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A BRIEF OVERVIEW OF COVID-19 DIAGNOSIS BY REAL-TIME RT-PCR

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Abstract. During the pandemic, key priorities included diagnosis, prevention, and elimination of the disease. Numerous test systems with varying levels of sensitivity were developed for diagnostic purposes. Among these systems, molecular-genetic assays hold a particularly important place, as they enable accurate identification of infectious agents. Within this approach, the most widely used method for diagnostic purposes in scientific research and clinical settings is real-time RT-PCR.

In this work, we provide a brief overview of the real-time RT-PCR method. The fundamental principles of the method, its variants, as well as its advantages and limitations, are outlined.

Keywords: real-time RT-PCR, virus, SARS-CoV-2, method

Аннотация. Пандемия учурунда негизги артыкчылыктарга ооруну диагностикалоо, алдын алуу жана жок кылуу кирген. Диагностикалык максатта ар кандай деңгээлдеги сезгичтиги бар көптөгөн тест системалары иштелип чыккан. Бул системалардын арасында молекулярдык-генетикалык анализдер өзгөчө маанилүү орунду ээлейт, анткени алар жугуштуу агенттерди так аныктоого мүмкүндүк берет. Бул ыкманын алкагында илимий изилдөөлөрдө жана клиникалык шарттарда диагностикалык максаттарда эң көп колдонулган ыкма-реалдуу убакыт режиминдеги РТ-ПЦР.

Бул иште биз реалдуу убакыт режиминдеги РТ-ПЦР ыкмасынын кыскача баяндамасын беребиз. Методдун негизги принциптери, анын варианттары, ошондой эле анын артыкчылыктары жана чектөөлөрү көрсөтүлгөн.

Ачкыч сөздөр: реалдуу убакыт RT-PCR, вирус, SARS-CoV-2, ыкмасы

Introduction

The COVID-19 pandemic caused by SARS-CoV-2 has resulted in more than 778 million confirmed cases and over 7 million deaths worldwide as of 2 November 2025 [1]. The consequences of the pandemic have profoundly disrupted living and working conditions for billions of people across the globe due to various forms of social distancing and quarantine measures imposed in many cities. The global economy has also been significantly affected by business closures and strict travel restrictions. To understand the complex dynamics associated with SARS-CoV-2 infection, it is essential to develop and implement accurate and rapid diagnostic systems to mitigate and eliminate these adverse impacts [2]. One such diagnostic approach is reverse transcription followed by real-time polymerase chain reaction (real-time RT-PCR) [3, 4]. This method originated from scientific research in the late twentieth century, when the enzyme reverse transcriptase was combined with PCR for RNA analysis [5, 6]. The RT-PCR assay for detecting SARS-CoV-2 was first developed at the Charité Institute of Virology in Germany and introduced by the WHO on 13 January 2020 [7].

The aim of this work is to provide a brief overview of the real-time RT-PCR method as one of the key diagnostic tools for COVID-19.

Principle of real-time RT-PCR

The fundamental principle of the real-time RT-PCR reaction is the conversion of the viral genomic RNA into DNA. This process is carried out by an RNA-dependent DNA polymerase (reverse transcriptase). The reaction employs short oligonucleotide primers specific to DNA sequences that are designed to precisely recognize complementary regions within the RNA viral genome. The reverse transcriptase enzyme synthesizes

a short complementary DNA copy (cDNA) from the viral RNA template.

In the real-time RT-PCR format, DNA amplification is monitored continuously as the reaction proceeds. This is achieved through the use of fluorescent dyes or sequence-specific DNA probes. Probes are labeled with a fluorescent reporter molecule and a quencher, as in TaqMan assays. The automated system then performs amplification for approximately 40 cycles, during which virus-specific complementary DNA is tracked until it is detected via a fluorescent or electrical signal [2, 8]. An illustration of the real-time RT-PCR process is provided below (Fig. 1).

