Digital PCR: Analytical Chemistry Method to Detect Viruses with High Sensitivity

Shiori Senoo

Our world currently faces a number of major challenges that threaten the future of humankind. One of the major problems facing 2019 is new coronavirus infectious disease, COVID-19. Frighteningly, it is generally known that infection occurs by droplets or contact. There is a risk of spreading the infection without symptoms, such as talking to many people at close range. To prevent infection, people have been restricted from free life that they want and required to change their lifestyles. The spread of the new coronavirus has a great impact on social systems and people's lives. In this situation, virus detection has become an important issue.

Recently, people are crazy about testing to determine if they are infected with a coronavirus. People with suspicious symptoms who are likely to be infected should be tested as soon as possible. This is because finding a positive person can provide sufficient preventive measures against infection in the surrounding area. In addition, there is an increasing number of cases of testing as etiquette for safety and security. It seems that they may work or participate in competitions after being tested to prove they are not infected.

Do you know that there are various methods of test for virus? The following three methods are known well. Ther are PCR test, antigen test and antibody test. First is PCR test. PCR is an abbreviation for Polymerase Chain Reaction. It is one of the well-known

techniques in molecular biology. This is a test method that amplifies and detects the gene of the virus you want to test using a special chemical solution. It is mainly used to check whether a virus is present in the body at the time of testing. Second is antigen test. This is a test method that uses the antibody of the virus you want to test to detect the antigen, which is a unique protein of the virus. The detection rate is inferior to that of the PCR test, but the results are obtained in a short time. It is used when prompt judgment is required because no special equipment is required. Third is antibody test. It is a test to check if you have been infected with the virus in the past. Check for the presence of antibodies in the blood, which are proteins that are formed when infected with a virus. It takes time for antibodies to form in the body. So, it is difficult to use them for testing that we are not currently infected with the virus.

The PCR test can detect the most sensitive of the three tests. Why? You can understand it if you know the principle. The PCR is a method of amplifying a specific target region on a deoxyribonucleic acid (DNA) sequence using a thermostable DNA polymerase. Thermostable DNA polymerase is an enzyme that synthesizes and replicates DNA, which is relatively stable even at high temperatures. If the template DNA is present even in a very small amount, the target region is amplified. DNA is increased exponentially with changes in temperature by repeating the three steps (heat denaturation [95 ℃], annealing [55 ℃], and elongation reaction [72 ℃]). As a result, it is possible to amplify DNA that was initially undetectable by a machine to an amount that can be detected. However, the new coronavirus, SARS-Cov-2 is an RNA virus. It is detected by the RT-qPCR method, in which complementary DNA (cDNA) to RNA is synthesized using reverse transcriptase and then PCR is performed. A huge number of virus genes are

copied in the reaction by using this method, so that the virus can be detected with high sensitivity. However, normal PCR continues to report a relatively high percentage of false negatives. This poses an important challenge for the management of hospitalized patients and the accurate monitoring of infectivity. Now, "digital PCR" is attracting attention as a new analytical method for PCR.

Digital PCR uses an unprecedented approach to detect and quantify DNA, and estimates the absolute number of molecules through statistical methods (Fig.1). This technique counts the number of individual molecules contained in a sample for absolute quantification. In digital PCR, DNA or cDNA samples are distributed to a large number of wells, and PCR is performed simultaneously in each well. Some wells contain one or more target molecules, while others do not contain any target molecules. After the completion of PCR, the presence or absence of PCR amplification is analyzed for each well. Wells with amplification is counted as a positive reaction containing the target molecule, and wells without amplification is counted as a negative reaction not containing the target. By the presence or absence of a signal in each well is detected, the absolute quantification of the target molecule in the sample is statistically performed based on the rate of negative reaction.

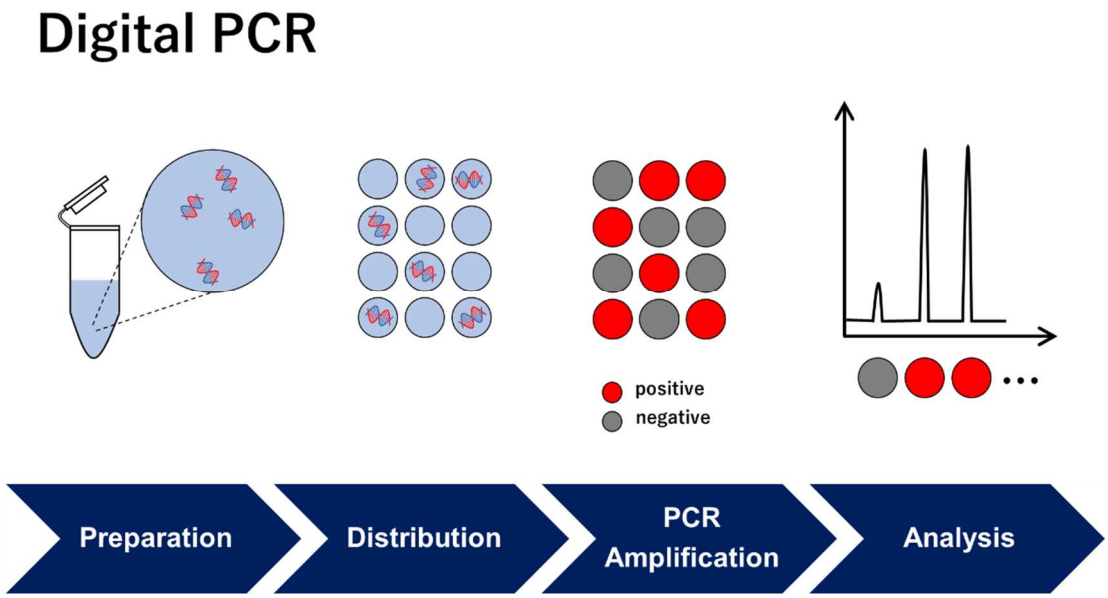

Fig.1 Workflow schematic of degital PCR.

In fact, the results of a digital PCR test on patients with COVID-19 pneumonia were reported in the paper [1]. Approximately 28% (18/64) of patients tested negative by regular PCR, but approximately 61% of negative patients (11/18) were found to be positive (false negative) by digital PCR. Amplification by digital PCR improved the overall sensitivity of viral molecule detection from approximately 72% (46/64) to 89% (57/64). Therefore, this method enables higher sensitive virus detection in SARS-Cov-2 as well.

This new statistical test method may provide one of the most useful methods in the fight against COVID-19. The development of new "analytical chemistry methods" will save the world.

Reference

[1] Paolo Poggio, *et al*, *scientific reports*, **2021**, *11*, 4310