Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/23/20231065

Application of lateral flow immunochromatography assay, CRISPR/Cas12, and QDs-based biosensing for SARS-CoV-2 diagnosis

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Abstract. During the COVID-19 pandemic, scientists have developed many diagnostic techniques, such as biosensors, to detect whether a patient is infected by SARS-CoV-2. This could help the governments to plan an effective method to decrease the rate at which the illness is spreading across the globe. These diagnostic techniques are developed after the research of this foreign viral pathogen, SARS-CoV-2, by investigating the potential analytes that could be used and the various ways that the virus could infect a person after exposure. The three diagnostic techniques will be discussed in this paper, including lateral flow immunochromatography assay (LFIA), CRISPR/Cas12, and quantum dots (QDs)-based biosensing system. In addition, this research will also systematically discuss the advantages and disadvantages of these three monitoring and analysis methods. Although these three diagnostic techniques have very high selectivity and sensitivity, establishing a reliable result, there are pathogens that could result in similar symptoms of respiratory pneumonia just like SARS-CoV-2. The diagnostic techniques explained in this paper can't diagnose more than one pathogen or disease, therefore, it is essential to further develop a multiplexing assay to produce a more detailed result.

Keywords: diagnostic techniques, biosensors, SARS-CoV-2.

1. Introduction

Since the COVID-19 pandemic outbreak in late 2019 or early 2021, many people have died and were forced to be separated from their families and friends due to the infection of this foreign virus, SARS-CoV-2. During this period, scientists have done many research or investigations on this pathogen to understand its structure and mechanism. By doing so, it would be more feasible for the researchers to develop biosensing techniques that can be used to detect SARS-CoV-2 from different samples extracted from patients.

To start with, CoV is an enveloped virus and it is also where the surface viral proteins are embedded [1, 2]. A protein that could be found on the surface of the membrane is the spike protein (S-glycoprotein) [1, 3], and it could bind with the angiotensin-converting enzyme 2 (ACE2) [3] that is found on the surface of the lower respiratory tract of humans [1]. In addition, all CoVs have their genome stored in a single-stranded RNA in the 5' to 3' orientation [1]. The length range of the RNA strand could vary from 26.4-31.7 kilobases, which allows the CoV to have the largest RNA genome amongst other known RNA-based viruses [1, 2]. In SARS-CoV-2, the genome of this coronavirus had high similarity to other CoV,

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specifically to Cov RaTG13, which is found in bats [3]. Therefore, suggesting the virus originated from bats and has slowly transmitted to humans through a variety of animal hosts (mediums) [1]. It has also been reported the incubation period is around 1-14 days, where 3-7 days is the most common [1].

SARS-CoV-2 belongs to a part of the coronavirus family. The infection of SARS-CoV-2 is highly contagious, resulting in a fast spread of infection across the globe. One of the earliest diagnostics used in COVID-19 is the real-time reverse transcriptase polymerase chain reaction (RT-PCR) [4]. However, this diagnosis technique is highly reliant on the type of sample that is taken, where the accuracy of each test could vary a little [4]. In addition, RT-PCR is not a point-of-care diagnosis, thus, it could only be utilized in specific areas, such as laboratories that have professionals who could run the test. This leads to the designing of biosensors that are integrated with techniques that could be used to detect SARS-CoV-2 without too much limitation in accessibility, such as POCTs. In this research, three effective methods for virus detection and analysis, including lateral flow immunochromatography assay (LFIA), CRISPR Cas, and quantum dots (QDs)-based biosensing system will be explained regarding the steps of diagnosing SARS-CoV-2. And the advantages and disadvantages of these methods in the detection application process will also be further analyzed.

1.1. LFIA analysis

The production of antibodies is stimulated when the immune system has detected a foreign antigen. To this end, the LFIA method was introduced to detect SARS-CoV-2 in patients during the pandemic. When a person's immune system is invaded by SARS-CoV-2, specific antibodies are produced in response to combat foreign species. In this process, the immunoglobulins that are primarily made include IgA, IgM, and IgG [5, 6]. Each of these antibodies varies in life span due to their conformation. In theory, any combination of the produced immunoglobulin could act as the analytes in the lateral flow assay to diagnose COVID-19 [6]. In the first few days of infection, the concentration of IgM would increase rapidly in the body [5]. Whereas IgG would take a slightly few more days to increase its concentration since the start of the incubation period, however, closely after the peak of IgM [5]. This nature of the immune response allows IgG and IgM to become easily detectable regarding SARS-CoV-2 once the symptoms start to appear.

