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A Study on the Trend of Molecular Diagnosis of COVID- 19 through Reviewing of the Review Method

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***Abstract*[1]**

Background/Objectives: The purpose of this study was to review the molecular diagnosis of coronavirus disease as COVID-19 was a worldwide pandemic from 2019. In addition, by providing accurate information on the molecular diagnostic tools currently in use, it was intended to be helpful in determining the timing of treatment and in public health policies. **Methods/Statistical analysis**: In this study, to examine the use of diagnostic tools related to COVID-19, "Omicron", "Diagnosis", "RT-PCR", "COVID-19", and "Analysis" were searched as keywords through Google and the literature on the latest diagnostic techniques was used. **Findings**: Recently, it was announced that the fatality rate of Omicron, which has a high epidemic rate and high infection rate, was 1/4 of that of Delta. SARS-CoV-2 Omicron variant RT-PCR laboratory developed assay was said to be sensitive and specific for detecting Omicrons in nasopharyngeal and nasal swabs. The closed tube Penn-RAMP can be used with minimal equipment and training. **Improvements/Applications**: The purpose of this study was to investigate the types and characteristics of accurate molecular diagnostic tests. These molecular diagnostic tests are considered to be important for prompt and accurate diagnosis, distinguishing infected persons, and coping with appropriate isolation and treatment. In addition, this study was intended to help the general public understand molecular diagnostic testing methods.

Index Terms

Omicron, Molecular Diagnosis, RT-PCR, COVID-19, Analysis

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I. INTRODUCTION

According to the Food and Drug R&D Issue Report [1], real-time RT-PCR (standard test method), real-time isothermal amplification method, Genetic Scissors method, and immunochemical diagnosis method are used as diagnostic testing methods for COVID-19. The COVID-19 started in 2019 and has spread to the present, and the spread of infection is continuously increasing. The top priority in preventing the spread of infection was to accurately and quickly diagnose infected patients. The domestic and foreign epidemics continue, and a rapid and accurate diagnosis method was required due to the occurrence of various mutations. Accordingly, as group infections due to high-risk respiratory viruses continue to rise, the development of diagnostic kits capable of early detection of viruses becomes important, and the demand for diagnostic kits is also increasing. Figure 1 shows the diagnostic methods for the COVID-19 virus, divided into powder diagnosis and immunodiagnosis.

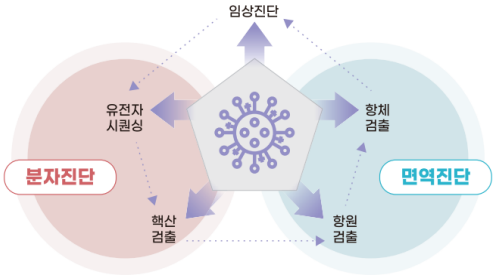

Figure 1. Diagnosis of COVID-19.

According to the Korea Disease Control and Prevention Agency [2], 87.1% of the first dose, 86.1% of the second, and 56.4% of the third were vaccinated against COVID-19. And the number of confirmed cases of COVID-19 was 627,889 males and 611,398 females. By age, 0-9 years old 116,385, 10-19 years old 160,735, 20-29 years old 220,852, 30-39 years old 182,905, 40-49 years old 187,644, 50-59 years old 157,237, 60-69 years old 128,748, 53,737 people aged 70-79 years and 31,044 people aged 80 years or older. Looking at the fatality rate by age of the confirmed cases, 0.85% for those aged 60-69 and there was not much population over the age of 70, but the fatality rate for those aged 70-79 was 3.55% and 11.29% for those over 80. The fatality rate was a value obtained by converting the number of deaths/number of confirmed cases * 100. The number of daily confirmed cases per 100,000 people was 53,926. (As of '22.2.11, 00:00, the number of confirmed cases of COVID-19 reported to the KDC A integrated disease and health management system, subject to change depending on the progress of epidemiological investigations, etc.).

