

COVID-19 Diagnosis — A Current Review of Its Developing Trend

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Abstract. The COVID-19 pandemic has been confirmed as a public health emergency by the WHO in 2020. In order to deal with it, the first generation of diagnostic method such as the RT-PCR test has been developed. However, there is obvious shortcoming of these methods considering efficiency and patient's feeling problems. Therefore, a series of SARS-CoV-2 diagnostic technique has been developed based on the basic PCR test or migrating from the existing virus detection method. With reference to the issued literature, this paper aims at presenting the developing history of these SARS-CoV-2 diagnostic methods and make analysis in extracting the logic behind it and make predictions about the future of these methods under the large-scale virus screening background nowadays.

Keywords: COVID-19, Diagnostic method, RT-PCR, CRISPER, VOC.

1. Introduction

COVID-19 is a serious pandemic caused by novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that spread throughout the world in 2019. It was so such a serious public health event that the World Health Organization (WHO) emergently acknowledged COVID-19 as a public health emergency on January 30, 2020. From then on, the virus has continuously developed and raised high levels of concentration in diagnostic field. The research in this field experienced boosting in the early stage of the pandemic to fulfill the need in identifying the patients and potential transmitters which aims at controlling the spreading scale. These methods are mainly divided into two categories: the gene-level analysis such as Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test and the molecules-level analysis such as antigen test and antibody test. However, both of these two kinds of methods have their own defects and inconvenience, where much of further research has been made to overcome and improve them. This review aims at combing through this logical chain, mainly outline the updating motivation of the RT-PCR test. First of all, an analysis of the problems of the existing front line diagnostic methods, especially the RT-PCR test will be given. In the next stage, focusing on the disadvantages of the RT-PCR test, the CRISPER/Cas diagnostic method will be provided and so do its improvement based on the shortages of the RT-PCR test and its own advantages. Finally, a novel non-invasive diagnostic method relied on the volatile organic compounds (VOCs) is introduced, as well as its advantage-disadvantage analysis. These three parts of analysis combine together to effectively predict the future trend of the diagnosis development in the COVID-19 background.

2. The front-line detection method

2.1. The RT-PCR test

The RT-PCR test is authorized for use worldwide in 2020, its accuracy and reliability make PCR test become the standard global COVID-19 diagnosis.

The RT-PCR test first collected samples from the potentially infected areas, such as oropharyngeal or nasal swabs, upper and lower respiratory tract aspirates, bronchoalveolar lavage, and the sputum. As an RNA virus, the RNA of SARS-CoV-2 are then extracted from the samples and being processed following two steps: (a) The RNA is reversely transcribed to complementary DNA (cDNA) and (b) The obtained cDNA is amplified by polymerase chain reaction through gene-specific primers and fluorescently labeled hydrolysis probes until a detectable amount. Among this process, the primers promise the amplification are done in the prescribed genome area and the probes not only make the genome being detectable by the machine but also quantifies the concentration of virus [1].

2.2. Analysis of the genomic methods

To be more specific, the RT-PCR test are done by two methods in practice: The one-step PCR and the two-step PCR. Their differences are that the two-step PCR the two steps described above are done in different holders which those in the one-step PCR are combined to one. Consequently, it raised a balance to be considered between accuracy and efficiency. The two-step method takes longer than a one-step method but allows for the optimization of both reactions.

RT-PCR is a mature diagnostic method. However, it has several shortcomings. First of all, there are only limited access to the RT-PCR testing equipment and the professional staffs needed to run the test are also limited. Next, the complex process such as the temperature controlling steps needs a lot of time to implement, which makes the result too slow for patients usage. Also, the complex process will make the probability of mistakes happening increase. To solve this problem, the loop mediated isothermal amplification (LAMP) method was developed, where only isothermal condition is needed to avoid the heterothermic requirements. Moreover, the LAMP methods have a very fast amplification speed, under which amount of 10^9 copies are amplified in one hour [2]. Finally, the material using has raised heated controversies. For example, there is evidence showing that the widely used nasopharyngeal samples are less effective than the saliva. However, there are also threats in using saliva [3].

