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Development of Rapid Aptamer-Based Screening Assay for the Detection of Covid-19 Variants

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Cite This: *ACS Omega* 2023, 8, 32877−32883

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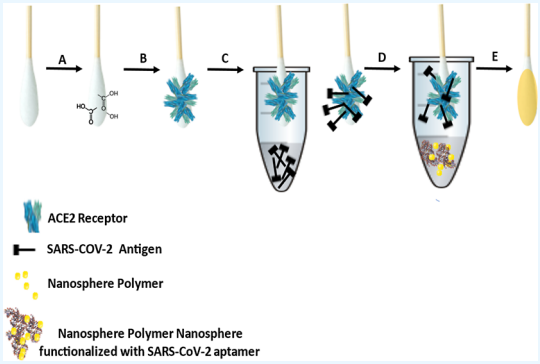
ABSTRACT: The development of a colorimetric severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection assay with the WHO published ASSURED criteria is reported, in which the biosensor should have the following characteristics of (i) being affordable for low-income communities, (ii) sensitive, (iii) specific, (iv) user-friendly to be used by non-skilled personnel, (v) rapid and robust, (vi) equipment-free, and (vii) delivered to the end-users as a simple and easy to use point-of-care tool. Early viral infection detection prevents virus spread and controls the epidemic. We herein report the development of a colorimetric assay in which SARS-COV-2 variants can be detected by colorimetric observation of color on the sensing cotton swab surface. Using the developed biosensor assay, it is possible to discriminate between the various SARS-CoV-2 variants with a LOD of 100 ng/mL within 4 min including sample preconcentration and incubation step. The results illustrated the development of a SARS-CoV-2 colorimetric biosensor that can be mass produced, with low-reagent cost, and can be read-out visually in the field by nonskilled personnel.



1. INTRODUCTION

The lockdown of the central Chinese city of Wuhan on February 23, 2020 alerted people worldwide to the novel corona virus disease 2019 (COVID-19) disease, which poses a serious challenge to public health and governance. The causative virus is currently classified as severe acute respiratory syndrome corona virus-2 (SARS-CoV-2)[1,2] To date, COVID-19 infected more than 613 million confirmed cases worldwide, resulting in 6.5 million deaths.[3] Despite unprecedented scientific efforts by several pharmaceutical entities and academic laboratories to develop vaccines and antivirals against SARS-CoV-2, epidemic control is still limited by the emergence of mutant variants labeled as variants of concern by the World Health Organization[4−6]

As of June 2021, six different variants of SARS-CoV-2 have been identified including the UK variant (B.1.1.7 alpha), the South African variant (B.1.351, Beta), the California variant (B.1.429, Epsilon), New York Mutant (B.1.526, Iota), Brazilian Mutant (P.1, Gamma), and Indian Mutant (B.1.617.2, Delta).[7,8] According to recent studies, SARS-CoV-2 variants are more infectious, of higher risk for hospital and intensive care unit admissions, and able to evade antibodies induced by vaccination.[9,10] Furthermore, various consequences including neurological, cardiovascular, respiratory, and psychiatric disorders have been reported following SARS-CoV-2 infection.[11,12] Thus, effective quarantine and isolation measures are essential to control COVID-19 pandemic and maintain the quality of life after the early detection of SARS-CoV-2.[13]

In the face of this pandemic, versatile diagnostic assays, able to detect single-nucleotide mutations in viral RNAs, confer a short sample-to-result turnaround time, and provide a multiplexing capability to identify SARS-CoV-2 variants, are in need.[14] Today, SARS-CoV-2 detection is based on reverse transcription-polymerase chain reaction (RT-PCR) which is an accurate and gold-standard method.[15,16] However, this technique is expensive and laborious and requires well-equipped laboratory facilities and specialized personnel. Also, the use of RNA as a target molecule can lead to misleading results due to mutations.[17] Alternatively, protein-based detection assays received considerable attention due to the stability of the target protein.[18,19] For early detection, antigen-detection strategies were more suitable than antibody-detection strategies, which can take few days to produce the antibodies after the onset.[20,21] Enzyme-linked immunosorbent assay (ELISA) is one of the commonly used techniques for the detection of proteins, yet its low sensitivity limits its application for the detection of SARS-CoV-2 physiologically relevant subpico-molar concentrations (∼10 pM).[22,23]