In Korea, the diagnostic standard for COVID-19 was based on the test standards for diagnosis, regardless of the clinical aspect, by setting the test standards for diagnosis for patients who have been confirmed to have COVID-19 infection, so that the detection of the COVID-19 and virus isolation were PCR performed. More recently, more attention has been paid to the impact on omicron mutations than COVID-19. Omicron was more easily transmitted than the COVID-19 prototype virus or delta mutation. The CDC said it was expected that individuals infected with Omicron will spread the virus to others, regardless of vaccination status or symptoms. Virus testing checks a sample taken from your nose or mouth to determine if you were currently infected with the virus that causes COVID-19. Virus testing can be done in a laboratory, laboratory, or at home or other location. Two types of virus testing were used: Nucleic Acid Amplification Tests (NAAT) and Interim Guidance for Antigen Testing for SARS-CoV-2 [3].

In order to monitor and respond to mutated viruses appearing at home and abroad, the Inspection and Analysis Team (New Pathogen Analysis Division) of the Diagnostic and Analysis Team of the Central Quarantine Countermeasures Headquarters reflects the WHO's mutant virus classification system (February 25, 2021) into major mutations and other mutations. It was said that the mutation system was classified and actively monitored. Mutant viruses were divided into Variant of Concern (VOC) and Variant of Interest (VOI). It was said that the mutant virus was named with the Greek alphabet (alpha, beta, gamma, etc.) to prevent the use of regional names and facilitate communication [4].

According to the CDC [5], omicron mutations are more easily transmitted than COVID-19 protovirus or delta mutations. The CDC said it expects people infected with Omicron to spread the virus to others, regardless of their vaccination status or symptoms. As for symptoms, it was explained that those infected with the Omicron mutation also showed symptoms similar to those of the previous mutation. In addition, the presence and severity of symptoms may vary depending on the status of COVID-19 vaccination, the presence of other underlying medical conditions, age, and previous infection history.

Recently, with the rapid spread of Omicron infection, the need for more accurate and purifying diagnostic tools has emerged.

This study aims to find a diagnostic test method through a faster and simpler method by collecting references on various diagnostic methods, and to inform more infected people of a rapid and accurate diagnostic tool method. In addition, by analyzing the status of rapid and accurate diagnostic kit development and diagnostic analysis methods, it was intended to identify analysis and diagnosis trends in each country.

II. Research Method

A. Research literature criteria and data selection method

In this study, in order to examine the application of COVID-19 and diagnostic techniques, Omicron, diagnosis, RT-PCR, COVID-19, and analysis were searched as keywords through Google, and references about the latest diagnostic techniques were used.

B. Definition of Major Mutant Viruses

It refers to a mutant virus that has increased transmission power or negative epidemiologic changes, increased pathogenicity or clinically confirmed disease severity, and reduced effectiveness of diagnosis, vaccine, and treatment. On November 30, 2021, the US Government SARS-CoV-2 Related Organizations Group (SIG) classified Omicron as a variant of concern (VOC). The SIG variant classification scheme defines four classes of SARS-CoV-2 variants. The variants being monitored (VBM) were alpha (B.1.1.7 and Q strains), beta (B.1.351 and derivative strains), gamma (P.1 and derivatives), Epsilon (B.1.427, B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), and zeta (P.2) [6].

C. Variant classification method[6]

The US Department of Health and Human Services (HHS) was making various efforts by improving cooperation among ministries such as the CDC, FDA, and NIH and establishing SARS-CoV-2 SIG-related (DoD) organizations. This group of institutions rapidly characterizes new mutations and monitors vaccines, therapeutics, and diagnostics for SARS-CoV-2. The SIG meets regularly to assess the risks posed by the SARS-CoV-2 mutation that was prevalent in the United States and to make recommendations on mutation classification. In this evaluation, each subject expert group evaluates the data. Available data include mutation rates at national and regional levels, effects of mutation populations on medical measures, disease severity, and potential or known effects on human-to-human transmission. Given the continued evolution of SARS-CoV-2 and the public health impacts of the mutation, the mutation may be reclassified based on its nature and prevalence in the United States.

