Application And Comparative Analysis In SARS-Cov2 by PCR And CRISPR/Cas9

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Abstract. COVID-19, a global large-scale epidemic caused by the novel coronavirus, is detrimental to people's mental and physical health, and it also destruct the economic environment. In the process of the study of COVID-19, fast and accurate detective tools are necessary. There are plenty of clinical and laboratorial methods for detection. Nevertheless, due to various of realistic limitations, these methods cannot be fully applied clinically. This paper reviewed the some kinds of current polymerase chain reaction (PCR) technologies and the latest CRISPR/Cas9 in the detection of SARS-Cov2, compared the differences among these methods, and summarized the major achievements in cutting edge and limitations. In order to provide new ways to diagnose and cure the disease efficiently, and help doctors and researchers to investigate the complex pandemic and control the diffusion. PCR technology is the golden standard in the field of detecting COVID-19, while the high false negative rate and high requirements need to be improved. Many detective methods have been developed based on the CRISPR/Cas9 technology, each has its advantages, but also limited. In the future, the existing technology will be improved to explore the methods of mutual reference and combination between technologies, and find ways to improve the diagnostic efficiency and help clinical application, so as to provide help for the management of COVID-19 infection.

Keywords: SARS-Cov2, PCR, CRISPR/Cas9, Nucleic Acid Testing.

1. Introduction

COVID-19 is a worldwide epidemic disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-Cov2). As to 8 December 2022, the number of confirmed cases has reached 642379243, while the deaths has exceeded 6.6 million [1]. However, in November 2002, it reported an unexplained outbreak of cluster pneumonia which known as SARS-Cov in Guangdong Province. In the next year, SARS cases increased significantly in China and subsequently spread all over the world. It infected at least 8,096 people and killed 774 people at that time [2].

At present, researchers have developed multiple of new diacrisis methods for COVID-19. PCR and relative technologies derived from PCR have the advantages of high specificity and accurate results. In addition, RT-PCR is the most accurate in diagnosis, but it is also limited by the rigor requirements to technicians so it could hardly serve as detection measures at every given place or time. The CRISPR/Cas9 system can perform rapidly, efficiently, visibly in targets timely and with high specificity in SARS-Cov2 diascisis. However, the given researches showed that the sensitivity of CRISPR/Cas9 was evaluated differently. As this technology is still in developing stage, so there are few applications in clinical area. One of the reasons of the alarming number of infections and deaths in COVID-19 is the difficulty of ensuring adequate medical devices and diagnosis measures [3]. At present, researchers are looking for ways to diagnose and treat SARS-Cov2 because of the rapid development of public health and medical technology.

Real-time PCR belongs to the second generation of PCR which can detect nucleic acids quantitatively, and it is one of the earliest detection technologies [4]. Now, RT-PCR is still the golden standard in diagnosing COVID-19. However, the process of RT-PCR is more complex and with high cost, harsh requirements for technicians. Therefore, it may be limited in detection range in some less

developed areas. In addition, it requires about 4~6 hours to output the results, generally needs about 3.4~4.5 copies per milliliter [5,6]. These factors are not suitable in rural areas with inadequate resources. As for a pandemic like SARS-Cov2, it is necessary to find an approach to get the diagnose result faster and more precise. Droplet digital PCR (dd PCR) is the most commonly used methods in the third generation of PCR which emerged in recent years. Compared with traditional RT-PCR, it calculates the copy number in the template, instead of relying on the standard curve and Ct value. Therefore, the results are more precise, faster and absolute quantification. At present, dd PCR has been used in the detection of various of virus such as HIV, etc. [5]. The discovery of CRISPR/Cas9 system provides new ideas to COVID-19 testing. With the in-depth research, the advantages of flexible, accurate and low-cost have great potentials in diagnosing and applications. Scientists are now digging out the potency [7]. According to current researches, CRISPR/Cas9 can reduce detection cost and elevate detection sensitivity significantly [8]. This essay introduced existing technologies like PCR and CRISPR/Cas9 respectively, summarized them, and explained their developments and applications in SARS-Cov2 diagnose. Some horizontal comparisons were made to explore the methods of mutual references and combinations among these technologies, and tried to find ways to improve diagnostic efficiency to help clinical application, in order to offer help to the control of COVID-19 epidemic.