3. The updating detection methods

3.1. The CRISPER/Cas System

3.1.1 Principle of the method

Offering an adaptive immunity is CRISPR-Cas. It is present in a wide range of bacteria and archaea to guard against gene-invading bacteriophages, viruses, and other external organisms. It is made up of an array of CRISPR repeat spacers. It is then changed into trans activated CRISPR RNA, CRISPR RNA with CRISPR activity, and a set of CRISPR connected to the CAS genes that encode endonuclease-active CAS proteins. The CAS protein can cut foreign DNA into small fragments when prokaryotes impose intrusive genetic elements. It subsequently becomes a new spacer in the CRISPR array. [4]. The CRISPER/Cas system has been regarded as an effective genome editing tool to make contributions in works such as gene therapy. Also, recent research focusing on guide RNA and RNA-targeted CRISPR effectors has accelerate its utilization in diagnosing RNA viruses based on based on CRISPR/Cas13 system [5]. Moreover, the Specific High Sensitivity Enzymatic Reporter Unlocking technique, which offers a high sensitivity in nucleic detection are then developed based on the CRISPER/Cas system. To be more specific, it combines the recombinase polymerase amplification technique to convert DNA to RNA and Cas 13 to detect the target RNA and hence achieve the diagnostic purpose [6]. This makes the CRISPER method a more efficient one.

3.1.2 Analysis of the method

The CRISPER method, combining with amplification process such as the LAMP, reduces time that is consumed in operating the machine. Moreover, the CRISPER-derived genome editing technique offer another way of designing the diagnostic method, changing the thought from measuring the RNA quantity on the DNA itself, to isolating RNA from DNA, which may originate

dozens of derived methods from it. Finally, the high differential selection increases the sensitivity in the performance of the CRISPER/Cas system.

Firstly, CRISPR is not efficient, this is because the performance of any genome editing project can be significantly hampered by variance [7]. The laboratory edition is often done in culture dish, in which the number of cells being effectively edited is represented by editing performance. The results should be carefully examined even though editing effectiveness of less than 100% is by no means a failure [9]. The unsuccessful cells in the population can show any undiscovered effects of genome manipulation. Secondly, many entities do not already own a genome editing process in place. Although the CRISPER/Cas technique is regarded as an ideal and effectiveness method and worth further studying, lack of corresponding equipment or pipeline in the laboratory will require more communication and regulation work to be done. Thus, the research progress will be delayed so that the expectation and interest on SRISPER/Cas system will be sharply decreased [10].

Moreover, a major concern for implementing CRISPR/Cas, to be more specific the CRISPER/Cas9, for gene cutting is its relatively high frequency of off-target effects (OTEs) [10] [11]. The OTEs consist of the majority of concern in complex eukaryotic cells, most frequent in vivo gene therapeutic operations. The OTEs represent the random mutants that may happen in the modifying process of CRISPER/Cas9 method, which leads to failure of it. As a result, although the CRISPER/Cas9 is a precise technique theoretically, in practice this promise is not so much [11]. The use of Cas proteins may be restricted or have negative consequences when genome editing methods break DNA, in the situation when not only at the intended target site but also in unintended locations [11]. Chromosome rearrangements brought on by off-target effects might unintentionally affect some inadequately matched genome locations hence restrict CRISPR/Cas editing techniques usage for therapeutic reasons. In addition to its negative effect on chromosomes stability, OTEs can also cause deficiency in crucial and basic function by over restrain or stimulate the activities of crucial human genes, which is likely to cause a variety of physiological or signaling disorders. From the fact that Cas protein are monomers and can luckily assist in the identification of shorter target sequences, studies have shown that CRISPR/Cas tools may be more susceptible to OTEs than many of the other existing genome-editing technologies. In contrast, the dimeric TALEN and ZFN constructions are less likely to raise such concerns [12]. OTEs are often caused by guide RNA recognizing mismatches and Cas enzymes cleaving unintended bystanders. Cas9 can cause duplex fracture which then activates the nature DNA repair mechanism, which is the principle of the often-used molecular process to insert DNA segments (such as cDNAs) [11]. Because of this, the targeted alleles frequently have unanticipated changes including deletions, several partial or full combinations of the targeting vector, and even repetitions. Although the 20-nt sgRNA guiding sequence and a PAM next to the target sequence in the genome are believed to tightly regulate Cas9's targeting specificity, it is still possible to experience off target cleavage activity on the DNA sequence if there are 3–5 mismatched base pairs at the far end of the PAM. Furthermore, earlier research has shown that various gRNA architectures have the ability to influence the cleavage happened at on-target and off-target sites [12]. However, although the CRISPER/Cas9 are of high controversy, the relative-frequent-used CRISPR-associated: Cas 12 or Cas 13 are more reliable. Therefore, the prospect of CRISPER/Cas diagnostic system are good.

