Hunting severe acute respiratory syndrome coronavirus 2 (2019 novel coronavirus): From laboratory testing back to basic research

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Abstract: The rapid spread of coronavirus disease 2019 (COVID-19) in many countries causes citizens of daily inconvenience and even life-threat for elderly population. The invasion of the main pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; 2019 novel coronavirus [2019-nCoV]), into human body causes different levels of impact to various patients. One of the most important issues for COVID-19 is how to defend this virus with the ability to foresee the infected targets. Thus, we maintain the quarantined essentially as for as others saved from COVID-19. So far, the routine laboratory test to confirm whether infected by SARS-CoV-2/2019-nCoV or not is through real-time reverse transcription polymerase chain reaction (rRT-PCR; quantitative polymerase chain reaction [qPCR]) with certain sequence regions that recognize SARS-CoV-2/2019-nCoV RNA genome. The heavy loading of rRT-PCR (qPCR) machine and handling labor have tight-packed the instruments as well as the manpower almost in every country. Therefore, the alternative approaches are eagerly waiting to be developed. In this review article, we sort out some state-of-the-art novel approaches that might be applied for a fast, sensitive, and precise detection of SARS-CoV-2/2019-nCoV not only to help the routine laboratory testing but also to improve effective quarantine.

Keywords: Clusters of regularly interspaced short palindromic repeats; Fluorescence resonance energy transfer biosensing; Rapid diagnostic tests; Real-time reverse transcription polymerase chain reaction/quantitative polymerase chain reaction; Severe acute respiratory syndrome coronavirus 2/2019 novel coronavirus

1. INTRODUCTION

The coronavirus-induced respiratory disease 2019 (COVID-19) caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; 2019 novel coronavirus [2019-nCoV])

outbreak at the end of 2019 in Wuhan, China.[1] It soon continuously impacts our daily life in all aspects worldwide. Alone the increasing infected numbers, the death rates (death person versus confirmed cases within selected area) seem to be less than 3% but much higher in new burst out countries. Most of the aged people are in dangerous status especially the one also has served medical history. The clinical symptoms of COVID-19 may be found, but not all, in infected patients include fever (Infrared thermo-sensor can be used to detect high temperature persons), coughing, myalgia, fatigue, some with productive cough, headache, hemoptysis, and even diarrhea. Some patients will have severe pneumonia with difficulty in respiration (18% calculated from record in China).[1] More recently, some patients were found to be with a disability to distinguish smell and/or tests (neurological disorder). So far, there is no effective way to distinguish healthy one from infected targets, even they do have some flu-like symptoms.

Moreover, lots of SARS-CoV-2 infected patients are found asymptomatic. Since no one knows who has already been infected and becomes infectious to others, they are at the same time freely to go out for their daily activities which, unfortunately, easily causes COVID-19 transmission. This situation causes a serious problem during the establishment of prevention network in every country. In Taiwan, to avoid the false-negative case, it requires at least three sequential negative tests of the SARS-CoV-2 before the case can be confirmed negative (combined with the positive or negative result to influenza virus). According to the Taiwan model, to quarantine any suspicious individuals for a period time according to the activities and contact history of the confirmed cases can efficiently slow down the transmission of COVID-19. However, it could profoundly disturb the daily life of a large population if related cases are confirmed positive for SARS-CoV-2. In general, for personal epidemic prevention of COVID-19, avoiding contact from hands, nose, and eyes and droplets is the primary task. How to foresee the SARS-CoV-2 infected targets and to just isolate selected population for advanced treatments while keeping others healthy (and free from quarantine) is one of the important goals during this global war against SARS-CoV-2.

To develop a SARS-CoV-2 detection system that can accurately identify viral macromolecules (either the RNA genome or viral proteins), the full understanding on the specific infection process of this virus is the first step (also will benefit to the development of anti-SARS-CoV-2 drugs). According to the current data, the SARS-CoV-2 virus has similar infection route to the SARS-CoV.[2–4] Both viruses utilize the surface protein angiotensin-converting enzyme 2 (ACE2), which expresses in certain types of human cells such as type 2 alveolar cells, as a receptor for its spike protein to attach to the host cells.[3] The entry of the attached virus requires endocytosis through priming the S protein by host transmembrane protease, serine 2 serine protease,[4] and is regulated by the AP2-associated protein kinase 1 (AAK1). Disruption of AAK1 might interrupt the entry of the virus into host cells and the intracellular assembly of virus particles.[5] The structures of several SARS-CoV related proteins, including the spike proteins, main protease of SARS-CoV-2, and ACE2,[6–8] have been resolved to provide valuable information for the following development of new detection sensors or even the design of anti-SARS-CoV-2 drugs.

In this review article, we present available approaches that can identify SARS-CoV-2, from the routine laboratory tests to the rapid diagnostic tests with great application potential to help reducing the labor of routine laboratory test. Hopefully with proper applications of these tools, we can finally stop the fast transmission of COVID-19 in the whole world.