2. PCR Technique

A variety of neo-coronavirus nucleic acid detection methods have emerged both domestically and internationally, with PCR being the dominant method. Based on the basic PCR process, researchers have made improvements and integration with other techniques.

2.1. Real-time Quantitative PCR

Real-time quantitative PCR(RT-qPCR) is a second-generation PCR technique for the quantification of nucleic acids. It is a technique derived from the classical PCR technique and was the first technique used for the diacrisis of SARS-Cov-2. The fluorescent dye technique is based on the principle that a fluorescent dye that binds non-specifically to the double-stranded nucleic acid is added to the reaction system, and with each cycle the dye binds to the new strand generated by amplification and fluoresces. The presence or absence of the target nucleic acid molecule can be determined by the number of amplification cycles (Ct value) required for the fluorescence signal to reach a predetermined fluorescence threshold. COVID-19 is an RNA virus which contains a single-stranded RNA as genetic material. First, reverse transcriptase catalysis the viral RNA reverse transcribing into cDNA, and then the cDNA is amplified by DNA polymerase. Based on the Ct value of the amplification curve, it can be determined whether the sample contains COVID-19 nucleic acid and thus whether the sample contains COVID-19 virus. The criteria recommended by the National Health Council of China are: Ct value <37 is reported as positive; no Ct value or Ct ≥40 is regarded as negative results; Ct value between 37 and 40 is repeated [4].

2.2. Droplet Digital PCR

Droplet digital PCR (ddPCR) is the third generation PCR technique with high accuracy and sensitivity for absolute quantification of targets, and is also used in the diagnosis of SARS-CoV2 now. ddPCR analyzes the signal generated by each "single molecule amplification" of the template. The more droplets and the smaller the size of the droplets generated during sample dispersion, the lower the detection limit of ddPCR and the higher the sensitivity. It has been shown that ddPCR can correct the results of RT-PCR assays and further improve the accuracy rate of nucleic acid detection [4].

Prior to amplification, the sample is microtitrated by being divided into thousands of nanoliter-sized microdroplets, each of which contains either none or one to several of the target nucleic acid molecules to be analyzed. Following PCR amplification, each droplet is individually analyzed. If there is a fluorescence signal, it is viewed as 1, and if not, it is regarded as 0. Instead of using the Ct

value, the beginning copy number or concentration of the target molecule can be calculated using the Poisson distribution principle and the quantity and proportion of positive droplets. This approach, unlike RT-qPCR, does not rely on standards to accomplish absolute quantification, nor does it rely on Ct values or internal reference genes to establish the absolute quantity of target molecules to be detected down to single copy, assuring accurate findings [9,10].

2.3. Multiplex PCR

The multiplex PCR technique has been developed based on conventional PCR. This is a nucleic acid detection technique that allows simultaneous detection of multiple pathogens. By adding primers and fluorescent probes specific for multiple pathogens in a single tube, the genomic fragments of multiple pathogens present in the system can be amplified and detected simultaneously, increasing the throughput of the targets to be tested compared to single-target PCR. Multiplex PCR relies on the principle of melting curve by adding primers and corresponding fluorescent probes for multiple targets in the same reaction system. If there is a target complementary to a probe in the system, the probe will not generate a melting curve after amplification because it is consumed. Multiplex PCR breaks the limitation of detecting only one pathogen at a time, improves the speed of clinical diagnosis, and is used for the combined detection of multiple respiratory pathogens, which has long-term guiding significance for infection-like disease surveillance and triage treatment [4].