3.2. The VOC System

3.2.1 Principle of the method

The idea behind the theory, which is that volatile organic molecules may be found in the exhaled breath using a number of sensing methods, is that they can be used to detect Covid-19. The production of this volatile may occur during cellular metabolic processes or by inhalation/absorption of foreign materials [13]. There are several sensing methods that may be used to identify volatile organic molecules in exhaled breath. This volatile could come from foreign sources or come from cellular metabolic processes that create them. Research into volatile organic compounds (VOCs), which may be found in sweat or exhaled air, is a cutting-edge field for treating respiratory disorders [14]. Either

the mass spectrometry method, which allows for the identification of specific VOC in exhaled air, or the electronic nose method, which is quicker and simpler and provides an indication of the general profile of the VOC without actually identifying them, can be used for VOC analysis [14]. The VOC has shown interest in some cases when employed as a diagnostic or prognostic tool in individuals being watched for thoracic tumorous pathology or bronchial or pulmonary vascular problems.

The bulk of exhaled breath is composed of inert gases, including water, carbon dioxide, nitrogen, and oxygen. The remaining portion of the breath is made up of trace components, which are volatile substances ingested from the environment or produced by the body [15]. These substances range in concentration from nmol/l to pmol/l (parts per billion volumes to parts per trillion volume) and are present in trace amounts. Exogenous volatiles are either inhaled into the lungs or absorbed via the skin [15]. They originate from a number of petroleum-based products and solvents. The endogenous volatiles are produced by the body's cellular metabolic processes. Monitoring VOC levels in the breath can therefore provide insight into how the body is biochemically operating [16].

3.2.2 Analysis of the method

The currently available research on VOC-based breath analysis took into account a wide variety of elements, including various populations, analytical methods, and surroundings. The research employed RT-PCR as the index test and several breath analyzer products as the reference standard. To obtain the overall results, data from the six included research were combined. The reported sensitivity value is 98.2% (with the 97.5% confidence interval being 93.1%-99.6%). Due to the great sensitivity rate of this screening method, more SARS-COV-2 carriers may be discovered and fewer may be missed. Both investigations consistently showed high sensitivity in tests involving several sets and variables. Despite the fact that specificity values varied across all investigations, VOCs-based breath analysis performed only fairly well in identifying non-SARS-COV-2 diseases, with an aggregate specificity value of 74.3% (with the 97.5% confidence interval being 70.3%-83.9%). The COVID-19 fast antigen test, a currently used diagnostic technique, has a minimum threshold of 90% sensitivity and 97% specificity [17]. With a good specificity level, VOCs-based breath analysis demonstrated a relatively satisfactory function in differentiating non-SARS-COV-2 infections, despite the fact that all studies revealed variation in specificity values. The minimal criterion is 90% sensitivity and 97% specificity for the COVID-19 fast antigen test, the currently used conventional diagnostic technique [17].

Screening for SARS-CoV-2 occurs in a series of contexts, including the initial encounter with medical professionals and public spaces that are vulnerable to spreading the virus throughout communities [18]. The need for mass screening stems from the fact that, in addition to infected patients' increased risk of experiencing negative outcomes, asymptomatic infected individuals may also pose risky factors to hospital staff, particularly during procedures that produce aerosols, and to other neighboring people in community [19]. Breath tests may be applied to detect SARS-CoV-2 infection, according to recent research. Viral respiratory infections alter the VOC profile and cause metabolic alterations. As a result, sensor arrays or electronic noses may be used to produce noninvasive diagnostic tools using infection-related volatile organic compounds (VOCs) [19].

4. Conclusion

At present, a series of COVID-19 diagnostic methods have been developed, among which the gene-based test and the serological test are the two mainline. However, there remains several practical problems to be resolved, relating to detection efficiency and degree of comfort in the test experience. For the first problem, updating represented by the LAMP and CRISPER/ Cas system have been done. On the one hand, they simplified the machine operation, hence decrease the human resource and time input. On the other hand, the new methods purified the test sample to some extent by high level of differential selection and usage of materials that has relative consistent test wise quality. As a result, the testing outcome will become more accurate to avoid false positive or false negative results. Also, the new choice of methods will likely shorten the needed waiting time, which is especially friendly

to the citizens with high demand of the professional test, such as a scholar who is going to attend senior conferences. For the second problem, the non-invasive diagnosis such as VOCs will make the diagnosis process more comfortable so that it will be more acceptable by the youngsters, which can potentially decrease the administrative cost for the governmental entities. In conclusion, the pharmaceutical industry has made great efforts in developing novel diagnosis which have positive importance for the COVID-19 pandemic prevention work. The prospects for the mentioned detection techniques will be excellent in the large-scale test background nowadays.

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