Received: June 11, 2023
Accepted: August 16, 2023
Published: August 31, 2023

To overcome ELISA limitations, several detection methods have been developed including optical (e.g., colorimetric),[24,25] electrochemical (e.g., voltammetric sensors),[26] and electrical (e.g., field-effect transistor).[27] Although some of these sensors have demonstrated femtomolar sensitivity, they lack an adequate limit of detection (LOD) and a wide dynamic range with good linearity, which makes accurate and reliable quantification of target proteins difficult.

An emerging method to detect SARS-CoV-2 is based on the use of nucleic acid aptamers. In comparison with antibodies, aptamers can be produced *in vitro*, at lower cost, and can be modified with little batch-to-batch variation. Aptamers have been previously demonstrated in therapeutic and diagnostic applications for various pathogenic analytes including parasites, bacteria, and viruses due to their reported high affinity and specificity.[28−30]

Several studies selected aptamers that target SARS-CoV-2 structural proteins such as the spike (S) protein, nucleocapsid (N) protein, envelope (E) protein, and membrane protein.[31] As a result of this, a number of aptameric-based detection tools have been developed including enzymatic oligonucleotide assays, lateral flow assay (LFA), and aptasensors.[28,30]

This work presents a colorimetric, simple, cost-effective, and user-friendly portable SARS-CoV-2 detection platform, in which SARS-COV-2 variants can be identified upon optical observation of the color over a cotton swab surface (Figure 1).

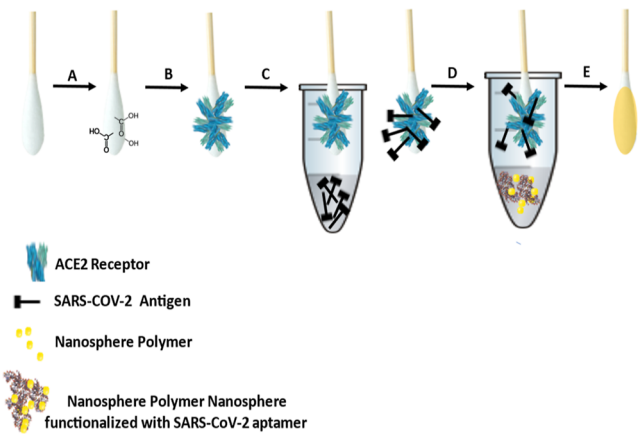


Figure 1. Development of the SARS-CoV-2 aptasensor. (A) Cotton swab activation. (B) Cotton swab functionalization with the ACE2 receptor. (C) Preconcentration of SARS-CoV-2 antigen over sensing swab surface. (D) Sandwiching of SARS-COV-2 antigen with the colored nanosphere-functionalized aptamer for the COVID variants. (E) Visual detection of positive result.

Using the developed biosensor assay, it is possible to discriminate between positive and negative and the various variants of SARS-CoV-2 samples in a few minutes including sample preconcentration and incubation step. The assay can be applied easily to other viruses detection.

2. MATERIALS AND METHODS

2.1. Materials. SARS-CoV-2 alpha, beta, delta, gamma, and omicron antigens were obtained from BiosPacific (BiosPacific, Emeryville, CA, USA) and stored at −80 °C. Amino-functionalized COVID-19 aptamers were synthesized by Metabion International (Planegg, Germany), and stored at −20 °C. 50 nm blue, red, orange, and yellow nanobeads were obtained from Polysciences Inc. (Taipei, Taiwan) and stored at 4 °C. ACE2, phosphate buffer saline (PBS), 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from Merck Life Science UK Ltd (Dorset, UK). Finally, the cotton swabs were purchased from a local pharmacy in Riyadh, Saudi Arabia.

