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Literature Review

Amplification genetic engineering strategy by Crispr-Cas 13 Enzymes for detection and treatment COVID-19 mediated with gold nanoparticle (AUNP)

Salsabila Farah Rafidah[1] Ilham Rahmanto[2], Ardellia Bertha Pratiska[2]

1) Faculty of Public Health, Airlangga University, Indonesia
2) Faculty of Medicine, Airlangga University, Indonesia

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Correspondence:

ns.firman92@gmail.com

ABSTRACT

WHO declared the disease outbreak due to the COVID-19 coronavirus a global pandemic. Indonesian government's efforts to eradicate the pandemic through mass screening have not been effective due to the limitations of the three main modalities used to detect COVID-19, including Rapid Test Diagnostic (RTD) antibodies, RTD antigens, and Reverse Transcriptase-Polymerase Chain Reaction (RT-RTD PCR), In addition, other detection tools are sometimes used, such as Enzyme-Linked Immunosorbent Assay (ELISA) and rapid molecular tests. To eradicate this pandemic, the government needs COVID-19 detection tools that are effective, cheap, fast, and accessible. To determine the application of the genetic engineering strategy of amplification by the CRISPR-cas13 enzyme for detecting and treating COVID-19 mediated by gold nanoparticles (AuNP). This research uses a qualitative literature study with content analysis, observation development, and literature study; an alternative solution to this problem is CRISPR-Cas13, achieved by the SHERLOCK method. This method designs and screens a targeted group of CRISPR RNAs based on the identification of functional crRNAs of SARS-CoV-2. Amplification of CRISPR-Cas13 by SHERLOCK and PAC-MAN enzymes has the potential to be the latest detection and treatment method for gold nanoparticle-mediated COVID-19 (AuNP) in Indonesia.

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INTRODUCTION

On March 11, 2020, WHO (World Health Organization) declared the disease outbreak due to the COVID-19 coronavirus a global pandemic. The virus is a contagious disease caused by *SARS-CoV-2*, one of the newly discovered types of coronavirus. In its development, the COVID-19 disease outbreak, which first occurred in Wuhan, China, from December 2019 to April 2020, has spread to 210 countries and reached 2.7 million positive cases (Worldometers 2020). With the characteristics of its very rapid spread between humans, coupled with very high human mobility and across national borders, this virus becomes even more dangerous. Based on data from Satgas Covid (2021) at covid19.go.id, the total positive COVID-19 in Indonesia on the last update on May 3, 2021, was 1.682.004 people, and the mortality case was 1.535.491. Detection of COVID-19 can be carried out by anamnesis, physical examination, and supporting examinations. Clinical symptoms can vary depending on the degree of disease. Therefore, a significant step is needed for massive, effective, comprehensive, and sensitive diagnostics and treatment to eradicate the COVID-19 pandemic (Handayani et al, 2002).

The Indonesian government's efforts to eradicate the pandemic through mass screening have not been effective due to the limitation of the three main modalities used to detect COVID-19, including antibody Rapid Test Diagnostic (RTD), Antigen RTD, and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) (Yanti, Ismida, and Sarah 2020). Therefore, other detection tools, such as Enzyme-linked Immunosorbent Assay (ELISA) and rapid molecular test, are sometimes used. These modalities have their respective advantages and disadvantages. Although RT-PCR is the gold standard, this modality has several limitations, such as being time-consuming, requiring a very sophisticated thermolysis machine, and skilled laboratory assistants (Mustafa and Makhawi., 2021). On the other hand, the urgency for rapid detection is very high. However, antibody RTD and antigen RTD are less effective to use because there are still high false positive and false negative rates, so they must be confirmed using RT-PCR (Wahjudi., 2020). To eradicate this pandemic, the government needs a COVID-19 detection tool that is effective, cheap, fast, and easy to use (does not require special skills).