3. CRISPR/Cas9 Technique

However, many additional tests have been developed since RT-qPCR is expensive, needs highly skilled testers, and demands expensive testing equipment. One of the newest technologies is CRISPR/Cas9, which allows for gene editing. The RNA-guided Cas nucleic acid endonuclease and the CRISPR-RNA (Cr-RNA) carrying the complementary sequence are able to identify the RNA transcript containing the target sequence. When target RNA is present, the creation of a tertiary Cas-CrNA-RNA-transcripts complex activates collateral cleavage activity, resulting in the appearance of fluorescence signals (As shown in figure 1. CRISPR-based nucleic acid detection) [9]. For DNA recognition and ligation of gold nanoparticles (AuNP)-DNA probes, Cas9/sgRNA can be employed. Specifically, a position for the bingding for the recruitment of AuNP-DNA probes is present in the scaffold sequence in the sgRNA loop region. The AuNP-DNA probe attach to the sgRNA and build up on the testing line when the Cas9/sgRNA complex specifically recognizes the biotinylated amplicon.With the naked eye, it is possible to see this auNP-DNA signal transduction probe deposition. This ensures that the arduous nucleic acid hybridization step is avoided, and specificity is increased [5].

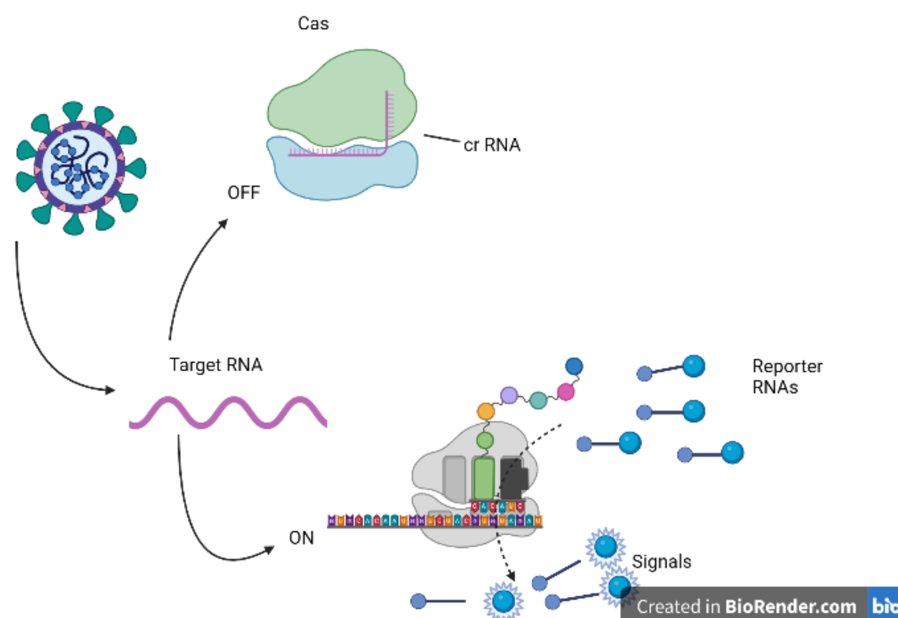

Fig. 1 CRISPR-based nucleic acid detection

Currently, based on CRISPR/Cas9 gene editing technology, researchers have developed several new coronavirus detection methods that have yielded very significant results.

One strategy aimed to integrate the CRISPR/Cas9 system with VirD2 relaxase. VirD2dCas9 complexes were created by combining inactive Cas9 nuclease and VirD2 relaxase to create engineered nuclease-deficient Cas9 (dCas9). Lateral flowmetry assay (LFA) is utilized in conjunction with reverse transcription with isothermal nucleic acid amplification of SARS-CoV-2 for nucleic acid detection. The Vigilant(VirD2-dCas9 guided and LFA-coupled nucleic acid test) assay method is used for this . Extremely precise, the sensitivity can reach 2.5 copies per μL [11].

Combining the CRISPR/Cas9 system with recombinase polymerase (RPA) technology is another approach. This is a multiplex reverse transcription-recombinase polymerase (RT-RPA) amplification-mediated trilineage lateral flow assay (TL-LFA) enabling quick duplicate gene identification in a single band experiment. In a single test, the approach simultaneously identifies the SARS-CoV-2 RNA envelope gene and the open reading frame 1ab(Orf1ab) gene in cell culture. For a 25 μL system, the sensitivity is 100 RNA copies per reaction. Such a dual gene test is crucial for increasing the assay's precision and is anticipated to offer a more precise and practical technique to identify SARS-CoV-2 or other infectious illnesses in locations with limited resources [5].