III. Results

A. Current status of COVID-19 gene analysis

The Inspection and Analysis Team (Novel Pathogen Analysis Division) of the Central Quarantine Countermeasures Headquarters was continuously operating the analysis rate to respond to the occurrence of COVID-19 mutations, and announcing the analysis results every week. The analysis exceeds the recommended standard for genome analysis of WHO and ECDC of 5 to 10%. Genetic information that has been analyzed was sequentially registered in the overseas GISAID (Global Initiative for Sharing All Influenza Data). Recently, it was announced that the fatality rate of Omicron, which has a high epidemic rate and high infection rate, was 1/4 of that of Delta. When the third dose was completed, the fatality rate of Omicron was equal to 0.08% and the seasonal flu fatality rate was the same as 0.05~0.1%. Without vaccination, the fatality rate of Omicron was 0.5%, which was 5 to 7 times higher than that of seasonal flu. Therefore, it was analyzed that if vaccinated, the fatality rate of Omicron would be similar to or lower than that of seasonal flu [2].

B. One-step qualitative RT-PCR assay[7]

A fast and accurate molecular biological method was used to effectively distinguish Omicron from other SARS-CoV-2 variants. In this study, a clinical specimen sample was detected in the upper respiratory tract and the SARS-CoV-2 Omicron variant was revealed by RT-PCR analysis. In particular, this assay did not show cross-reactivity with other SARS-CoV-2 variants, including Delta (B.1.617.2). This SARS-CoV-2 Omicron variant RT-PCR laboratory developed assay was said to be sensitive and specific for detecting Omicrons in nasopharyngeal and nasal swabs. As shown in figure 1, 2 were alignment of the SARS-CoV-2 Omicron variant RT-PCR assay's primers and probes sequence.

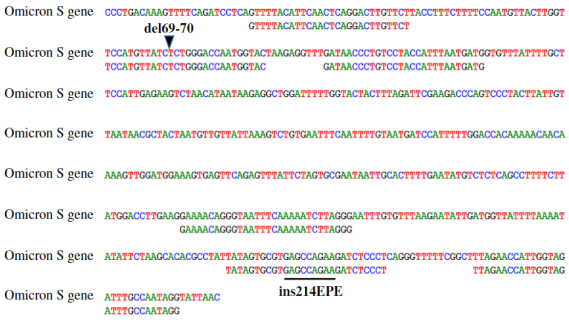

Fig 1. Alignment of the SARS-CoV-2 Omicron variant RT-PCR assay's primers and probes [7]

Primers & Probes	Sequences (5' - 3')	Targets
S-del69-70-F	GTT TTA CAT TCA ACT CAG GAC TTG TTC T	Spike gene del69-70
S-del69-70-R	CAT CAT TAA ATG GTA GGA CAG GGT TAT C	
S-del69-70-P	56-FAM/TC CAT GTT A/ZEN/T CTC TGG GAC CAA TGG TAC /3IABkFQ	
S-ins214EPE-F	GAA AAC AGG GTA ATT TCA AAA ATC TTA GGG	Spike gene ins214EPE
S-ins214EPE-R	CCT ATT GGC AAA TCT ACC AAT GGT TCT AA	
S-ins214EPE-P	56-FAM/TA TAG TGC G/ZEN/T GAG CCA GAA GAT CTC CCT /3IABkFQ	
RP-F	AGA TTT GGA CCT GCG AGC G	RNase P
RP-R	GAG CGG CTG TCT CCA CAA GT	
RP-P	56-FAM/TT CTG ACC T/ZEN/G AAG GCT CTG CGC G/3IABkFQ/	