Therefore, no evidence recommends an effective anti-COVID-19 drug. The pharmacological management of COVID-19 as of April 2020 in Indonesia uses chloroquine, hydroxychloroquine, favipiravir, and remdesivir (Instiaty et al., 2020). Clinical studies limited to these four drugs demonstrated some efficacy of COVID-19 treatment with tolerable side effects. Potential serious side effects occur with chloroquine and hydroxychloroquine in the form of cardiac arrhythmias. Based on a clinical pharmacological review, the decision to use these drugs should consider the potential benefits and the risks to the patient (Instiaty et al., 2020). Other considerations in using these drugs include effectiveness, safety, availability, and accessibility (relatively cheap); until now, researchers are still looking for new drug candidates that can treat COVID-19 effectively and comprehensively.

The use of vaccines launched by the government to control this pandemic is only designed for disease prevention, and their effectiveness is still uncertain (Ophinni et al., 2020). Based on their content, several COVID-19 vaccine models exist, including mRNA vaccine models, inactivated viruses, virus vectors, and protein subunits. The Indonesian government chose Coronavac, made by a Chinese company, Sinovac, an inactive virus containing the protein S (Spike) antigen from *SARS-CoV-2* (Ophinni et al., 2020). This vaccine has been

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tested on at least 30.000 participants in several countries, such as Indonesia, Turkey, Brazil, and Chile, to assess its effectiveness. However, vaccination in Indonesia needs to reach at least 67% of the country's population to achieve herd immunity to stop the pandemic. This will take a long time, considering that Indonesia is the fourth most populous country in the world. In addition, vaccines can only prevent disease manifestations by creating antibodies and not curing the disease (Ophinni et al., 2020).

On the other side, genetic and nano-based diagnosis and treatment are widely developed by health analysis; one of the most accurate is CRISPR-Cas13. The implementation of this technology in the form of SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing), which integrates with Cas13 to detect single-molecule RNA (Gootenberg et al. 2017) by quantitative and accurate input measurement up to 1uL (2 aM), 3.5 times more precise signal sensitivity, multiplex, portable, fast, visual nucleic acid detection platform, and can distinguish different inputs by single nucleotides at low concentrations (Myhrvold et al., 2018).

PAC-MAN (CRISPR Antiviral Prophylaxis in human cells). This method performs viral inhibition that can effectively degrade the *SARS-CoV-2* sequence in human lung epithelial cells by antivirus modification which will divide up more than 90%. Gold nanoparticles (AuNPs) are also used as a lipid inhalation CRISPR RNA (crRNA) to target lung cells precisely. AuNP has stable properties, high sensitivity, and good bio capability to facilitate PAC-MAN placement to pulmonary epithelial cells. The purpose of this literature review is to analyze the potential of CRISPR-Cas 13 and gold nanoparticles as methods of detection and treatment of Covid-19 and as an effort to inform readers about the latest methods based on eradication technology in the form of detection and treatment for *SARS-CoV-2* (Abbott et al., 2020).

LITERATURE REVIEW

Genetic engineering CRISPR-Cas13 has the potential to become the latest technology in detecting and treating COVID-19. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are defense mechanisms owned by bacteria to cleavage foreign genetic material such as from plasmids and viruses by creating specific RNA guides. Meanwhile, CRISPR-associated 13 (Cas13) isolated from the *Leptotrichia wades* bacteria plays a role in cutting off the foreign genetics (Mustafa and Makhawi 2021; Shihong Gao, Zhu, and Lu 2021). Several studies have proven that Cas13 can be used as a new diagnostic tool for COVID-19 that is no less sensitive and specific than RT-PCR called SHERLOCK (Patchsung et al., 2020). Therefore, researchers are developing PAC-MAN, which utilizes Cas13 so that it can be used as a new step in the treatment of COVID-19 in the form of aerosol drugs (Lotfi and Rezaei., 2020). As the transporters, SHERLOCK and PAC-MAN are combined with gold nanoparticles (AuNPs) which have stable properties for diagnostics and treatment of COVID-19 (Draz and Shafiee., 2018). It is predicted that this genetic engineering technique can be used to eradicate this prolonged pandemic in Indonesia.

