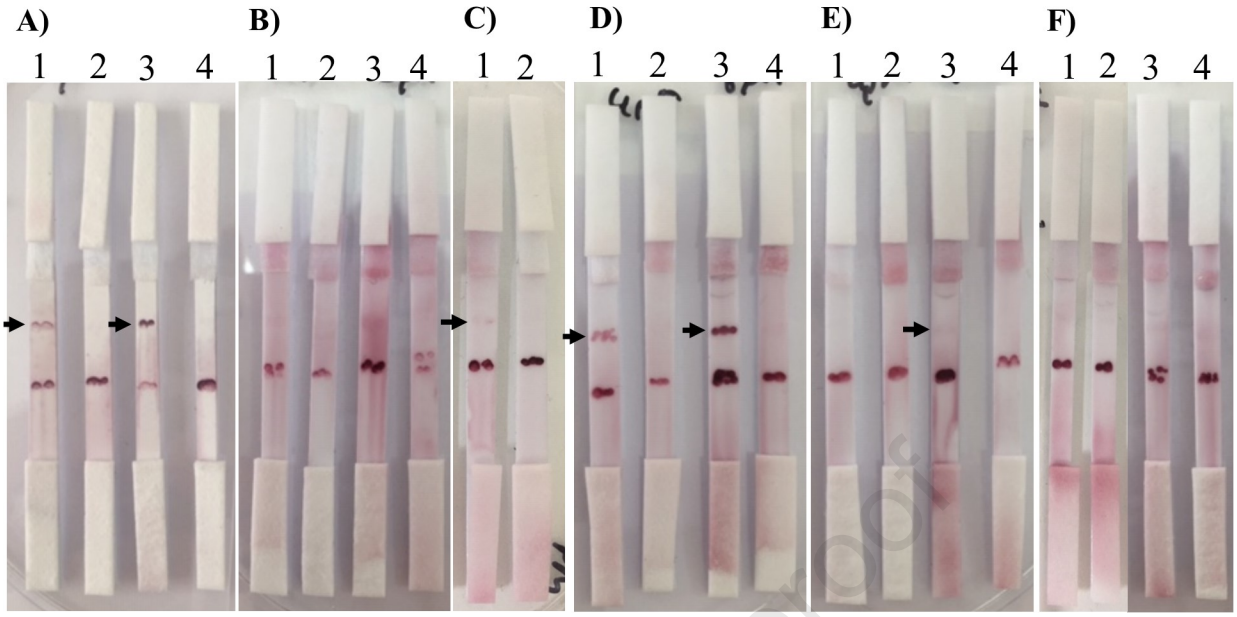
Fig. S4. Agarose gel electrophoresis of 2019-nCoV_N3. 1) Ultra low DNA ladder (Thermoscientific), 2-9) PCR products of 2019-nCoV_N3 (72 bp).











Highlights

- 2019-nCoV_ N3 was detected by developed LFA, rapidly
- This is the first sandwich assay shows the hybridization models of PCR products for the detection of 2019-nCoV_N by LFA
- Developed LFAs can be an alternative method to the conventional and real time PCR
- Designed LFAs can be adoptable to the molecular detection of SARS-CoV-2 mutants