Original Article

Positive Concordance between Commercially Available SARS-CoV-2 Rapid Antigen Test and RT-PCR during the Omicron Wave in Thailand

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Background: Rapid SARS-CoV-2 antigen tests are widely used throughout the world. False positive antigen tests have been reported.

Objective: To evaluate the positive percentage concordance (PPC) between a rapid antigen test and the nucleic acid amplification testing (NAAT).

Materials and Methods: The present study was a retrospective laboratory-based study on rapid antigen test-positive nasopharyngeal or throat swabs sent for confirmation by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) between January 1 and April 8, 2022. The primary outcome was a PPC between antigen-positive samples and PCR-positive samples.

Results: Of the 22,808 samples received, there were 3,656 or 16.04% of the samples with documented positive rapid antigen tests sent for confirmation by rRT-PCR. Overall, PPC was 92.67%, 95% CI 91.82 to 93.51. A higher PPC was found during the BA.2-dominant Omicron variant period at 96.08% (95% CI 95.2 to 96.95).

Conclusion: The PPC between the rapid antigen test used and rRT-PCR was very high, especially during the BA.2-dominant period.

Keywords: Rapid antigen test; Omicron; rT-PCR; Positive percentage concordance

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During the Omicron variant wave, there was a steep rise in COVID-19 cases. Test and isolate strategies using rapid antigen tests play an important role during the widespread infection in the community suggested by the World Health Organization (WHO)[1]. They provide a rapid diagnosis of SARS-CoV-2 infection with low cost compared to nucleic acid amplification testing (NAAT). According to a recent meta-analysis, the sensitivity of rapid antigen tests used during the Omicron variant compared to the Delta variant was 37.1% with a range of 23.3% to 53% versus 81% with a range of 65.2% to 90.6%, respectively[2]. Despite having a lower sensitivity, the specificity remains as high as 100%[2]. However, false positive results of antigen tests have been reported, citing up to 42% false positive antigen test results[3]. Positive percentage agreement (PPA) between rapid antigen tests and NAAT may be as low as 51.9% according to a previous report[4].

In the present study, the authors examined the positive percentage concordance (PPC) between rapid antigen tests used in the present study community versus NAAT as a confirmation test.

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Materials and Methods

Study design, setting, and data collection

The authors performed a retrospective laboratory-based study at the center of the molecular laboratory, Suddhavej Hospital, Faculty of Medicine, Mahasarakham University, between January 1 and April 8, 2022. The study samples consisted of all nasopharyngeal or throat swabs with documented positive rapid antigen tests by saliva, nasal, or nasopharynx, or nasal plus saliva samples. These samples were obtained by self-swabbing or health care provider-swabbing. Additional samples from antigen-positive patients were obtained and subsequently sent to the authors' molecular laboratory for confirmation

of SARS-CoV-2 detection by polymerase chain reaction. The data were retrieved from the present study molecular laboratory records and computer database in collaboration with the Ministry of Public Health Co-Lab 2 system.

Nasopharyngeal or throat samples were collected in viral transport medium (VTM) and sent to the molecular laboratory within working hours. DNA extraction was performed per the manufacturer's instructions. Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) was performed using four different detection kits as follows, 1) regular DaAn Gene 2019-nCoV kit (Guangzhou, China), 2) Fast DaAn Gene 2019-nCoV kit (Guangzhou, China), 3) Uni-medica Real-time PCR kit for 2019-nCoV (Guangdong, China), and 4) STANDARD™ M10 SARS-CoV-2 multiplex rRT-PCR (SD Biosensor, Gyeonggi, Republic of Korea). For the regular and Fast DaAn Gene 2019-nCoV kit, the primers target the *N* gene or open reading frame 1ab (*ORF1ab*), while the Uni-Medica real-time PCR kit was used for the 2019-nCoV target *E* gene, *ORF1ab* gene, and *N* gene. The cycle threshold cut-off per the manufacturer's recommendation for each detection kit was as follows: 1) 40 or less, 2) 30 or less, 3) 40 or less, and 4) 38 or less. The limit of detection (LOD) for each kit was 500 copies/milliliter (mL) for regular and Fast DaAn Gene, 200 copies/mL for Uni-medica Real-time PCR kit for 2019-nCoV, and 6.63×10^{-4} $TCID_{50}$/mL for STANDARD™ M10 SARS-CoV-2 multiplex rRT-PCR. Interpretation of the rRT-PCR results was classified as positive, negative, and invalid or inconclusive per each kit's instructions.

The decision of which PCR detection kit would be used depended upon the availability of the detection kits during each period and per the technicians' discretion. As of April 23, 2022, there were 293 and 186 rapid antigen test kits approved for self-antigen and health care provider use, respectively, and 11 kits approved for semi- or fully automated antigen tests[5-7].

