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ORIGINAL ARTICLE

Comparison of Positive/Inconclusive Xpert Xpress SARS-CoV-2 with the Standard M nCoV Real-Time Detection

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SUMMARY

Background: **COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can be diagnosed using rapid real-time PCR, real-time reverse transcription PCR (rRT-PCR), or rapid antigen testing. Among these, rRT-PCR is considered the gold standard assay. The Xpert Xpress SARS-CoV-2 assay is a rapid real-time PCR test, approved by the Korean Disease Control and Prevention Agency in 2020. The overall concordance and positive concordance rates of the Xpert assay with the STANDARD M nCoV Real-Time Detection kit were determined.**
Methods: **All samples with positive or inconclusive Xpert test results from July 2021 to February 2023 that underwent confirmatory testing using the reference rRT-PCR assay were included in the analysis.**
Results: **Samples from 224 patients (93 men and 131 women) with a median age of 59 years (range 15 - 90 years) were included. Of 212 samples that tested positive using Xpert, 112 (52.8%) were true positves and 100 (47.2%) were false positives on rRT-PCR testing. The overall concordance and positive concordance rates were 52.8% (112/212) and 54.5% (112/224), respectively. In the Xpert positive group, the samples had a lower Ct value for the *E* gene than the *N2* gene. The Ct values for the *E* and *N2* genes were significantly lower in the positive group than in the inconclusive group.**
Conclusions: **Positive or inconclusive Xpert results should be confirmed by the gold standard rRT-PCR for early control of this disease. Furthermore, Korea's policy should be reconsidered given the high false-positive rate of the rapid real-time PCR Xpert Xpress SARS-CoV-2 assay.**
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KEYWORDS

Coronavirus disease 2019 (COVID-19), Xpert Xpress SARS-CoV-2 assay, real-time reverse transcription PCR, concordance rate

INTRODUCTION

COVID-19 is a highly infectious respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The COVID-19 pandemic started in December 2019 [1-6]. COVID-19 can be diagnosed using various methods, including rapid real-time, real-time reverse transcription PCR (rRT-PCR), or rapid antigen tests [7-9]. Among these, rRT-PCR is regarded as the gold standard assay [3,10,11]. The Xpert Xpress

SARS-CoV-2 assay (Xpert; Cepheid, Sunnyvale, CA, USA) is a rapid real-time PCR assay approved by the Korean Disease Control and Prevention Agency (KDCA) in 2020 [11-13].

We determined the overall concordance rate and positive concordance rate of the Xpert assay with the STANDARD M nCoV Real-Time Detection kit (SD; SD Biosensor, Suwon, Korea) as the reference test. Several studies have compared other types of SARS-CoV-2 tests. However, few studies have performed confirmatory testing of samples using SD within 24 hours after testing them using Xpert. The aim of this study was to compare the results of samples with positive and inconclusive results using the Xpert assay with the results of the gold standard test for SARS-CoV-2.

MATERIALS AND METHODS

Sample selection

The target samples were all samples tested with Xpert from July 2021 to February 2023. Samples were collected with a nasopharyngeal swab by trained personnel following standard procedures. The swab was inserted in universal transport medium (Seegene Inc., Seoul, Korea) and transported to the laboratory in triple-layered packaging. We analyzed the distribution of positive, inconclusive, and negative results of all samples tested for SARS-CoV-2 using the Xpert assay. All samples that tested positive or inconclusive using Xpert that underwent confirmatory testing using the SD assay within 24 hours after the Xpert test were included (Figure 1). Samples with negative results on Xpert were excluded because most of these samples did not undergo confirmatory testing. Samples that underwent confirmatory testing more than 24 hours after the Xpert test were also excluded.

This retrospective study used deidentified electronic medical data collected by an information technology team. The protocol was approved by Institutional Review Board of Korea Caner Center Hospital (2023-04-004), and the study was carried out in accordance with the Declaration of Helsinki. The requirement for informed consent was waived because of the retrospective study design. Samples used for Xpert and confirmatory tests were stored at 4℃ in a refrigerator, and repeat samples were analyzed as soon as possible, without storage.