In the Type II system, a trans-activated crRNA (tracrRNA) hybridizes with crRNAs to trigger their processing and utilization by the help of Cas9. The CRISPR-Cas system employs crRNAs to detect foreign genetic material. Researchers discovered that tracrRNAs hybridize with cellular RNAs to create atypical crRNAs by studying the Cas9-RNA complex of Campylobacter jejuni. Based on this, scientists created LEOPARD (leveraging engineered tracrRNAs and on-target DNAs for parallel RNA detection). The LEOPARD diagnostic technology can differentiate SARS-CoV-2 virus and its D614G mutation in patient samples with single-base resolution and concurrently detect RNA from many viruses in a single test [12].

4. Comparison and Discussion

Crispr/Cas9 technology is mainly used in gene editing, mechanisms of disease researching, animal models establishing, new drugs screening and virus researching. Das reported that the Crispr/Cas9 technology would inhibit the replication of human immunodeficiency virus [13]. However, the virus would also usually escape from the inhibition. Li used Crispr/Cas9 technology studying hepatitis B virus and discovered the Crispr/Cas9 could target the virus surface antigens and inhibit the virus

replication [14]. Readlerdiscovered the receptor that mediates adenoviral infection and they also used the Crispr/Cas9 technology [15]. Cui combined Crispr/Cas9 technology with next generation sequencing technology and tracked the path of influenza A virus infection [16]. In the field of SARS-CoV-2, Crispr/Cas9 technology also plays an important role. Sun constituted a model of mouse for SARS-CoV-2 [17]. The model would provide a tool for further SARS-CoV -2 research. Xiong, Mrasic, Moon and Jiao all developed tests for SARS-CoV-2, which have high sensitivity [18]. These new tests would provide a new direction for diagnosing COVID-19. Xiong considered the false negatives was possible due to the test efficiency. Therefore, the test needed further elaboration and design in order to keep satisfactory combination efficiency. Maisic regarded the high sensitivity and the non-specific binding of gene would lead to high wrong positive rate. Tester should extract RNA carefully and eliminate contamination as much as possible to restrict the false positive result. Moon believed the colorimetric diagnosis based on Crispr/dCas9 could combine with antibody so as to optimize the operating steps, sensitivity and quantification.

PCR technology is the golden standard in the field of detecting COVID-19. However, the high false negative rate is an important problem. The overall sensitivity was just between 45~60% in the RT-PCR test of nasal and oropharyngeal swabs. There were some researches reporting that the false negative rate between 2~33% in suspected or confirmed COVID-19 patients with typical respiratory symptom and the chest CT scan. The high rate of false negative is an extreme limitation of the RT-PCR to detect and diagnose COVID-19. Some researchers have traced back the reason why there was such a high rate of false negative. Then the researchers found that the time of sampling was important. The false negative rate would change over sampling time after symptom onset. Droplet digital PCR offers a better explanation of the COVID-19 test repositive after patient hospital discharge due to its large increase in sensitivity. The reason for the high rate of false negative of RT-PCR was the testing result in relation to the sample viral load. If the sample viral load was deficient, the RT-PCR technology would be hard to detect the SARS-CoV-2 [19].

Besides, PCR technology requires high-caliber laboratory equipment. It is difficult to use PCR out of the laboratory because of the tedious testing process. Some companies launch highly sensitive droplet digital PCR kit, but it still requires precise laboratory equipment and complicated operation. Moreover, the droplet digital PCR kit is prohibitive. All of these problems impose restrictions on the application of PCR technology in the field of detection and diagnosis of COVID-19 [4].

5. Conclusion

Numerous advances have been made in the diagnosis and management of SARS-Cov-2 up to this point. Real-time PCR, digital PCR, multiplex PCR, and other technologies all offer benefits and drawbacks that vary depending on the situation. The CRISPR/Cas9 technology has been used to construct some detective techniques. Although each of them has advantages, there are still limitations that prevent their widespread use in COVID-19 detection.

Controlling the spread of the COVID-19 infection is necessary. Research on COVID-19's diagnosis and management is still required. People require technology that is more convenient and quick in addition to having increased sensitivity and specificity. A growing number of investigative technologies that fulfill clinical needs and increase diagnostic effectiveness will be recognized along with the quick development of modern science and technology. They will support medical professionals and academics as they investigate and manage the complex pandemic.

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