The Mechanism of SHERLOCK for Detecting COVID-19

For detecting COVID-19, Cas13 in SHERLOCK is very specific and sensitive in detecting single-stranded RNA (ssRNA), such as in the *SARS-CoV-2* virus, compared to DNA. When the target viral RNA is detected, CRISPR RNA (crRNA) will immediately recognize and guide Cas13 to a suitable spacer and the base pair with the complementary sequence in the target viral RNA. Then, Cas13 will cleavage the distal part to the target RNA sequence paired with a spacer (Shihong Gao

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et al., 2021). Cas13 will also collaterally cleavage the RNA sensor to relieve the fluorescence color, indicating the virus's presence in the sample **(Figure 1)** (Shihong Gao et al., 2021). During its development, the SHERLOCK modality is divided into two forms: fluorescence assay readout and lateral flow strip readout. Compared to the fluorescence assay readout, lateral flow strip readout has several advantages because it uses visual colorimetric readout in commercial lateral flow, does not require special tools without purifying and isolating nucleic acid, and is highly sensitive for multiplex signal detection on lateral flow strips, making it more effective to use as a candidate for the latest COVID-19 detection tool in Indonesia (Mustafa & Makhawi., 2021).

The Mechanism of Lateral Flow Strip Readout and Gold Nanoparticles for Detecting COVID-19

The commercial lateral flow strip as a mediator for Cas13 can be used for single target detection. Simply put, the detection reaction in SHERLOCK is applied to a portable strip using a fluorescein (FAM)-biotin RNA sensor (Shihong Gao et al., 2021). The full-length RNA sensor will accumulate in the first streptavidin line (first band) if a negative result is obtained. A band is visualized because anti-FAM gold nanoparticle-conjugated antibodies were added for the detection reaction. Meanwhile, a positive test result indicates that the sensor RNA has been cleavage. The FAM antibodies can flow down the strip to bind to the second band and provide visualization **(Figure 2)** (Shihong Gao et al., 2021). In other words, the results of this test are as accessible and straightforward as a pregnancy test. AuNPs were chosen due to their stability, biocompatible, and unique optical properties, as well as their ability to produce colorimetric signals that can be seen in the test line (Patchsung et al., 2020).

Research Evidence of SHERLOCK as A New Method for Detecting COVID-19

The use of SHERLOCK CRISPR-Cas13 has been approved by the food and drug administration (FDA). It effectively detects the S (Spike) gene in the *SARS-CoV-2* virus (Lotfi and Rezaei, 2020; Patchsung et al., 2020). Research conducted by (Patchsung et al. 2020) using a lateral flow strip readout on nasopharyngeal and throat swab samples collected in 154 patients in Thailand showed a specificity of 100% and a sensitivity of 88% compared to RT-PCR. More than that, the limit of detection (LOD) reached 100% specificity and 97% sensitivity with the lateral flow readout. They also developed a lateral flow strip that can detect foreign agent contamination using AuNPs. Research by (Myhrvold et al., 2018) showed that SHERLOCK is also proven to be effective in detecting *Zika Virus* (ZIKV) and *Dengue Virus* (DENV). Therefore, researchers have also created SHERLOCKv2, which offers a sensitivity of up to 3.5 times with the addition of Csm6 (supporting type III CRISPR effector nuclease) (Mustafa and Makhawi., 2021). This method can also detect a single mutation in the virus and provide detection results in about one hour. This evidence shows that this method can be a breakthrough in virus detection tools, especially the *SARS-CoV-2* virus (Lotfi and Rezaei, 2020; Wang et al., 2021).