In the IgG and IgM antibodies test kit, there will be a sample port, reaction, IgM test zone, IgG test zone, and control zone. Starting off with the reaction zone, there will be 2 types of molecules loosely attached to the test strip, gold COVID-19 antigen conjugate and gold rabbit IgG conjugate [7]. Then moving on to the IgM test zone, there will be anti-human IgM antibodies immobilized in the test strip. This is exactly the same in the IgG test zone, where it'll be anti-human IgG antibodies immobilized in the strip [7]. Finally, the control zone will contain immobilized anti-rabbit IgG antibodies [7]. When a sample of either blood or serum is loaded into the sample port [6, 7], the COVID-19 IgM antibody and COVID-19 IgG antibody in the sample will bind with the gold COVID-19 antigen conjugate. The location of binding in this antibody-antigen complex occurs at the fragment antigen binding (Fab) region of the antibody with a site on the antigen. Then, all molecules from the reaction zone will migrate toward the test zones and control zone by capillary action [6, 7]. At the IgM test zone, the antibody-antigen complex that has COVID-19 IgM would bind to the Fab region of the immobilized anti-human IM antibodies with its fragment crystallizable (Fc) region [5]. The same occurs to the COVID-19 IgG antibody-antigen complex at the IgG test zone, however binding to the immobilized anti-human IgG antibodies. To ensure the assay is working properly, the gold rabbit IgG conjugate from the reaction zone would bind to the Fab region of the anti-rabbit IgG antibodies at the control zone. If the patient is positive, then either two or three red bands will appear, one of which is the control zone [5, 7]. Whereas if the patient is negative, then only the control zone will have a red band [5, 7]. A point to add is the red colour band comes from the colloidal gold that is on the gold COVID-19 antigen conjugate and the gold rabbit IgG conjugate [3]. The red colour appears once the antibody-antigen complex binds to the immobilized anti-human IM and IgG antibodies, or the gold rabbit IgG conjugate binds to the immobilized anti-rabbit IgG antibodies.

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The strength of using IgM IgG immunochromatography is that it has high accessibility because it could be utilized in a variety of locations, whilst holding user-friendly and portable properties (POCT). This is succeeded by the nature of this diagnosis technique, where it will only require available samples and prepared test strips. In addition, regarding the mechanism of immunochromatography, the testing sample could be of various types, such as serum, blood, or oral fluids, therefore bringing higher flexibility to the diagnosis while keeping the diagnostic minimally non-invasive [8]. Another advantage of using both IgG and IgM antibodies as analytes could increase the detectable rate in patients from 94.1% to 100% [3, 8] after the symptoms have appeared. This is because the IgG and IM concentrations would increase during the acute phase of infecting SARS-CoV-2. In the study of Li et al., they designed the IgG and IgM combined LFIA that could be used for diagnosis at any stage of SARS-CoV-2 infection, as shown in Figure 1. This device had been tested by doing clinical trials on 525 patients by collecting their samples, where 397 of them are confirmed to have COVID-19 and the rest had tested negative at 8 or more different clinical sites [7]. After doing multiple trials with multiple different types of samples, such as serum and plasma, it has been concluded that the sensitivity and specificity of using IgG and IgM LFIA are 88.66% and 90.63% [7]. This result evaluates the combined LFIA has good sensitivity compared to single IgG or IgM assay. However, the limitations of this specific biosensing technique are the quantitative data is limited. Lateral flow assay provides qualitative results (positive or negative) because of the colloidal gold [3], but it could not directly provide precise quantitative information, such as the antibody concentrations. Knowing what the concentration of antibodies is in the body, would help to deduce the stage of infection [8]. In addition, because the IgG and IgM concentration would only increase as the symptom onset, it would be hard to avoid false negatives if the LFIA is taken before the symptoms have appeared [5].

