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Molecular Assays for the Detection of COVID-19 andFuture Prospects

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Abstract

COVID-19 disease is the current challenging global public health concern infectious human pandemic disease, caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). In December 2019, this COVID-19 disease outbreak form Wuhan city, China and easily spread to several countries around the world. The causative agent of the COVID-19 disease, SARS-CoV-2 coronavirus can transmit from an infected person having a respiratory tract symptom to a non-infected person during the incubation period of 2 days to 14 days through coughing, exhaling, sneezing with small droplets from nose or mouth. Humans with pre-existing health problems and also aged people are more vulnerable to acquire the coronavirus COVID-19 disease. The recommended measures to reduce the transmission of this deadly human coronavirus are interrupting human-human contact or apply social distance, taking face mask, stay home and isolate patients at early stages or self-quarantine. Early diagnosis of SARS-CoV-2 coronavirus is the key for quick management of COVID-19 cases and control of the spread of the coronavirus based on the appropriate sample collection. Nowadays, the rapid, accurate and promising molecular laboratory diagnosis assay (such as RT-PCR, microarray, LAMP, point-of care, CRISPR) for detections of SARS-CoV-2 RNA of pathogenic coronaviruses would be valuable to controlling the sources of infection, prevent subsequent secondary spread, saving people's lives and help patients to prevent the illness evolution.

Keywords: COVID-19; Coronaviruses; Molecular assay; SARS-CoV-2

Introduction

The novel, pandemic Coronavirus Disease-19 (COVID-19) is the current challenging global public health concern infectious human disease, caused by the severe acute respiratory Syndrome Coronavirus-2 (SARS-CoV-2). SARS-CoV-2 is a positive-sense, single-stranded RNA virus. Initially, this coronavirus outbreak from Wuhan city, China, in December 2019 and speedily spread to other regions of china and other countries around the world [1]. On March, 2020 the WHO upgraded the status of the Coronavirus Disease (COVID-19) outbreak from epidemic to a global pandemic. The causative agent of COVID-19 disease, coronavirus (SARS-CoV-2) is contagious and can transmit from an infected person having a respiratory tract symptoms (such as high fever and headache, cough, muscle pain, fatigue, shortness of breath, weakness) to a non-infected person during the incubation period of 2 days to 14 days *via* coughing, exhaling, sneezing with small droplets from nose or mouth and then settle on air in the environment or in inanimate surfaces like metal, glass or plastic for up to 9 days and further infecting humans who breathe or touch these places and then touch their body parts such as mouth, nose, eye [2,3]. However, some infected patients show few to no signs of illness during the early phase of infection but can still transmit the virus to non-infected persons. Since December 2019 up to 13 June 2020, many people were infected with COVID-19 cases, and many people were died worldwide at the time of writing this review [4], which implies that the virus has a great ability to spread, adapt and survive in different environmental conditions. Some finding suggests that aged people and humans with pre-existing health problems including cancer, HIV/AIDS, diabetes, heart disease, lung disease are more vulnerable to acquire the COVID-19 and develop serious illness more often than others [5,6]. The WHO recognized the battle against this deadly human coronavirus *via* interrupting human-human contact or apply social distance, taking face mask, home care or stay home and isolate patients at early stages or self-quarantine measures to reduce the transmission and keep the virus from spreading to others. But some cases call for more complex of keeping the outbreaks of the virus due to improper disposal of used materials masks, some infected persons with no signs; peoples have no staying at home due to lack of daily meals, inadequate risk assessment regarding the urgency of the situation. So that early diagnosis of COVID-19 *via* molecular diagnostic assay is vital to minimize its spread. This review briefly describes the molecular diagnostic testing approaches for detection of novel COVID-19 infection.