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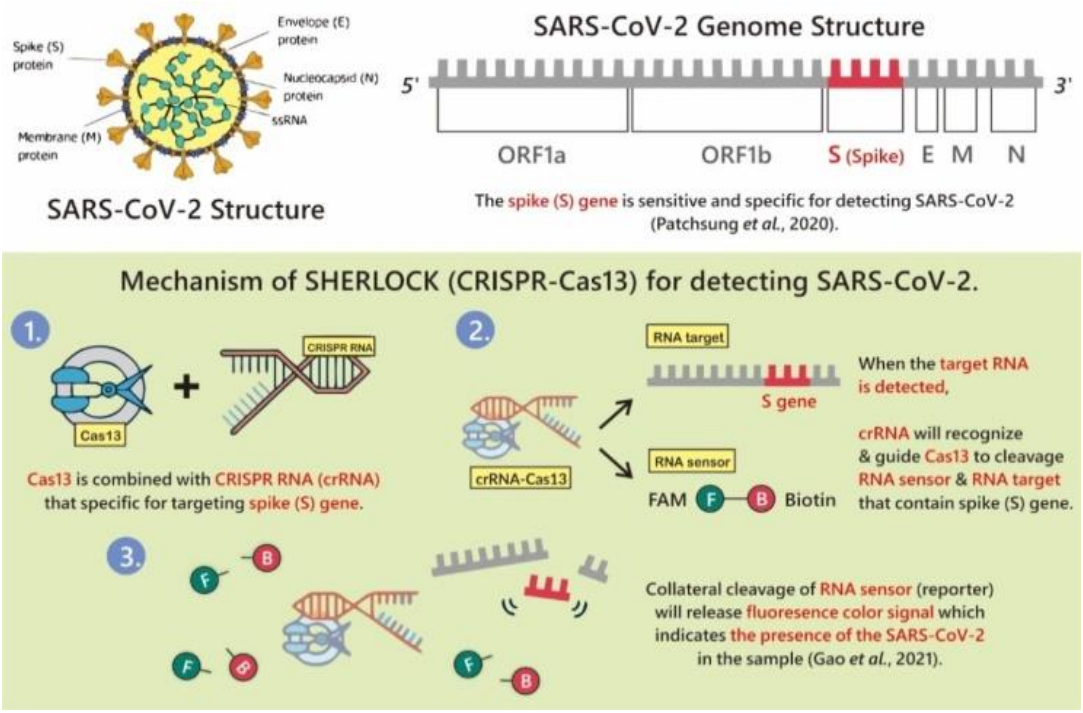

Figure 1. The *SARS-CoV-2* Structure & Genome Structure, and The Mechanism of SHERLOCK (CRISPR-Cas13) for Detecting *SARS-CoV-2*. (Author's Illustration, 2021)