2.2. Methods. *2.2.1. Preparation of Activated Cotton Swabs.* The cotton swab materials were immersed in a mixture of 100 mL of 2 mM potassium periodate (KlO_4) and 1 mL of sulfuric acid (H_2SO_4) overnight at room temperature (Figure 1A) to oxidize the alcohol groups to aldehyde groups. Then, the cotton swabs was extensively washed with cold water to remove the excess of the oxidizing agent. The activated cotton swabs were then stored dry at room temperature for later use. The oxidation of the cellulose hydroxyl groups to aldehyde was confirmed using FTIR by the appearance of a new peak at 1730 cm^{-1}.

3.1.2. Cotton Swab Functionalization with ACE2

Immobilization of ACE2 on activated cotton swabs was achieved by immersing the activated cotton swab in 100 μL of a solution of 100 ng/mL ACE2 (Figure 1B) overnight. Then, it was washed with PBS and kept at 4 °C until further use.

2.2.2. Nanosphere Functionalization with the SARS-CoV-2-Specific Aptamer. 300 μL of 50 nm (nanobeads 2.5% aqueous suspension, ∼1.69 × 10^{10} beads/mL) of the carboxy-functionalized colored (blue, yellow, red, and orange) nanosphere suspension was pipetted in a 1.5 mL micro-centrifuge tube and washed with distilled water three times. The colored nanospheres were incubated with 500 μL of freshly prepared EDC (1 mg/mL)/NHS (1 mg/mL) solution and stirred for 30 min at room temperature. After that, the nanospheres were washed five times with PBS buffer. Then, 100 μL of the 50 ng/mL the aptamer solution was added to the washed nanospheres and incubated at 4 °C overnight. At the end, the active sites on the nanosphere were blocked by incubating them in 1 mg/mL BSA in PBS for 1 h, followed by thoroughly four times washing with PBS to remove any unbound blocking agent. Nanosphere-aptamer conjugates were stored at 4 °C for future use.

2.2.3. Colorimetric Detection Platform. Semiquantitative detection of SARS-COV-2 alpha, beta, delta, and gamma variant antigens was observed by the color change of the cotton swab surface. Generally, the detection process includes three steps; (1) ACE2-conjugated cotton swaps were immersed in the tested SARS-COV-2 variant dilutions (10−10^7 ng/mL) for 2 min to preconcentrate the antigen (Figure 1C); (2) after that the swabs were washed with PBS buffer three times to remove the unbound antigens; and (3) the sensing swabs were immersed in the developing solution which contains colored nanosphere functionalized with SARS-COV-2-specific aptamer solution for 2 min (Figure 1D). Then, the swabs were washed with PBS to remove the unbound nanosphere. In this assay protocol, the SARS-COV-2 variant antigens were sandwiched between the cotton-immobilized primary ACE2 and the SARS-COV-2 aptamer conjugated to the colored nanosphere, which in turn results in a change in the color of the cotton swab surface, indicating the specific binding to the target SARS-COV-2 variant antigen.

3. RESULTS AND DISCUSSION

Pandemic infection can be controlled by developing a colorimetric detection assay that meets the ASSURED WHÒs criteria. The biosensor has to be rapid, highly sensitive