Outcome

The primary outcome of the study was a PPC between antigen-positive samples and PCR-positive samples. Secondary outcomes included Ct values and PPC comparison and analyses for each detection kit and during each month.

Statistical analysis

Categorical data were expressed as frequencies and percentages and compared using the chi-square test. Ninety-five percent confidence intervals (95% CI) were calculated using a binomial model. Continuous variables were expressed as the median and interquartile range (IQR) and were compared using Kruskal-Wallis and Dunn's correction for multiple comparisons. The median difference was calculated using the Mann-Whitney test. IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9 were used for statistical analysis. A p-value less than or equal to 0.05 (two-sided) was considered significant.

Ethical approval

Exemption for informed consent was granted according to Mahasarakham University's Ethics Committee for research involving human subjects (No. 160-183/2565).

Results

Of the 22,808 samples received, there were 3,656 (16.04%) samples with documented positive rapid antigen tests sent for confirmation by rRT-PCR. Samples were sent from five community hospitals from five districts in Maha Sarakham Province and from Suddhavej's acute respiratory tract infection clinic (ARI clinic) as follows, Phayakkhaphum Phisai 1,825 (49.92%), Na Dun 625 (26.2%), Wapi Pathum 146 (3.99%), Chiang Yuen 54 (14.8%), Kae Dam 48 (1.31%), and Suddhavej's ARI clinic 625 (17.1%).

Of the 3,656 samples, 3,388 samples with a PPC of 92.67% (95% CI 91.82 to 93.51) tested positive for SARS-CoV-2 by rRT-PCR. There were 247 or 6.76% of the samples with negative rRT-PCR, eight or 0.22% with rejected specimens due to incomplete identification or leaked specimens, one or 0.03% with invalid or inconclusive results, and 12 or 0.33% with missing data. PPC was significantly lower among samples sent from Chiang Yuen at 53.7% (29/54) and significantly higher among samples sent from Suddhavej's ARI clinic at 95.9% (589/614), followed by Kae Dam at 95.8% (47/48), Wapi Pathum at 95.2% (138/145), Na Dun at 94.3% (901/955), and Phayakkhaphum Phisai at 92.6% (1,685/1,819).

Among the 3,388 positive samples tested, the Uni-Medica Real-Time PCR kit for 2019-nCoV accounted for 53.16%, followed by 40.47%, 6.23%, and 0.15% for the Fast DaAn Gene 2019-nCoV kit, regular DaAn Gene 2019-nCoV kit, and STANDARD™ M10 SARS-CoV-2 multiplex rRT-PCR, respectively. Three detection kits performed similarly except for the Fast DaAn Gene 2019-nCoV

Table 1. Positive percentage agreements for each PCR detection kit from January to April 2022

Month	PCR detection kits; Positive percentage concordance (95% CI)				
	All detection kits (n=3,656)	Uni-medica Real time PCR kit (n=1,877)	Fast DaAn Gene 2019-nCoV kit (n=1,559)	regular DaAn Gene 2019-nCoV kit (n=215)	STANDARD™ M10 (n=5)
Janurary	80.71 (76.09 to 85.34)	-	80.07 (75.32 to 84.83)	100 (100 to 100)	100 (100 to 100)
February	87.85 (85.66 to 90.04)	-	87.75 (85.54 to 89.96)	100 (100 to 100)	-
March	96.08 (95.2 to 96.95)	96.77 (95.79 to 99.94)	93.17 (90.81 to 95.53)	98.04 (96.14 to 99.94)	-
April	94.25 (92.4 to 96.1)	94.25 (92.4 to 96.1)	-	-	-

PCR=polymerase chain reaction; CI=confidence interval

Table 2. Percent positive of samples categorized by the cycle threshold value ranges between each PCR detection kit and target gene

Ct	PCR detection kits; Percent positive by Ct range (%) (95% CI)		
	Uni-medica Real time PCR kit (n=1,801)	Fast DaAn Gene 2019-nCoV kit (n=1,371)	regular DaAn Gene 2019-nCoV kit (n=211)
N gene			
0 to 9.99	0.1 (0.0 to 0.2)	23.1 (20.9 to 25.5)	0.0 (0.0 to 0.0)
10 to 19.99	56 (53.6 to 58.2)	64.5 (61.9 to 67.0)	4.7 (1.9 to 8.1)
20 to 29.99	31.3 (29.3 to 33.4)	12.4 (10.8 to 14.3)	80.6 (75.4 to 85.8)
30 to 39.99	12.7 (11 to 14.3)	-	14.7 (10 to 19)
ORF1ab gene			
0 to 9.99	0.1 (0.0 to 0.2)	10.6 (9 to 12.3)	0.0 (0.0 to 0.0)
10 to 19.99	50.6 (48.4 to 53)	74.3 (71.9 to 76.4)	23.7 (18 to 29.4)
20 to 29.99	35.3 (33.1 to 37.4)	15.1 (13.3 to 17)	67.8 (61.6 to 73.9)
30 to 39.99	14.0 (12.3 to 15.7)	-	8.5 (4.7 to 12.3)
E gene			
0 to 9.99	0.0 (0.0 to 0.0)	-	-
10 to 19.99	33.8 (31.7 to 35.9)	-	-
20 to 29.99	47.2 (44.6 to 49.6)	-	-
30 to 39.99	19.0 (17 to 20.8)	-	-