Test methods

The Xpert system is a fully automated and integrated PCR-based nucleic acid detection system that takes only 45 minutes and has a hands-on time of less than 5 minutes. The Xpert assay targets the nucleocapsid (N2 region of the *N* gene) and envelope (*E*) genes of SARS-CoV-2, and the result is positive when the cycle threshold (Ct) value is less than 45 cycles for both nucleic acid targets (*N2* and *E*) or for *N2* alone [13,14]. If the Xpert result was positive or inconclusive, the sample was tested using SD, the gold standard method, and the results of Xpert and SD were compared. SD tests were conducted using the STANDARD M nCoV Real-Time Detection kit (SD Biosensor, Suwon, Korea) and CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) according to the manufacturer's guidelines. SD targets the RNA-dependent RNA polymerase (*RdRP*) and *E* genes.

Analysis of results

The Xpert-positive rate referred to all tests with a positive Xpert result, regardless of the SD result. A concordant result was defined as a test result that had the same result using the Xpert and SD tests when the Xpert results were positive or inconclusive. A discordant result referred to a test result that differed using Xpert and SD tests. We analyzed rRT-PCR results according to whether both genes or only the *N2* gene was positive using Xpert.

Statistical analysis

Continuous data were compared using *t*-tests, analysis of variance, and Tukey's post-hoc analysis. Normality tests were performed on the data with Kolmogorov-Smirnov test. p-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 29 (IBM Corp., Armonk, NY, USA).

RESULTS

Of the 5,280 samples tested using Xpert, 224 satisfied the inclusion criteria (Figure 1). The majority of the samples (n = 4,832, 91.5%) tested negative (Table 1). The 224 samples were obtained from 93 men and 131 women with a median age of 59 years (range, 15 – 90 years). The overall concordance and positive concordance rates were 52.8% (112/212) and 54.5% (112/224), respectively (Table 2). The overall-positive and false positive rate on Xpert were 50.0% (112/224) and 47.2% (100/212), respectively. The Ct results of Xpert *E* gene and *N2* gene tested when Xpert results were positive or inconclusive are shown in Figure 2.

The positive/positive (Xpert/SD), positive/inconclusive (Xpert/SD), and positive/negative (Xpert/SD) samples had significantly different Ct values on the Xpert assay ($p < 0.001$) (Table 3.). However, there were no significant differences between the Ct values of the positive/inconclusive and positive/negative subgroups ($p = 0.668$).

We analyzed the rRT-PCR results according to both gene positivity and *N2* gene positivity using the Xpert assay (Table 4). When the *E* gene was detected using Xpert, the SD result tended to be positive, and when the *E* gene was not detected using Xpert, the SD result tended to be negative. Only 12 inconclusive results were obtained on Xpert testing (Table 4). Detailed statistical analysis was not possible because of the small

Table 1. Results of the Xpert Xpress SARS-CoV-2 assay results.

Results	n (%)
Positive	320 (6.1)
Inconclusive	128 (2.4)
Negative	4,832 (91.5)
Total	5,280 (100.0)

Table 2. Comparison of the Xpert Xpress SARS-CoV-2 assay (Xpert) and STANDARD M nCoV Real-Time Detection kit (SD) test results.

Xpert	SD			
	Positive	Inconclusive	Negative	Total
Positive, n (%)	112 (52.8)	12 (5.7)	88 (41.5)	212 (94.6)
Inconclusive, n (%)	2 (16.7)	0 (0.0)	10 (83.3)	12 (5.4)
Total, n (%)	114 (48.7)	12 (5.4)	98 (43.8)	224 (100.0)

SD - STANDARD M nCoV Real-Time Detection kit, Xpert - Xpert Xpress SARS-CoV-2 test.

Table 3. Real-time assay Ct values of envelope (*E*) and nucleocapsid (*N2*) genes in SARS-CoV-2-positive and inconclusive results using the Xpert assay.

SD								
Xpert	Positive (n = 113)		Inconclusive (n = 19)		Negative (n = 90)		Total (n = 234)	
	Xpert E	Xpert N2	Xpert E	Xpert N2	Xpert E	Xpert N2	Xpert E	Xpert N2
Positive (n = 212)	23.0 ± 7.6	25.6 ± 8.6	34.5 ± 3.6	39.7 ± 3.1	38.4 ± 4.9	41.7 ± 2.3	25.1 ± 8.9	33.1 ± 10.2
Inconclusive (n = 12)	NA	42.6 ± 0.2	NA	NA	NA	42.3 ± 0.5	NA	42.3 ± 0.5
Total (n = 224)	23.0 ± 7.6	25.6 ± 8.6	34.5 ± 3.2	39.7 ± 3.1	38.4 ± 4.9	41.7 ± 2.3	25.1 ± 8.9	33.6 ± 10.2