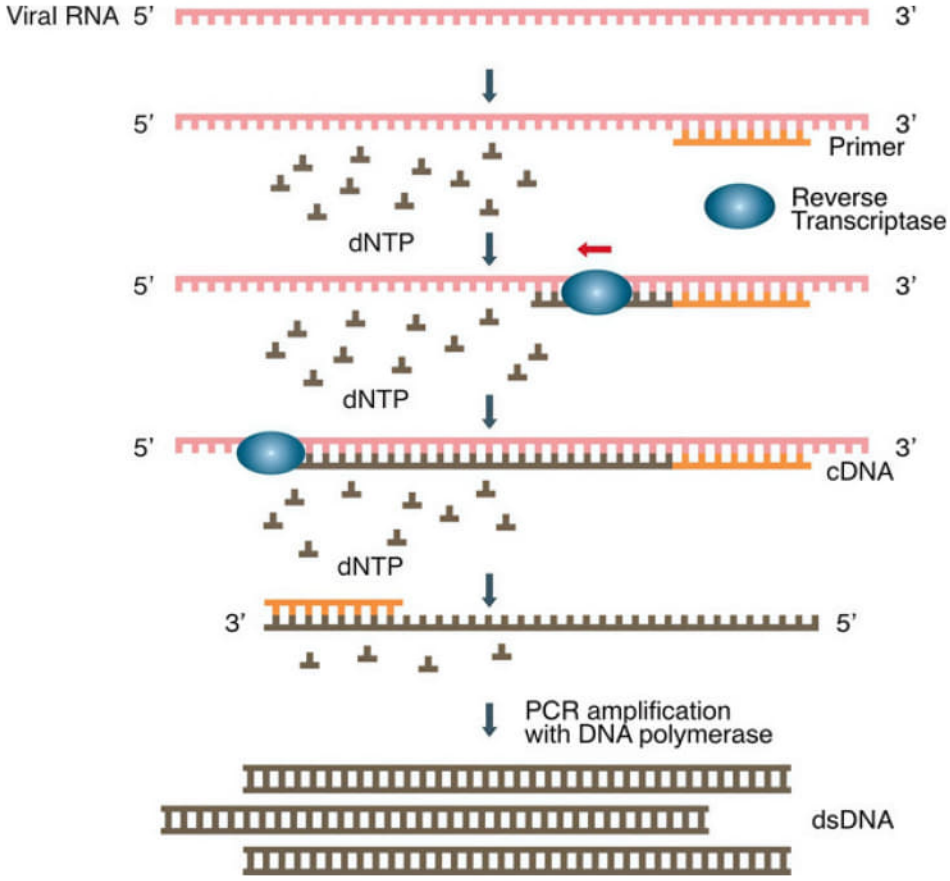

Figure 1. Illustration of the real-time RT-PCR method [2].

Types of real-time RT-PCR

Carter L.J. et al. describe that reverse transcription PCR (RT-PCR) is traditionally performed in either a one-step or two-step format. One-step real-time RT-PCR is carried out in a single tube containing all the necessary primers for the entire reaction. In contrast, two-step real-time RT-PCR requires multiple tubes to separately perform the reverse transcription and amplification reactions, but it offers greater flexibility and enhanced sensitivity. Additional advantages include the need for smaller amounts of starting material and the ability to store cDNA for the quantitative analysis of multiple targets [2, 9, 10, 11].

Advantages of real-time RT-PCR

Real-time RT-PCR offers several advantages, including its ability to analyze RNA, high sensitivity and specificity, quantitative capability, simplicity, and accessibility [8, 12], as well as a wide range of applications [8]. At present, real-time RT-PCR is one of the most widely used methods in molecular biology for epidemiological surveillance and in vivo diagnostics in humans [8].