Fig 2. Sequences of the primers and probes used in the SARS-CoV-2 Omicron variant RT-PCR assay [7]

C. Four RT-qPCR assays[8]

This study described four RT-qPCR assays that can rapidly identify the continually emerging SARS-COV-2 Omicron (B.1.1.529) strain. This assay targets micron-specific mutations in the nsp6 (Orf1a), spike and nucleocapsid genes. The four diagnostic assays described here are designed to aid in the rapid identification of Omicrons in the laboratory. The protocol presented in this study was said to be inexpensive, rapidly diagnosed, and easily modified. Compared to the analysis that detects general mutations such as the spike gene 69-70 deletion or the N501Y mutation, it was very accurate in identifying suspected Omicrons. Although the results of this study were a preliminary process, it was said that the results were presented quickly to make an additionally optimized method later. At this point, it seems to be the most important time to disclose it to the international community and find a way to respond quickly. Figure 3 showed the primer sequences used in this study.

Primer name	Sequence 5'->3'	Reference
N_{del} positive detection		
28322 Fwd	TTTGGTGGACCCTCAGATTC	This study
28424 Rev	CGCAGTATTATTGGGTAAACCTTG	This study
28354 probe	CCAGAATGGTGCGGCGCGATC	This study
N_{del} negative detection		
2019-nCoV_N1-F1a	CTAAACGAACAAACTAAAATGTCTG	US CDC[3,a]
2019-nCoV_N1-R1a	GCCCCACTGCGTTCTCCATTC	This study
2019-nCoV_N1-P	ACCCCGCATTACGTTTGGTGGACC	US CDC[3]
S_{indel} reaction		
22174 Fwd	GTTATTTTAAAATATATTCTAAGCACACG	This study
22248 Rev	TAAAGCCGAAAAACCCTGAG	This study
22206 probe	ATTATAGTGCGTGAGCCAGAAGATCTCC	This study
Nsp6 deletion reaction		
11248 B529 Fwd	ATATGGTTGATACTAGTTTTAAGC	This study
COV19_11310_P	CTGTGTTATGTATGCATCAGCTGTAGT	This study
COV19_11344_R	ACACAGTTCTTGCTGTCATAAGG	This study
E-sarbeco reaction		
E_Sarbeco_F1b	GTTAATAGCGTACTTCTTTTTCTTGC	Corman *et al.*[1,a]
E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	Corman *et al.*
E_Sarbeco_P1	ACACTAGCCATCCTTACTGCGCTTCG	Corman *et al.*

[a]This sequence was modified as described in Reference 2, to render it more suitable for multiplexing

Fig 3. Sequences of the primers [8]

D. RAMP

The recent rapid spread of the novel coronavirus (SARS-CoV-2, COVID-19) was seriously putting pressure on the capabilities of the global health community. As a result, many countries needed rapid lockdown and prompt diagnosis. Here, we will describe various LAMP methodology as an approach for rapid and robust analysis for on-site diagnosis of COVID-19 [9]. Loop mediated isothermal amplification (LAMP) is a novel isothermal nucleic acid amplification method. The molecular diagnostic method of LAMP uses a DNA polymerase and a primer set that recognizes four specific sequences that recognize a total of six different sequences of the target DNA. The inner primer containing the sequences of the sense and antisense strands of the target DNA begins with LAMP. The next strand primed by an external primer releases single-stranded DNA. Since LAMP recognizes a target with 6 different sequences initially and then with 4 different sequences, LAMP amplifies the target sequence with high selection [10].

According to a study by El-Tholoth *et al* [11], the closed tube Penn-RAMP can be used with minimal equipment and training. The study states that closed tubes, single-stage COVID-19 LAMPs and COVID-19 closed tubes for use in homes, clinics and entry points can be used for Penn-RAMP analysis. This analytical method is used to minimize the cost and time required for sample processing. The reaction tubes are incubated at 63℃ in the LAMP process and at 38℃ and 63℃ in the two-step Penn-RAMP process. Incubation does not require thermal cycling and can be performed with simple equipment. It was said that quantification was possible because the amplification process can be monitored in real time with a fluorescent dye. Table 1 was the primer sequence used in this study.