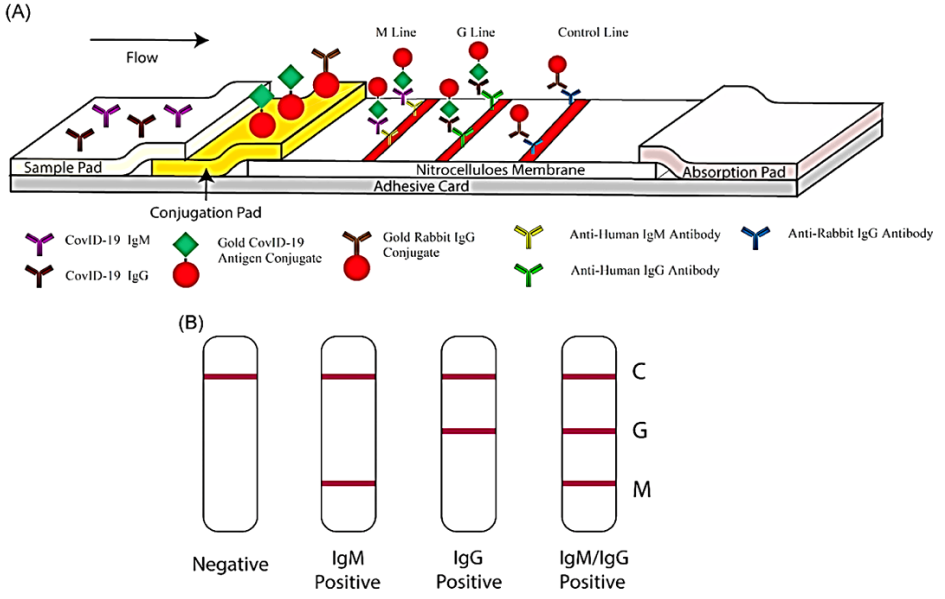

Figure 1. Principle of SARS-CoV-2 IgM-IgG combined antibody detection [7, https://doi.org/10.1002/jmv.25727]. (A) Illustration of the interaction of molecules that are present in the device during diagnostics; (B) The possible combination of a positive test and negative test.

1.2. CRISPR/Cas12 analysis

CRISPR is a widely utilized tool used for genome editing and is discovered in the 1980s [9]. CRISPR is an adaptive immune system found in bacteria or prokaryotes and archaea to protect itself from foreign, invasive genetic materials, and it is highly reliant on Cas proteins [9]. As technology develops, the CRISP Cas system has slowly integrated into the field of nucleic acids detection, and it could be broken into two major components. Cas endonuclease is used to break the target site of the gene, and then guide RNA is further used to analyze the Cas endonuclease to the target site [9]. However, there are different

DOI: 10.54254/2753-8818/23/20231065

types of Cas endonucleases which will have slightly different effects. For instance, Cas9 endonuclease has a very specific cleavage activity which brings high sensitivity and specificity in DNA detection and diagnostics [9]. Whereas, Cas12 endonuclease has a collateral cleavage activity that can produce a fluorescent signal as a result of nucleic acid detection [9].

The CRISPR Cas12 diagnosis technique was first developed by Chen et al. to detect the nucleic acids of human papillomavirus (HPV) in 2018 [10, 11], where the collateral activity of Cas12 has been discovered to have the potential to be used in diagnostic tools [10, 11]. For SARS-CoV-2 analysis with the CRISPR/Cas12 method, the first step is to prepare the sample by extracting and purifying the viral RNA from the patient by either using nasal swabs or saliva. Then, reverse transcriptase [12] is used to transcribe the viral RNA into complementary DNA (cDNA), followed by isothermal amplification of this cDNA. There are two types of isothermal amplification, one being recombinase polymerase amplification (RPA) and the other being loop-mediated isothermal amplification (LAMP). In this case, the LAMP is used [9, 12]. The amplification of cDNA will ensure minimal amounts of viral genetic materials could be detected. After the concentration of cDNA has increased, a single guide RNA (sgRNA) [11] that is specifically designed to recognize a unique sequence within the genome of SARS-CoV-2 is used to locate the sequence.