Despite its strengths, the method also has several limitations. For SARS-CoV-2 detection, the disadvantages of real-time RT-PCR include a restricted window of infection detection, false-negative results, prolonged turnaround time, false results in asymptomatic or recovered

individuals, high equipment costs, and a shortage of qualified personnel to perform and interpret real-time RT-PCR assays. The detection window is limited to the active phase of infection, as the number of viral particles in a sample decreases significantly after approximately three weeks following exposure; low viral loads negatively affect test performance and limit its diagnostic value. False-negative results may occur depending on the timing of sample collection or individual differences in the patient's immune response [2, 13, 14].These limitations complicate and restrict the use of this diagnostic method in hospitals and clinical laboratories. Consequently, many scientific organizations, companies, and laboratories worldwide are working to further improve the efficiency and rapidity of real-time RT-PCR technology, and these efforts play an important role in public health and everyday life.

Conclusion

Thus, during the pandemic, real-time RT-PCR test systems for the detection of SARS-CoV-2 were developed and implemented, and they were used in primary healthcare settings as well as in large-scale and screening studies. This, in turn, contributed to the rapid, efficient, and accurate identification and diagnosis of the virus.

1. COVID-19 Cases, World. https://data.who.int/dashboards/covid19/cases

2. Carter LJ, Garner LV, Smoot JW, Li Y, Zhou Q, Saveson CJ, Sasso JM, Gregg AC, Soares DJ, Beskid TR, Jervey SR, Liu C. Assay Techniques and Test Development for COVID-19 Diagnosis. ACS Cent Sci. 2020 May 27;6(5):591-605. doi: 10.1021/acscentsci.0c00501

3. Mackay IM, Arden KE, Nitsche A. Real-time PCR in virology. Nucleic Acids Res. 2002 Mar 15;30(6):1292-305. doi: 10.1093/nar/30.6.1292.

4. Artika IM, Dewi YP, Nainggolan IM, Siregar JE, Antonjaya U. Real-Time Polymerase Chain Reaction: Current Techniques, Applications, and Role in COVID-19 Diagnosis. Genes (Basel). 2022 Dec 16;13(12):2387. doi: 10.3390/genes13122387

5. Haddad F, Baldwin KM. Reverse transcription of the ribonucleic acid: the first step in RT-PCR assay. Methods Mol Biol. 2010;630:261-70. doi: 10.1007/978-1-60761-629-0_17

6. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 1988 Jan 29;239(4839):487-91. doi: 10.1126/science.2448875

7. Chung YS, Lee NJ, Woo SH, Kim JM, Kim HM, Jo HJ, Park YE, Han MG. Validation of real-time RT-PCR for detection of SARS-CoV-2 in the early stages of the COVID-19 outbreak in the Republic of Korea. Sci Rep. 2021 Jul 20;11(1):14817. doi: 10.1038/s41598-021-94196-3.

8. Mo Y, Wan R, Zhang Q. Application of reverse transcription-PCR and real-time PCR in nanotoxicity research. Methods Mol Biol. 2012;926:99-112. doi: 10.1007/978-1-62703-002-1_7.

9. Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. Biotechniques. 2005 Jul;39(1):75-85. doi: 10.2144/05391RV01

10. Arnaudova K.Sh., Yasenyavskaya A.L., Rostoshvili G.A., Samotrueva M.A., Bashkina O.A. Modern molecular-genetic technologies in the diagnosis of viral diseases. RMJ. Medical Review. 2021;5(7):497-502. DOI: 10.32364/2587-6821-2021-5-7-497-502.

11. Trotskaya O.S. Modern molecular-genetic technologies in the diagnosis of viral diseases. https://www.10gkb.by/uslugi/platnye-umlugi/otdelenie-meditsinskoj-reabilitatsii/sovremennye-molekulyarno-geneticheskie-tekhnologii-v-diagnostike-virusnykh-zabolevanij

12. Afzal A. Molecular diagnostic technologies for COVID-19: Limitations and challenges. J Adv Res. 2020 Nov;26:149-159. doi: 10.1016/j.jare.2020.08.002

13. Nguyen NNT, McCarthy C, Lantigua D, Camci-Unal G. Development of Diagnostic Tests for Detection of SARS-CoV-2. Diagnostics (Basel). 2020 Nov 5;10(11):905. doi: 10.3390/diagnostics10110905

14. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal. 2020 Apr;10(2):102-108. doi: 10.1016/j.jpha.2020.03.001