NA - not applicable, SD - STANDARD M nCoV Real-Time Detection kit, Xpert - Xpert Xpress SARS-CoV-2 test.

sample size. Among the Xpert-positive test results, the *E* gene had significantly lower Ct values than the *N2* gene ($p < 0.029$). Among the Xpert *N2* gene results, samples with positive results had significantly lower Ct values than those with inconclusive results ($p < 0.001$). The *N2* gene, but not the *E* gene, was detected in all the inconclusive Xpert test results. Detailed statistical analysis was not possible because of the small sample size.

DISCUSSION

We determined the positive-concordance rate, overall concordance rate, and false-positive rate for all Xpert SARS-CoV-2 tests compared with the gold standard SD rRT-PCR. Our data showed a 47.2% false-positivity rate on Xpert compared with SD rRT-PCR. The Xpert assay takes only 45 minutes, but positive results require confirmation, so false-positive results are time-consuming and labor intensive [7].

Some limitations of this study should be considered. This was a retrospective study; therefore, the available sample size was limited. We did not include Xpert data

Table 4. Characteristics of 12 cases of inconclusive test results using the Xpert Xpress SARS-CoV-2 assay.

Case No.	Gender/Age	Xpert			SD		
		E	*N2*	Result	*RdRP*	*E*	Result
1	F/48	ND	42.9	inconclusive	ND	ND	negative
2	F/84	ND	42.1	inconclusive	ND	ND	negative
3	F/68	ND	42.3	inconclusive	ND	ND	negative
4	F/74	ND	41.4	inconclusive	ND	ND	negative
5	F/79	ND	41.8	inconclusive	ND	ND	negative
6	M/67	ND	42.9	inconclusive	ND	ND	negative
7	M/62	ND	42.1	inconclusive	ND	ND	negative
8	M/30	ND	42.8	inconclusive	ND	ND	negative
9	M/35	ND	42.4	inconclusive	ND	ND	negative
10	M/44	ND	42.2	inconclusive	ND	ND	negative
11	F/61	ND	42.4	inconclusive	31.60	30.50	positive
12	M/63	ND	42.7	inconclusive	32.53	31.88	positive

ND - not detected, SD - STANDARD M nCoV Real-Time Detection kit, Xpert - Xpert Xpress SARS-CoV-2 test.

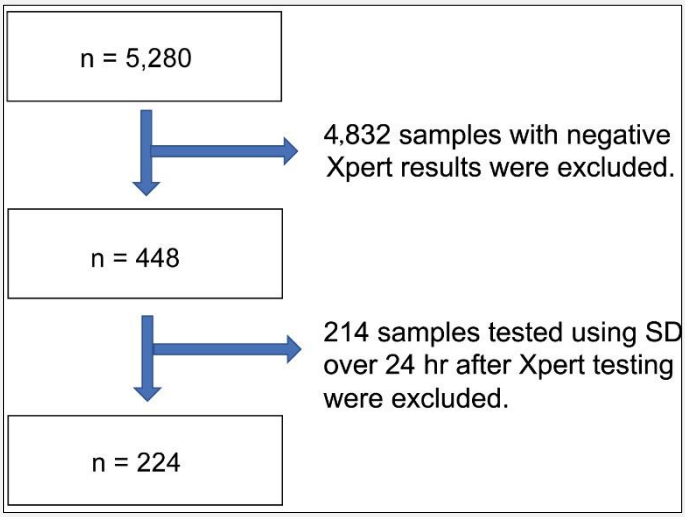

Figure 1. Total number of samples tested by the Xpert Xpress SARS-CoV-2 assay.

on the tests that were negative on Xpert because there was a policy change regarding Xpert results. In the early stages of the COVID-19 pandemic, the KDCA control guidelines specified that all Xpert-positive SARS-CoV-2 test results must be confirmed using rRT-PCR. However, the policy was later changed, and Xpert-positive test results alone could be used to make a definite diagnosis of COVID-19, without confirmatory testing. In addition, most patients with Xpert-negative test results did not undergo confirmatory testing using the SD test. Therefore, we were unable to determine the sensitivity of the Xpert assay. Future investigations involving multicentric studies with larger sample sizes may help to better understand the clinical relevance of Xpert SARS-CoV-2 testing in different healthcare settings and its diagnostic accuracy. We used de-identified medical data