Table 1. Sequences of primers used by El Tholoth *et al* [11]

Primer	Sequence (5′ – 3′)
F3	TGCTTCAGTCAGCTGATG
B3	TTAAATTGTCATCTTCGTCCTT
FIP	TCAGTACTAGTGCCTGTGCCCAC ATCGTTTTTAAACGGGT
BIP	TCGTATACAGGGCTTTTGACAT CTATCTTGGAAGCGACAACAA
Loop F	CTGCACTTACACCGCAA
Loop B	GTAGCTGGTTTTGCTAAATTCC

E. Korean Diagnostic Test Method

On January 15, 2020, the Korea Centers for Disease Control and Prevention and academic experts gathered to discuss the possibility of the introduction of COVID-19 and its impact on Korea. On January 20, 2020, the Korean Society of Laboratory Medicine organized a special team to respond to COVID-19 made up of specialists in laboratory medicine to prepare for the rapid introduction of the COVID-19 diagnostic test. The three preconditions for the introduction of the COVID-19 diagnostic test (diagnostic kit evaluation, inspection personnel training, external quality control program) were confirmed and agreed to cooperate. The evaluation was conducted by diluting the samples from 3 confirmed patients in Korea to 4 concentrations and repeating the measurement 3 times, and inserting two genes (E, RdRp) into the plasmid DNA to measure them. On February 4, 'PowerChek The 2019-nCoV Real-time PCR kit (Kogene Biotech, Korea)' was

approved for emergency use by the Ministry of Food and Drug Safety for the first time and was used [12].

IV. CONCLUSION AND DISCUSSION

As confirmed in various studies, fast and simple test and diagnosis methods are continuously being studied. As of May 18, 2020, the reagents approved for emergency use for in vitro diagnosis of COVID-19 announced on the Korea Centers for Disease Control and Prevention website are those of Kogen Biotech, Seegene, Solgent, SD Biosensor, Bioseum, and BioCore [2]

In Korea, there are three methods of diagnosing COVID-19, and there is a lot of interest. These three methods are based on sensitivity and specificity. The diagnostic tests used in the provisional selection laboratory are RT-PCR, RT-LAMP, and antigen-based tests. RT-PCR and RT-LAMP test time is less than 24 hours. In contrast, the antigen-based test has the advantage of being able to check the results in more than 30 minutes. However, in the sensitivity part, antigen-based test is 90% lower than RT-PCR and RT-LAMP. Recently, in Korea, anyone with no suspected symptoms can receive a corona diagnosis test. As the spread of Omicron has increased, recently, the saliva test method, which can be easily tested at front-line medical institutions, has been sequentially disseminated so that people can receive tests conveniently. This will be a measure to overcome the most difficult test equipment shortage and difficulty in collecting samples during diagnostic tests.

Administrative agencies in charge of quarantine plan to actively introduce antigen testing that allows immediate confirmation of test results at medical sites. Since this test has the advantage of being able to diagnose without separate diagnostic equipment, it is highly useful in emergency rooms and areas where PCR test is not possible, and it is judged to be highly useful for screening tests in nursing hospitals. To this end, rapid antigen testing and PCR testing using saliva samples are being promoted to dramatically expand testing capabilities and to improve preemptive testing methods in facilities susceptible to infection, such as nursing hospitals [4].

The purpose of this study was to investigate the types and characteristics of accurate molecular diagnostic tests. These molecular diagnostic tests are considered to be important for prompt and accurate diagnosis, distinguishing infected persons, and coping with appropriate isolation and treatment. In addition, this study is intended to help the general public understand molecular diagnostic testing methods.

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