https://doi.org/10.1021/acsomega.3c04137
ACS Omega 2023, 8, 32877−32883

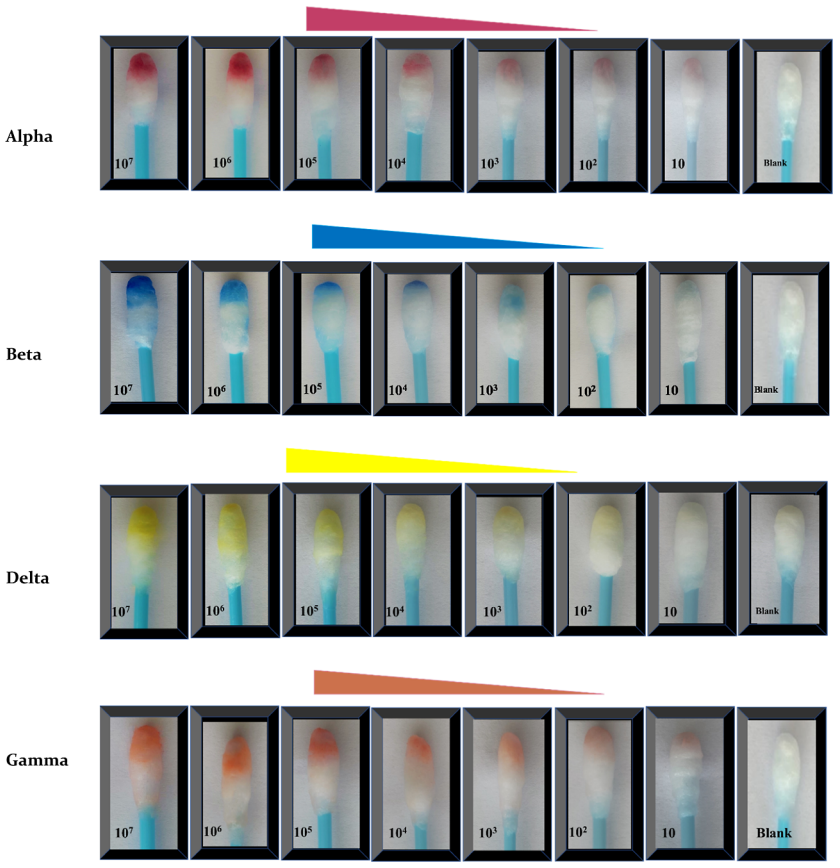


Figure 2. SARS-COV-2 virus aptasensor after the assay performance at different SARS-COV-2 variant antigen concentrations (10−10^7 ng/mL).

and specific, instrumentation-free, user-friendly, and can be run in the field by non-skilled personnel.[32−37] Early viral infection detection prevents SARS-COV-2 virus spread and controls the epidemic. Herein, the SARS-COV-2 virus has been screened out using an aptasensor-based technique, where the virus sandwiched between ACE2 immobilized on a cotton swab surface and an aptamer conjugated to the colored nanosphere. In this assay, the SARS-COV-2 was preconcentrated upon capturing by the ACE2 immobilized on the cotton swab (Figure 1C). In the next step, the secondary aptamer conjugated with the colored nanosphere will sandwich SARS-COV-2 (Figure 1D). As the secondary aptamer is specific to the target virus, the cotton surface will turn into the color of the beads, which can be blue, red, orange, or yellow depending on the variant (Figure 1E). It is a simple technique in which the pathogenic corona virus can be identified from the color of the cotton swab surface by the human naked eye. SARS-COV-2 sandwich complexes were formed on the cotton surface upon treatment with serial dilutions of virus antigen in the range (10−10^7 ng/mL) (Figure 2). The control sample was prepared by adding PBS buffer instead of the antigen so that we can notice the difference in color. The high intensity of the color means the higher formation of the SARS-COV-2 sandwich complexes on the cotton surface. In other words, the intensity of the color was directly proportional to the concentration of the virus present on the cotton surface. The LOD was determined to be the lowest concentration of SARS-COV-2 antigen, which resulted in a visually discernible color. The LOD of the sensor was determined to be 10 ng/mL, which satisfies the diagnostic requirements of point-of-care testing for COVID-19[38] and is comparable to the detection limits obtained by other COVID-19 biosensors (Table 1). The advantage of this diagnostic tool is that it serves as both a virus collection and concentration technique with no need for sending the diagnostic swabs to laboratory, thus saving time and effort. Moreover, since this aptasensor is specific for SARS-COV-2 variants, it can be used in some high-risk areas such as hospitals. Assay specificity was confirmed by its ability to detect SARS-COV-2 alpha, beta, delta and gamma variants at different concentrations, as shown in Figure 2.