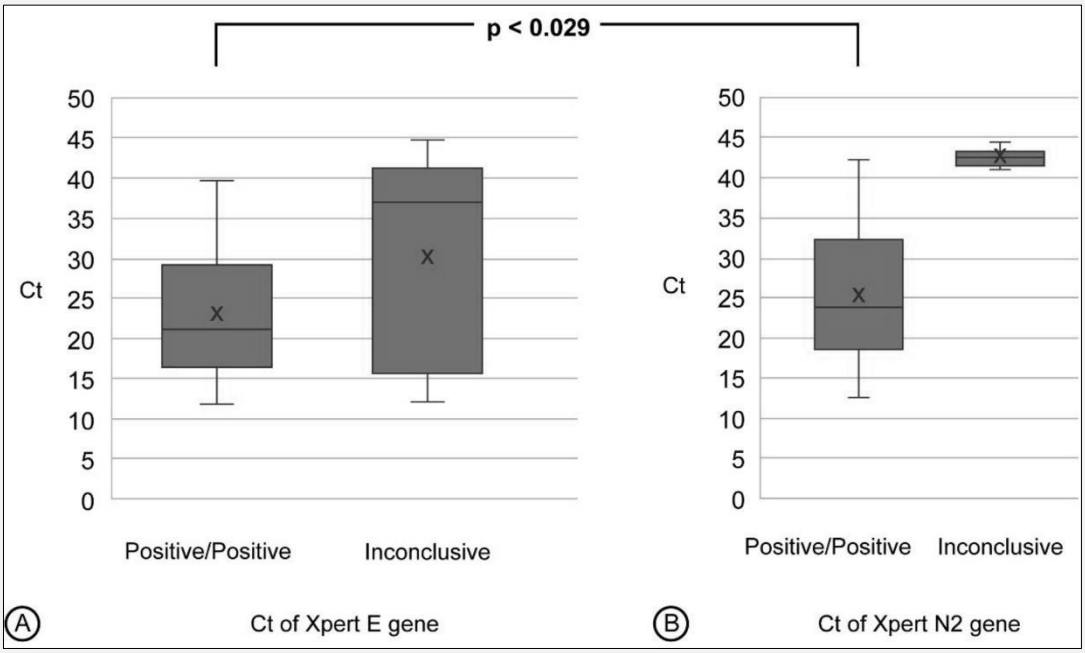

Figure 2. Box plot of Ct values of envelope (*E*) and nucleocapsid (*N2*) genes in SARS-CoV-2-positive and inconclusive results using the Xpert assay.

obtained from the information technology team and were unable to ascertain the time of sampling, or determine whether the sample used for SD testing was the same as the one used for Xpert testing, or a repeat sample. The SD samples were tested after Xpert within 24 hours. Several previous studies have found that the Xpert SARS-CoV-2 test is highly sensitive and specific, although in some studies the number of samples was limited [15-18]. Nonetheless, our data showed only a 52.8% positive-concordance rate and 47.2% of false-positive rate compared with SD. Other studies have reported that the diagnostic accuracy of Xpert is comparable to that of rRT-PCR [15-18], which was not confirmed by our study. Other studies have suggested that Xpert results should be confirmed using a gold standard method [20-23].

In conclusion, the overall concordance, positive concordance, and false-positive rates of Xpert with the gold standard SD assay were 52.8% (112/212), 54.5% (112/224), and 47.2% (100/212), respectively. In our study, among the Xpert positive samples, the *E* gene had a lower Ct value than that of the *N2* gene in contrast to another study [15]. The Ct values for the *E* and *N2* genes were significantly lower in samples with positive results than those with inconclusive results. The Ct values differed significantly according to whether the sample was positive/positive, positive/inconclusive, and positive/negative when tested using the Xpert and SD tests. However, there was no significant difference between the positive/inconclusive and positive/negative subgroups. Based on our results, we suggest that positive or inconclusive Xpert results should be confirmed using rRT-PCR as a gold standard test [20-23] for infection control purposes. Furthermore, we suggest that Korea's policy regarding Xpress testing should be reconsidered because of the high false-positive rate associated with this assay.

Declaration of Interest:

The authors have no conflicts of interest.

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