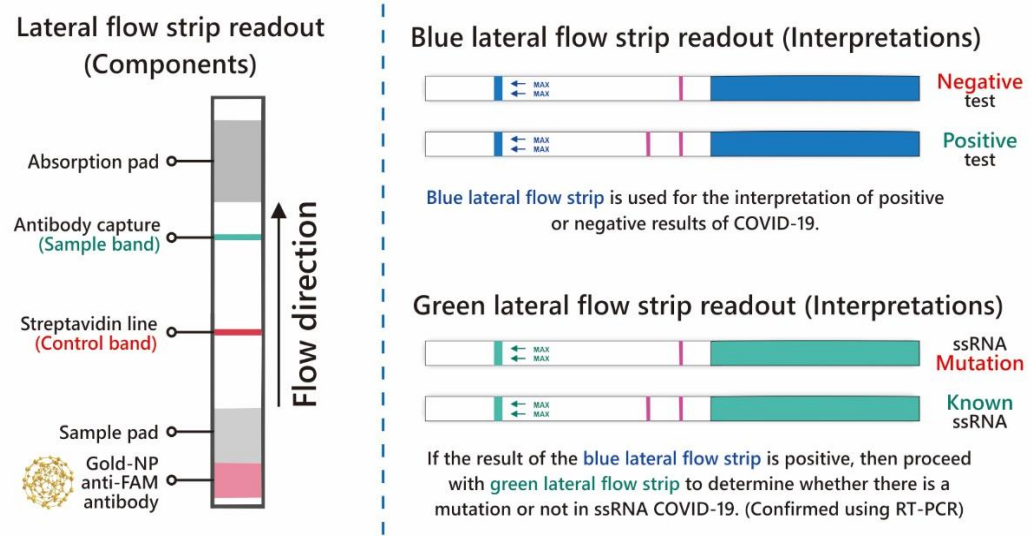

Figure 2. The Interpretations of Blue & Green Lateral Flow Strip Readouts (Patchsung et al., 2020).

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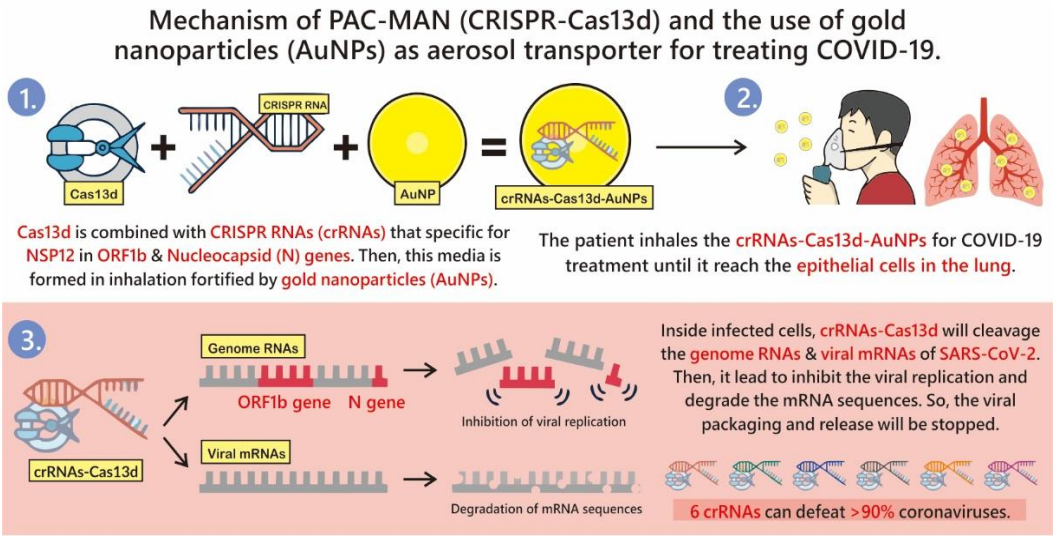

Figure 3. The Mechanism of PAC-MAN (CRISPR-Cas13d) and The Use of Gold Nanoparticles (AuNPs) as Aerosol Transporter for Treating COVID-19 (Author's Illustration, 2021).

Mechanism of CRISPR-Cas13 in the Treatment of COVID-19 (PAC-MAN) and The Use of Gold Nanoparticles for Aerosol Transporter

PAC-MAN (CRISPR Antiviral Prophylaxis in huMAN cells) performs viral inhibition by degrading the *SARS-CoV-2* sequence in human lung epithelial cells. This method designs and screens a group of targeted CRISPR RNA (crRNA) based on the identification results of the *SARS-CoV-2* cleavage functional crRNA (Abbott et al., 2020). This potential PAC-MAN approach was developed through the Cas13 enzyme in the form of collateral RNase activity and targeting viral nuclease in the following sequence.

