LETTER TO THE EDITOR

Surveillance of SARS-CoV-2 and RT-PCR-Based Assay Design

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KEYWORDS: Coronavirus, Diagnostics, COVID-19, SARS-CoV-2, RT-PCR, Assay kits.

How to Cite This:
Kuznetsova NA, Pochtovyy AA, Nikiforova MA, Guschin VA. Surveillance of SARS-CoV-2 and RT-PCR-based assay design. Biomedica. 2020; 36 (COVID19-S2): 56-7.

Dear Editor,

High population density in the cities with busy transportation systems and booming supermarket run can promote the global spread of a viral infection in a matter of days. The novel SARS-CoV-2 coronavirus has already infected over millions of people worldwide and still the myriad of fatalities is ongoing. Molecular techniques allow SARS-CoV-2 RNA to be quickly detected in clinical samples, aiding the differential diagnosis in severely ill patients and facilitating identification of asymptomatic carriers or pre-symptomatic individuals. Real-time PCR with fluorescent hybridization is the most available, highly sensitive and specific technique for SARS-CoV-2 RNA detection in biological samples. The efficacy of RT-PCR for SARS-CoV-2 detection depends on the specimen source and the applied sampling technique. It is reported that bronchoalveolar lavage fluids have the highest diagnostic value in terms of SARS-CoV-2 detection, followed by sputum, nasal swabs, fibro-bronchoscope brush biopsy, pharyngeal swabs, feces, and blood (1%)[1]. So far, the oligonucleotides and real-time RT-PCR kits for SARS-CoV-2 detection have been described in a few dozens of publications by international authors. In those studies, several different approaches can be identified to designing real-time RT-PCR assays for SARS-CoV-2 detection. Singleplex PCR assays, in which oligonucleotides are selected to target only one specific gene, are the simplest and the most available. Multiplex assays are more advanced and allow targeting a number of different genes simultaneously. Primers and probes for multiplex assays can have different specificity or enable discrimination between SARS-CoV-2 and the related coronaviruses or other respiratory infections. For SARS-CoV-2 detection, primers and probes are usually selected to target the nucleocapsid genes *N1* and *N2*, the RNA-dependent RNA polymerase gene (*RdRP*) and the E protein gene of the viral envelope. For example, CDC (Centers for Disease Control and Prevention) recommends that identification of COVID-19 patients should start with a screening test for the E protein gene, whose nucleotide sequence does not differ from that of SARS, and then proceed to differentiating SARS from SARS-CoV-2 using oligonucleotides for the *RdRP* target[2]. According to the World Health Organization protocol, the collected samples should be screened for *N* and *Orf1b*. However, the proposed oligonucleotides do not help in discriminating between SARS-CoV-2 and SARS; therefore, sequencing is advised to finalize the identification procedure[3].

Whole-genome sequencing has never been so

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close to adoption in the clinical setting as it is now. There are a few approaches to whole-genome sequencing of SARS-CoV-2. The classic approach consists in the extraction of nucleic acids from nasopharyngeal and/or oropharyngeal swabs, subsequent depletion of the host's ribosomal RNA for library preparation and sequencing itself carried out according to the protocols supplied by the manufacturer. However, this approach requires a fair amount of viral RNA and good read depth. With low viral loads, the virus can be replicated using cell cultures. For that, serial passages are performed in Vero V, Vero E6, LLC-MK2, and some other cell lines. This approach has been successfully used in some laboratories, including the Reference center for coronavirus infection (GISAID ID: EPI_ ISL_421275). Whole-genome amplification is an alternative to cell cultures. So far, a few panels have been designed for sequencing the entire genome of SARS-CoV-2. Among them is the Ion AmpliSeq SARS-CoV-2 Research Panel (Thermo Fisher Scientific; USA) and Paragon Genomics Inc (USA) multiplex PCR research panel with two primer pools is readily in market.

Another newer protocol for sample preparation and bioinformatic analysis was proposed by the ARTIC network.[4]It was developed for Oxford Nanopore sequencing platform and generates results within 8 h. Despite of a plethora of designs and assays, classic RT-PCR is still the preferred detection technique for SARS-CoV-2 infection. Refinement of isothermal amplification tools will make molecular analytical techniques more accessible in the future and improve their efficacy in monitoring and controlling biological threats. Sequencing allows accumulating more data about changes occurring in the viral genome and using it for RT-PCR primer optimization, vaccine development, study of the evolution of the virus, and reconstruction of epidemiological processes that drive the epidemic. Sequencing platforms make it possible to analyze collected samples in the clinical setting, outside the lab, thereby reducing the turnaround time. More RT-PCR assay kits are needed for mass screening, which will help to identify infected individuals and contain the current outbreak of COVID-19.

CONFLICT OF INTEREST

None to declare.

FINANCIAL DISCLOSURE

None to disclose.

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Author's Contribution

KNA, PAA, NMA, GVA: Conception of study, acquisition of data, drafting of manuscript.

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