The sgRNA could be designed to target the gene that codes are used for the envelope (E gene) of SARS-CoV-2 [10], or it could target the gene which codes for the viral nucleoprotein (N gene) [10]. As soon as sgRNA binds to its complementary target sequence, Cas12 protein would be guided towards the sgRNA to bind with it [12]. This triggers a conformational change in the Cas12, stimulating the nonspecific endonuclease activity to be activated. The activated Cas12 will cut the target viral DNA and cleaves the nearby single-stranded DNA molecules that are nonspecific, and this process is the collateral cleavage activity [12]. To generate signals and collect data during the CRISPR/Cas system, a reporter molecule is added to the experiment, which could be fluorescence or a colorimetric probe [12]. When the probe is intact, it will emit signals that are fluorescence. The nature of this designed probe will cause a change in the fluorescence signal as the collateral cleavage activity of Cas12 cleaves the probe, resulting in a loss of fluorescence colour. This change in fluorescence signal could be analyzed to deduce the presence or absence of the SARS-CoV-2 viral DNA in the sample, which will indicate positive or negative for the patient.

The strength of using CRISP/Cas12-based diagnostic tests provides quick and rapid results, which can be used for point-of-care testing in different situations or areas, such as in an emergency room or remote areas. The CRISPR/Cas12 system could also be modified to recognize specific sequences in nucleic acids to reduce the odds of false positives when diagnosing SARS-CoV-2, as the genes for specific proteins will have unique sequences. However, a possible limitation of using the CRISPR system is that there are possibilities in the nonspecific binding of the guided RNA to the viral genome may cause the results to be misunderstood [9]. This could potentially alter the result of the diagnosis where false positives or negatives may occur. As shown in Figure 2, in the study of Cao et al., they used fluorescence and cis-trans cleavage mechanisms in the CRISPR/Cas12 system to analyze SARS-CoV-2 [12]. The limit of detection of the present method is 0.25 copies/L with the odds of diagnosing a false positive or negative to be decreased. In addition, it has been reported that the sensitivity and specificity are 100% when compared with the RT-qPCR [12], thus establishing very accurate diagnostic results. However, one of the challenges that were faced during this experiment was to balance the competition between the polymerase and the polymerase that was used for Cas protein as the same DNA template was used [12], which could potentially affect the efficiency of the test.

DOI: 10.54254/2753-8818/23/20231065

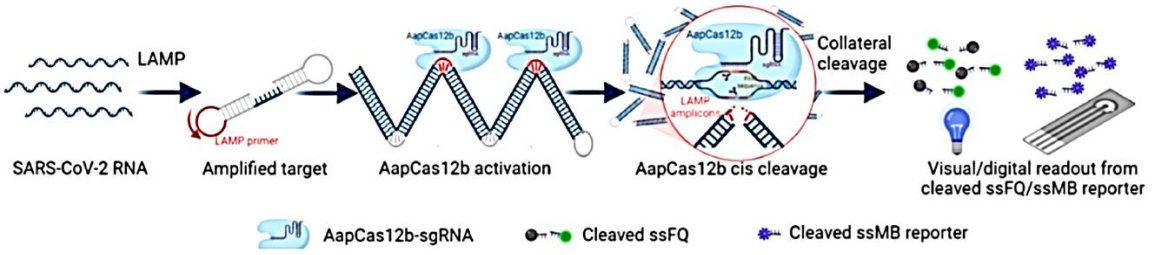

Figure 2. The steps of using CRISPR Cas12 in SARS-CoV-2 diagnosis. The RNA of SARS-CoV-2 is first amplified with the LAMP. The Cas protein will then be activated as it binds to the target section of the RNA (red region), which triggers the Cas protein to cleave the site. The ssFQ and ssMB could also be cleaved which will produce a fluorescence signal that could determine the sample to be positive or negative [12, https://doi.org/10.1016/j.bios.2023.115402].