The mechanism of PAC-MAN starts from the preparation of tools and materials, such as Cas13d (the smallest protein similar to Cas13 but has a strong target cleavage activity). Furthermore, these mechanisms are shown in **Figure 3**. The cells were transfected with gold nanoparticles (AuNP) until they reached the lung epithelial cells as the target cell. Integration with gold nanoparticles as an inhalation nanocarrier for local treatment is promising. Therefore, it has been widely regarded as candidate material.

Research Evidence of PAC-MAN as a New Method for Treating COVID-19

Based on a study of Scnn1b transgenic (Tg) mice treated with this treatment for 2 hours, inhaled AuNP rapidly binds to the alveolar epithelium. Therefore, epithelial targeting is required. It can be concluded that this is a further improvement, i.e., the PACMAN method corresponding to the defined crRNA and that this strategy for therapeutic targeting of epithelial cells is very successful. This method has been investigated and analyzed in various studies (Geiser et al., 2013; Yan et al., 2018). Geiser and Yan found that cas13d had the best strength in the cleavage process and was small in size, continued by Abbott et al. (2020) that the entire *SARS-CoV-2* genome of patients was sequenced based on the level of conservation found in RNA-dependent RNA polymerase (RdRP) and nucleocapsid (N) genes, (Metsky et al., 2020) also added in the results. The research recommends that the simpler approach is only one crRNA sequence

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instead of 2, especially in targeting *SARS-CoV-2* RNA.

Research Evidence of Gold Nanoparticles (AuNPs) as Transporter of CRISPR-Cas13 for Detecting and Treating COVID-19

Gold nanoparticles (AuNPs) have advantages over many other nanoparticles in chemicals. AuNP can form stable chemical bonds with groups containing S and N. This allows AuNP to attach to various organic ligands or polymers with specific functions. AuNP has a large role in eradicating a disease, especially diseases of the lungs. AuNP material in aerosol form has significant potential as a drug delivery system to treat COVID-19 due to its high stability, carrying capacity, and ability to provide water-soluble and insoluble drugs (Gelperina et al., in Anderson et al., 2020). Direct administration to the lung tissue also has the advantage of requiring a lower dose, minimizing side effects, and requiring less administration, which can result in better patient adherence (Rojanarat et al., 2012).

The Advantages of CRISPR-Cas13 as a New Method for Detecting and Treating COVID-19

SHERLOCK on the lateral flow strip readout provides convenience in terms of the detection of COVID-19 (Mustafa and Makhawi, 2021). This method is no less specific and sensitive than RT-PCR as the gold standard. Following WHO criteria regarding a good detection tool, lateral flow strips have several advantages: rapid, specific, sensitive, instrument-free, and cost-effective. PAC-MAN CRISPR-Cas13 offers advantages such as being easy to use, more cost-effective, sensitive, and specific than other therapeutic methods (Lotfi and Rezaei., 2020). This modality has minimal off-target effects on the host transcriptome in mammalian cells, thereby preventing unwanted and unpredicted mutations. The combination of PAC-MAN with transporter AuNP by aerosol (as nebulizer) can be a solution to the latest COVID-19 management in Indonesia. In other words, developing the SHERLOCK lateral flow strip readout and the PAC-MAN nebulizer can be a step forward for Indonesia in eradicating the COVID-19 pandemic.

CONCLUSION

The alternative solution to Covid-19 problem is CRISPR-Cas13, achieved by the SHERLOCK method, a proven detection method for detecting viruses. Next is PAC-MAN, which performs viral inhibition by degrading, integrated with gold nanoparticles (AuNPs) located at the tip of the biotin fluorescein (FAM) RNA reporter as a conductor and stabilizing reagent for the SARS-CoV-2 sequence in human lung epithelial cells. This method designs and screens a targeted group of CRISPR RNAs based on the identification of functional crRNAs of SARS-CoV-2 cleavage. As a result, the amplification of CRISPR-Cas13 by SHERLOCK and PAC-MAN enzymes has the potential to become the latest detection and treatment method for gold nanoparticle-mediated COVID-19 (AuNP) in Indonesia.

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