2. DETECTION STRATEGY

2.1. RNA level

The major laboratory assay to confirm whether patients are infected with SARS-CoV-2 or not is through the nucleic acid amplification tests (NAATs), that is, the real-time reverse transcription polymerase chain reaction (rRT-PCR) method carried out in the hospital or disease control center of the government. In Taiwan, such method is relatively mature since the breakout of SARS in 2003. However, it still takes around 4 hours to get the results, including 2 to 3 hours of reaction and around 1 hour of medical consultations and sampling. Moreover, to carefully confirm the infection, three regions of the SARS-CoV-2 RNA sequences are selected for the assay: coding region for spike protein, open reading frame 1 (ORF1), and ORF8. To shorten the reaction time, some company tried to develop new instruments and related kits for this purpose, for example, the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Inc., Van Allen Way, Carlsbad, CA,

USA), Xpert Xpress SARS-CoV-2 test (Cepheid, Caribbean Drive, Sunnyvale, CA, USA), and AutoSAT (an automated high-throughput platform that's compatible with the kit), Rendu Biotechnology, Shanghai, China with related kit, etc. These improvements may shorten the examination reaction within 1.5 hours.

2.2. Protein level (mostly antibody-based)

At protein level, the detection time might be shortened since there is no excess time needed to amplify the detected signal. On the other hand, such protein level detection needs precise recognition and high sensitivity. At least two groups claimed that they can detect the SARS-CoV-2 within 15 minutes.[9]

One group from Guangzhou Medical University, China developed the test for patient's serum to check if the patients have the specific antibody against SARS-CoV-2's antigen. Since this method is not a direct detection of SARS-CoV-2 virus, there could exist blind spot when the samples are found to be negative. Two possible situations should be clarified, one is that the case is not infected, the other is that the case has not produced antibody against SARS-CoV-2 yet.

The other group from Genomics Research Center, Academia Sinica, Taiwan developed a novel sensitive test with a specific monoclonal antibody (mAb, immunoglobulin G) against the nucleocapsid protein of SARS-CoV-2. The mAb does not cross react with other SARS related proteins from SARS-CoV or Middle East respiratory syndrome coronavirus (MERS-CoV). This method can be quickly developed for application by applying the specific mAb onto current commercially available test kit (such as replacing the anti-flu antibody with the specific mAb in flu test kit). Thus, a rapid diagnostic test kit for SARS-CoV-2 will be soon produced for large demands. Nevertheless, these new methods still need to be through clinical trials with real samples from suspected populations and infected cases through sampling from nasopharyngeal swab, sputum or lower respiratory track aspirate.

2.3. Novel approaches

In the meantime, scientists around the world continuously introduced alternative methods for the detection of SARS-CoV-2.[10–12] For example, a new powerful gene-editing tool clusters of regularly interspaced short palindromic repeats (CRISPRs) technique has been applied to successfully detect synthetic SARS-CoV-2 RNA sequences.[11,12] As described in the latest reports, the testing reaction can be restricted within 60 minutes with relatively low cost.[12] Such proof of concept idea has been emerged and will also be tested in the real samples to confirm its detection efficacy.

More recently, a unique idea that combines a modified RNA amplification (reverse transcription enzymatic recombinase amplification [RT-ERA]) and a fluorescence resonance energy transfer (FRET)-based probe was proposed.[13] The key to this new method is to use fluorescein amidites (FAM) and Black Hole Quencher-1 (BHQ-1) as a FRET quenching pair, which means that BHQ1 will quench the fluorescence of FAM when they are located close together. A specific exonuclease II (recognizing tetrahydrofuran site) will be utilized to cut the double-strand status due to targeted RNA sensed the RNA of SARS-CoV-2 (such exo FRET probe is designed to be located within the targeted amplicon). Once this cut happens, it allows the restore of the FAM fluorescence since the quencher BHQ1 leaves. The authors claim that the sensing limit of this ultrasensitive detection method can be as low as 0.32 aM (0.32×10^{-18} M) of RNA.[13]

3. FUTURE PERSPECTIVES

From the above descriptions on various types of detection methods for SARS-CoV-2, rRT-PCR is still taking the heavy works corresponding for the official confirmation. However, the increased

susceptive populations nearly exhaust the routine test procedure in many countries. Indeed, some of the new emerged approaches has potential for the future rapid diagnostics to release the heavy loading of rRT-PCR test, if their specificity and efficacy can be proved through clinical trial. For example, the antibody-based test developed by Academia Sinica has a great potential for fast exam since it can be adapted in the commercial flu-like test. The challenge will be the amplification of mAb, which is the rate-limit stage for large production of this kit. On the other hand, another novel method combining a modified RNA amplification technique with FRET quenching concept provide convincing results for future usage. Finally, in addition to the above example, the FRET-based strategy can also be applied to detect the binding of SARS-CoV-2 spike protein to human ACE2 receptor, or the activity of SARS-CoV-2 protease using specific peptide sequence as substrate between the FRET pair fluorescent proteins.

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