Molecular Diagnostic Assay for Detection of COCID-19 Disease

After the proper sample collection, clinical specimens should be sent to the core laboratory immediately for extraction of nucleic acid using approved viral isolation kits and for the diagnosis of COVID-19 infection. Early diagnosis of COVID-19 is crucial for the timely management and isolation of confirmed cases to prevent further transmission of coronavirus. The major challenges in the diagnosis of COVID-19 are ways of appropriate sample collection and transport, and kit or equipment validation. The rapid and accurate molecular assays for detections of SARS-CoV-2 RNA of pathogenic coronaviruses COVID-19 disease, which would be valuable to controlling the sources of infection, prevent subsequent secondary spread, saving people's lives, help patients to prevent the illness evolution and play a vital role in selecting appropriate preventions and treatments. Molecular biology, has given the various gold standard rapid, accurate and promising methods currently available for the molecular detection of coronavirus SARS-CoV-2 RNA.

Real-Time Reverse Transcriptase-PCR (RT-PCR) Based Method

Initially, the extracted genetic materials of coronavirus, single-stranded RNA is transferred into short complementary (cDNA) by reverse transcription process through the RNA-dependent DNA polymerase enzyme and subsequently the Polymerase Chain Reaction (PCR) is performed the detection of PCR produces double-stranded (dsDNA), which is a copy of the virus's RNA, gel electrophoresis and sequencing methods to analyzes the DNA sequences. Afterward, the DNA amplified by the DNA-dependent DNA polymerase enzymatic based PCR with a primer produce a numerous copy of a gene products through separating the double strands of the DNA containing the gene segment, and hereafter the PCR products analyzed by gel electrophoresis and sequencing for the molecular detection of human coronaviruses RNA of COVID-19 disease [10].

Real Time Reverse Transcriptase-PCR (RT-PCR) assay is a predominant and more sensitive PCR-based method for molecular diagnosis of coronavirus RNA in early COVID-19 infection. In real-time Reverse Transcriptase-PCR (RT-PCR), the amplification of DNA is monitored in real time as the PCR reaction using a fluorescent dye or a sequence-specific DNA probe labeled with a fluorescent molecule and a quencher molecule, as in the case of TaqMan assays. The novel TaqMan- probe based real-time RT-PCR was designed for detection of human coronaviruses RNA in clinical specimens from patients with respiratory tract COVID-19 infection. However, the molecular diagnosis of coronavirus using real-time RT-PCR becomes a serious problem for clinical lab technicians because of lacking the safe and stable External Positive Controls (EPC). To overcome this difficulty, the armored RNA was prepared as an external positive control for multiplex real-time reverse transcription-PCR molecular detection of severe acute respiratory Syndrome Coronavirus (SARS-CoV). Even if this Real-time (RT-PCR) is specific, rapid and economical, it cannot precisely analyze the nucleic acid sequence of the amplified gene fragments, and thus all target fragments are considered to be positive or false-negative results obtained by using real-time RT-PCR kits and must be carried out only by experienced technicians in large and qualified laboratories.

Nucleic Acid Amplification Testing-Based Method (NAAT)

The Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP)-based human coronavirus detection assays have been developed and used in clinical SARS-CoV diagnosis in the presence of primers to identify SARS-CoV-2 (COVID-19) virus RNA using RNA samples purified from respiratory swabs collected from COVID-19 patients, then the amplified products were analyzed by agars gel electrophoresis. The detection rates and the sensitivity for SARS-CoV-2 diagnosis in the LAMP assay are similar to that of conventional PCR-based methods. However, the LAMP based assay is a novel, rapid nucleic acid amplification method with high efficiency and also it does not require expensive reagents or dyes and help to reduce the cost for molecular detection of human coronavirus. The RT-LAMP assay was designed 5 full LAMP primers sets targeting SARS-CoV-2 RNA, with amp icon regions designed to the 5 region of the ORF1ab gene and Gene N and showed similar detection sensitivity and could consistently detect 120 copies. Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) has been developed as a rapid and cost-effective testing alternative for detection of SARS-CoV-2 RNA using a set of four primers specific for the target gene.

Microarray Hybridization-Based Method

Microarray assay is a no fluorescent, low-cost, low-density oligonucleotide array test used for rapid high-throughput detection of different emergent strains of SARS-CoV-2 as a result of mutational variation with a sensitivity equal to that of individual real-time RT-PCR [7]. The coronavirus SARS-CoV-2 RNA first produce complementary cDNA labeled with specific probes *via* reverse transcription process. Then these labeled cDNAs hybridize with solid-phase oligonucleotides on the microarray, followed by a series of washing to remove free DNAs and the coronavirus SARS-CoV-2 RNA can be detected by specific probes [8].