1.3. QDs-based biosensing system

Nanotechnology is a common strategy that can be designed and developed into nanovaccines, nanobiosensors and antiviral delivery systems with their special physiochemical properties [13]. Scientists have also taken this advantageous property of nanomaterials to develop it into diagnostics tools that hold high sensitivity and high selectivity [13]. Moreover, the antiviral delivery system property of a diverse of different nanomaterials can be used for reducing side effects during diagnosis whilst improving selectivity [13]. As shown in Figure 3, in the case of SARS-CoV-2, scientists have integrated quantum dots (QDs) into a variety of diagnostic tactics, such as noninvasive visualization of respiratory viral infection [13]. The designed functionalized QDs can inhibit or disrupt the activities of the targeted virus [13], thus, has the ability to be used in diagnosis. One of the applications of QDs in SARS-Cov-2 diagnosis is QD-based biosensing by using Förster resonance energy transfer (FRET) [13]. This technique is reliant on the minute size of QDs and their amenable surfaces for it to contribute to the effectiveness of the QDs-based biosensing technique [13].

The first step of this system is to functionalize the QDs with ligands or antibodies that can selectively bind to the SARS-CoV-2 viral component, such as the spike protein. These functional groups on the surface of QDs facilitate the recognition of specific target analytes. It is also important to point out that functionalized QDs are excited and will emit energy that creates fluorescence light. However, when the functionalized QDs are exposed to ACE2-conjugated gold nanoparticles (Au NPs) under close proximity, the energy emitted by QDs will be transferred to the ACE2-conjugated Au NP through FRET [14]. This is because QDs are donor molecules while Au NPs serve as the acceptor molecule, forming the donor-acceptor pair [14-16]. The transfer of energy from QDs to ACE2-conjugated Au NPs will quench the fluorescence light that is emitted by QDs [13] and how quenched the fluorescence light is determined by the presence of analyte which influe`nces the distance and orientation [14] between QDs and Au NPs. Therefore, if the SARS-CoV-2 viral component (analyte) is present in the sample that is tested, it can specifically bind to the functionalized QDs. This will disrupt the proximity between the QDs and ACE2-conjugated Au NP, resulting in a less efficient FRET between the two molecules, leading to the recovery of QDs fluorescence [13]. The fluorescence activity and fluctuations [15] occurring in the system can be analyzed by using a fluorescence spectrometer or fluorescence microscope. The relationship between the degree of fluorescence recovery and the concentration of the target analyte is proportional, and thus could be used to diagnose SARS-CoV-2.

The strength of using QDs in diagnosis is it could develop and design a nanosystem that is effective and promising. This is because QDs have low toxicity and high surface performance compared to other nanomaterials [13, 14]. Therefore, it is safe to deal with QDs during diagnosis and could interact with SARS-CoV-2 analytes adequately. In addition, functionalized QDs could have high functionality which guarantees high selectivity and stability in SARS-CoV-2 diagnostics. For instance, in the study of Garshkov et al., they demonstrated` QDs to have a high sensitivity while interacting with the SARS-CoV-2 target DNA under the FRET-based biosensor system [15] as they investigated the interaction

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between nanoparticles coupled to the ACE2 receptor (AuNP-ACE2) and QDs coupled to the recombinant spike receptor binding domain (QD-RBD) [15]. The QDs-based biosensing system also provides both qualitative and quantitative information because of the fluorescence [15], helping in analyzing the concentration of t`arget analyte from SARS-CoV-2 which could deduce how infectious the patient is. However, there are also limitations in this particular diagnostic system. For instance, for a successful FRET to occur, the distance between the donor and acceptor molecule is essential, preferably to be equal or less than 10nm [16]. Therefore, not all energy transfers between the functionalized QDs and ACE2-conjugated Au NP might be efficient enough to produce a detectable signal [16].