3.1. Cross-Reactivity Test. One of the main criteria to evaluate the sensor performance and its selection property for a specific antigen is the cross-reactivity study.

The same assay mentioned above was performed by testing the developed sensors with alpha, beta, delta, and gamma antigens to check sensor selectivity. Also, aptasensor clinical applicability was verified using samples obtained by spiking various concentrations of SARS-COV-2 virus antigens. Cotton swab sensors were functionalized with the ACE2, then individually incubated with alpha, beta, delta, gamma, and omicron antigens, followed by incubation with the colored nanospheres conjugated with the secondary specific aptamer. The color of the cotton swabs does not turn colored which indicates that the sensor has high selectivity to SARS-COV-2 virus (Figure 3).

3.2. SARS-CoV-2 Colorimetric Biosensor Stability. One of the significant challenges for the stability of colorimetric biosensors is their sensitivity to the external environment, particularly, temperature and humidity. High temperatures can denature the sensing protein molecules, while humidity can affect the surface of the biosensors, leading to a decrease in sensitivity and specificity. Developed colorimetric biosensors

https://doi.org/10.1021/acsomega.3c04137
ACS Omega 2023, 8, 32877−32883

Table 1. Performance of a Number of Up-to-Date Colorimetric Biosensors Designed for the Detection of COVID-19 Infection

method	targets	biological recognition element	LOD	time (min)	advantages	sensitivity and specificity	ref
paper-based polydiacetylene biosensor	spike protein	antibody	1 ng/mL	4 h	smartphone-assisted sensing; user-friendly	high specificity; minimally influenced by pH and temperature	42
paper-based fuchsine dye-loaded polymersome	spike protein	antibody	0.11 ng/mL	1 min	smartphone-assisted sensing can be performed at home by people which reduced the burden on the healthcare facilities. Furthermore, these tools can be used in underdeveloped regions	high specificity	43
half-strip LFA	nucleocapsid protein	biotinylated antibody	0.65 ng/mL	20 min	had a potential for manufacturing at large scale. Assay utilized only commercially available reagents and conventional protocols	84% sensitivity	44
colorimetric detection based on metallic nanozyme catalysis	spike protein	antibody	11 ng/mL	20 min	had a potential for rapid and accurate detection of SARS-CoV-2	high specificity and sensitivity	45
colorimetric detection based on gold nanoparticle	spike protein	antibody	48 ng/mL	10 min	had a potential for rapid and accurate detection of SARS-CoV-2	high specificity	46
colorimetric and fluorescent dual-functional lateral flow immunoassay biosensor	spike protein	antibody	1 ng/mL	30 min	had a potential for rapid and accurate detection of SARS-CoV-2	high specificity	47
nanoparticle-transfer biosensors	nucleocapsid protein	antibody rabbit IgG	3 ng/mL	<10 min	noninvasive; can be read with a smartphone; ideal for decentralized mass screenings	96.2% sensitivity and 100% specificity	48
colorimetric detection based on gold nanoparticle plasmonic sensing	nucleocapsid protein	antigen	150 ng/mL	5 min	observable by the naked eye	high specificity	49
naked-eye detection mediated by N gene-targeted antisense oligonucleotide-capped plasmonic nanoparticles	N gene	antisense oligonucleotides	0.18 ng/μL	~10 min	observable by the naked eye, no sophisticated instrumental required	high specificity	50
colorimetric and electrochemical detection based on gold nanoparticle	spike protein	antibody	1 pg/mL	10 min	observable by the naked eye; do not require sophisticated instruments	high sensitivity	46
colorimetric-based biosensor	spike protein	ACE2-labeled receptor	100 pfu/mL	5 min	observable by the naked eye; do not require sophisticated instruments	high specificity and selectivity	51
current assay	antigen	aptamer	100 ng/mL	4 min	colorimetric assay can be read by the naked eye, scalable and can be used in the field by nonskilled personnel	high specificity and selectivity	