Molecular Point-of-Care Testing-Based Method

On March 21, 2020, the US FDA granted emergency use authorization to a rapid, point-of-care diagnostic *in vitro* diagnostics test designed to detect SARS-CoV-2 COVID-19 infection in around 45 minutes, following clinical specimen collection from a nasopharyngeal swab, nasal wash or aspirate. The point-of-care testing SARS-CoV-2 is designed to detect nucleic acid from SARS-CoV-2 *via* quantitative real-time PCR [9]. It can detect and amplify two genomic targets, E and N2 genes in the case of COVID-19 coronaviruses [10]. Point-of-care testing means that results are delivered to patients in the patient care setting, such as hospitals, urgent care centers and medical emergency rooms, instead of samples being sent to a testing laboratory. The currently available rapid, novel molecular point-of-care assay, id now COVID-19 test, which is used for detecting SARS-CoV-2 viral RNA in upper respiratory swabs utilize real- time RT-PCR at a lower time of 13 min.

CRISPR-Based Molecular Assays

In general, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) assay is being used in another promising method for detection of DNA or RNA target sequences using nucleic acid pre-amplification combined with CRISPR-Cas associated enzymes of Cas9, Cas12, and Cas13. The Cas12 and Cas13 family enzymes can be automated to target and cut viral RNA sequences including coronavirus. The CRISPR-Cas assays can explore the possibility of gene-editing for detection of SARS-CoV-2 RNA sequences of the E and N genes. The CRISPR system is a recently potential point-of-care diagnosis tests for detection of SARS-CoV-2 RNA, which is low-cost and can be performed below 1 hour. A quantitative detection SHERLOCK (Specific High-Sensitivity Enzymatic Reporter Unlocking) platform of nucleic acids pre amplification with Cas13 to detect single molecules of RNA. Cas13 protein has a capable of editing or cleaves the reporter RNA sequences in response to activation by SARS-CoV-2 for specific guide RNA sensing.

Conclusion

Nowadays, Coronavirus Disease 19 (COVID-19) is a challenging task globally, when the virus spreads from infected person to non-infected person by droplet infection transferred in to air, environment and inanimate objects. Contamination of regular touch surfaces in healthcare and family settings, hotels and bedrooms, public transport vehicles are a potential source of viral transmission form person-to-

person. The WHO indorses to apply the environmental surface cleaning *via* disinfectant agents effectively and consistently. Some of the inanimate surface disinfectant detergents to decrease human coronavirus infectivity within 1 minute exposure time are 62%-71% ethanol, 0.5% hydrogen peroxide effectively. Early diagnosis of COVID-19 disease is the key for rapid management of the infection and also controls further spread of the virus. Molecular diagnostic assays such as Reverse Transcription-Polymerase Chain Reaction (RT-PCR), LAMP-based nucleic acid amplification assays, molecular point-of-care testing-based assays and CRISPR-based methodologies are the current essential and promising alternatives used for easily diagnosis of SARS-CoV-2 RNA. However, an ongoing research is critical to investigate the efficient and more cost-effective diagnostic tools for detection of COVID-19 infection.

Further Perspectives

Currently the number of confirmed cases of COVID-19 and death of humans rises rapidly throughout the world and remain an ongoing challenge in the fight against this deadly disease. To overcome these challenges, several rapid diagnostic tests have been developed. However, COVID-19 requires more highly specific rapid, inexpensive, non-invasive and easy-to-use point-of-care diagnostic tests with high sensitivity. Also the cost- effective, point-of-care test kits that must be produced widely in mass quantities in order to speed up the response time for treatment and distributed throughout the world especially resource-limited countries and developing countries to minimize the current COVID-19 pandemic related pressure on health systems or lower the risk of spreading infection, and used it at airports and national borders to screen individuals and avoid imported cases of the COVID-19 infection19. The COVID-19 diagnostic assay developers will continue the ongoing global efforts to facilitate another new promising molecular diagnostic approach development and worldwide test kit delivery into near the future for SARS-CoV-2 RNA virus detection.

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