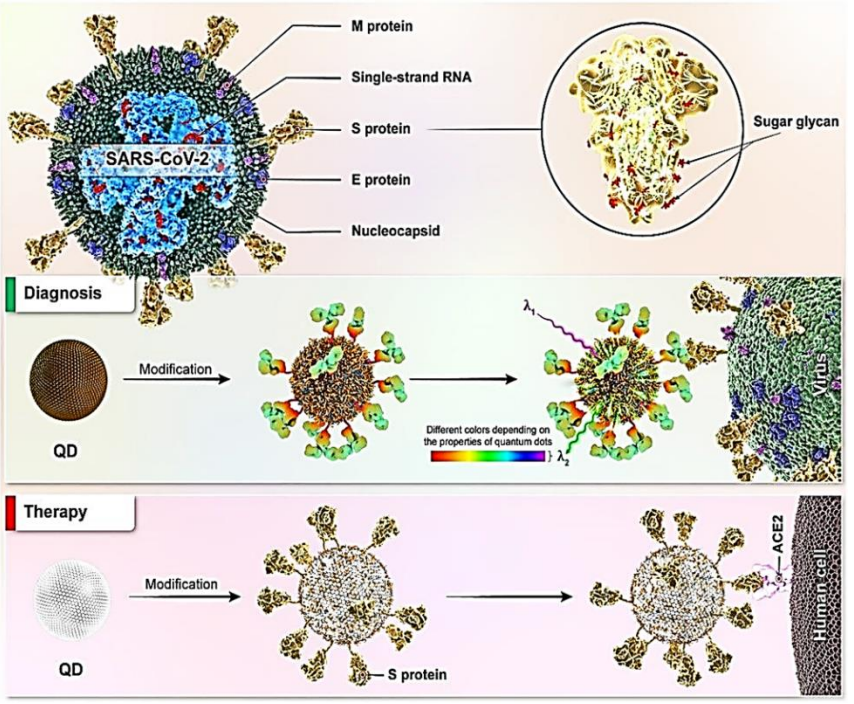

Figure 3. Labeled diagram of SARS-CoV-2 and potential of using QDs in the diagnostics of COVID-19; Diagnosis: The QD needs to be first modified into a functionalized QD in order for it to interact with the analyte of the virus. During their interaction, it triggers the FRET-based biosensing system, which will either emit fluorescence or cause the fluorescence to be quenched [13, https://doi.org/10.1002/jctb.7036].

2. Conclusion and outlook

The nature of SARS-CoV-2 is highly contagious, causing others to be easily infected under close contact with infectors, and this results in a global pandemic. To effectively control the spread of disease in the community, a good diagnosis technique needs to be done to lower the odds of sick people contacting healthy individuals. In terms of a diagnostic technique that uses biosensors with high sensitivity and selectivity, the three biosensing techniques that are introduced in this paper are LFIA, CRISPR/Cas12, and QDs-based biosensing system. Each of these diagnostic techniques has its own strengths and weaknesses. For instance, immunochromatography lateral flow assays are portable and can be done by anyone by following the steps written on the packet. However, the collected results are qualitative rather than quantitative, which does not establish the concentration of viral load in the patient. Whereas quantum dot and CRISPR/Cas12 could analyze the SARS-CoV-2 concentration in the sample through the fluorescence signal that is given off. Yet, these diagnostic techniques would be slightly more time-consuming when compared with lateral flow assay which only takes minutes.

The limitation of this paper in investigating the steps of SARS-CoV-2 diagnostics is that some of the strengths or weaknesses of the biosensor were difficult to find, while the mechanism was also uneasy to

DOI: 10.54254/2753-8818/23/20231065

research. This is because many of the papers do not provide a very straightforward answer to the steps on how the biosensors work to diagnose the presence of SARS-CoV-2. Therefore, the understanding of how the three different diagnostics may not be entirely accurate, which isn't very authentic. However, the information is based on recent publications, which indicates the information provided in this paper is not entirely out of date.

Although many SARS-CoV-2 diagnostics have been developed since the outbreak of the pandemic, where improved versions of the current existing biosensors are also invented to better adapt the selectivity and sensitivity of the analytes against SARS-CoV-2. However, there are also other pathogens that could cause similar respiratory pneumonia, such as the influenza virus. Therefore, it would be effective in developing multiplex testing, where there will be a simultaneous detection of other respiratory pathogens while detecting SARS-CoV-2. This could differentiate COVID-19 from other diseases that have similar symptoms when a patient is infected by SARS-CoV-2. Despite the fact that there are already some SARS-CoV-2 multiplex testing produced, however, there are still limitations. For instance, if the SARS-CoV-2 virus concentration in the sample exceeds a certain amount, then it could inhibit the detection of influenza and result in a false negative for the influenza test. Therefore, it is important to have developed a COVID-Flu multiplex assay kit that could overcome this limitation.

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