https://doi.org/10.1021/acsomega.3c04137
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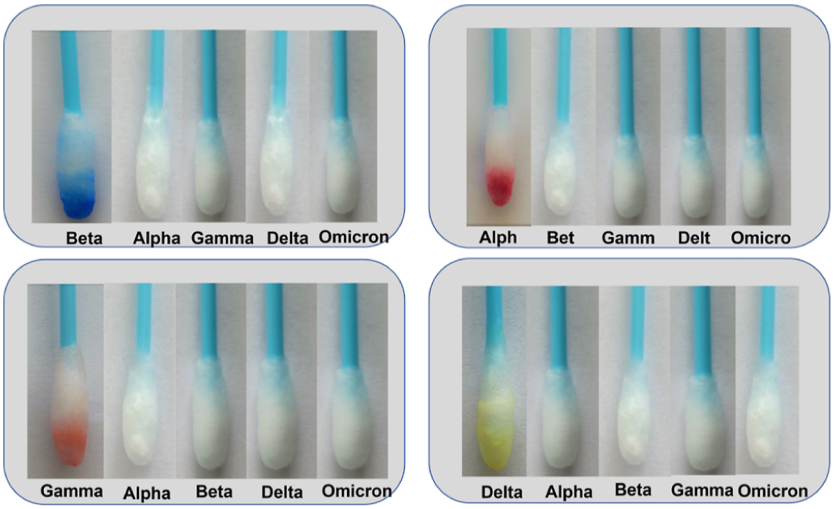

Figure 3. Cross-reactivity results of the alpha, beta, delta, and gamma colorimetric sensors. The assay takes around 4 min to be performed in the field and does not require any instrumentation and can be run by non-skilled personnel, which reduces the cost and turnaround time. This low-cost diagnostic tool can assist physicians and their associated teams to conduct interventional measures and control protocols as early as possible, which in turn will prevent the spread of COVID-19 infection.

proved stability for 6 months if stored under appropriate conditions, such as at low temperatures (5 °C) and low humidity levels.

Another crucial factor affecting the shelf life of colorimetric biosensors is the nature of the ACE2 sensing molecules utilized. In this assay, the SARS-CoV-2 aptamer is considered a stable biomolecule that will support biosensor stability.

3.3. Comparison with Alternative SARS-CoV-2 Colorimetric Biosensors. In emergencies, home care, and limited resources areas, colorimetric biosensors present promising diagnostic tools. Although each biosensor benefits from the privilege of being visible by the naked eye, sensitive, and specific, their practical use on-site may be constrained by the necessary pretreatment steps and large-scale availability. Researchers have developed a paper-based assay for the colorimetric detection of SARS-CoV-2 variants that allows the detection of single-nucleotide mutations in viral RNAs.[39] The assay detected the presence of SARS-CoV-2 as well as alpha, beta, and gamma SARS-CoV-2 variant mutations with 100% concordance with quantitative rt-PCR and RNA sequencing.[39] Another study has employed the pH indicator as colorimetric assay to detect the amplicon of viral RNA. As a result of this, the color will change from pink to yellow.[40,41] The performance of some of the up-to-date colorimetric biosensors designed for the detection of COVID-19 is summarized in Table 1.

4. CONCLUSIONS

A simple, easy-to-use colorimetric, instrument-free test for the detection of the SARS-COV-2 virus was developed. Cotton swab aptasensor was used as a diagnostic instrument for sample collection, preconcentration, and detection. Using yellow nanospheres, the sandwich immunoassay principle was applied to detect the SARS-COV-2 antigen. This assay showed good sensitivity and specificity. Although this biosensor is good in detecting virus antigens even at its lowest concentration, more research in this field and the use of clinical samples is needed. To summarize, this essay is rapid, simple, inexpensive, and easy to apply.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors would like to acknowledge the generous funding from the Saudi Ministry of Health under project number 1276.

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