PCR=polymerase chain reaction; CI=confidence interval

kit, which had the lowest PPC. The regular DaAn Gene 2019-nCoV kit had 98.1% (211/215), the Uni-medica Real-time PCR kit for 2019-nCoV had 96.8% (1,801/1,860), and the Fast DaAn Gene 2019-nCoV kit had 88.2% (1,371/1,555).

The PPCs were significantly higher in April and March at 94.25% (574/609) in April and 96.08% (1,836/1,911) in March versus 87.85% (752/856) in February and 80.71% (226/280) in January ($p<0.001$). These differences were also observed within each PCR detection kit during each month, as shown in Table 1.

After adjusting for months and type of PCR detection kits used (Fast DaAn Gene 2019-nCoV kit versus non-Fast DaAn Gene 2019-nCoV kit), a multivariate logistic regression model demonstrated an increase in odds ratios for a positive rRT-PCR during March at 1.99 (95% CI 1.26 to 3.12) ($p=0.003$) and a decrease in odds ratios using Fast DaAn Gene 2019-nCoV kit at 0.4 (95% CI 0.25 to 0.64) ($p<0.001$).

According to the detection kits with Ct values cut-off of 40 or less, most tested samples had Ct values ranging between 10 to 29.99 for all target genes (Table 2). For the Fast DaAn Gene 2019-nCoV kit, 64.5% (95% CI: 61.9 to 67.0) of the tested samples were positive, with Ct values ranging from 10 to 19.99 for the *N* gene and 74.3% (95% CI 71.9 to 76.4) with Ct values from 10 to 19.99 for the *ORF1ab* gene (Table 2).

For the Uni-Medica Real-time PCR kit, the *N* gene had significantly lower Ct values than the *ORF1ab* and *E* genes: median 19.22 (IQR 16.6 to 24.62) versus 19.91 (IQR 17.37 to 25.26) and 21.66 (IQR 19.13 to 26.99), respectively. These corresponded to a median difference of 0.69 (95% CI 0.37 to 0.98) for the *N* gene versus the *ORF1ab* gene ($p<0.001$) and 2.440 (95% CI 2.16 to 2.77) for the *N* gene versus the *E* gene ($p<0.001$), while the Ct value for the *E* gene was significantly lower than that for

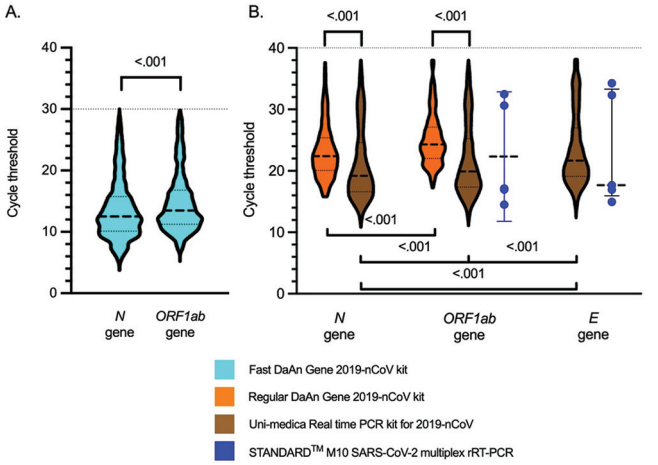

Figure 1. Truncated violin plot showing the Ct value for each target gene among the PCR detection kits. Each color labels different PCR detection kits as shown. (A) Violin plot demonstrating the Ct values of the *N* and *ORF1ab* genes derived from the Fast DaAn Gene 2019-nCoV kit. (B) Violin plots showing the Ct value derived from the Uni-Medica Real-Time PCR kit and the regular DaAn Gene 2019-nCoV kit. The blue-aligned dot plot shows the Ct value from STANDARDTM M10 SARS-CoV-2 multiplex rRT-PCR. The thick dashed line and thin dotted line within a violin plot represent the median and interquartile range (IQR).

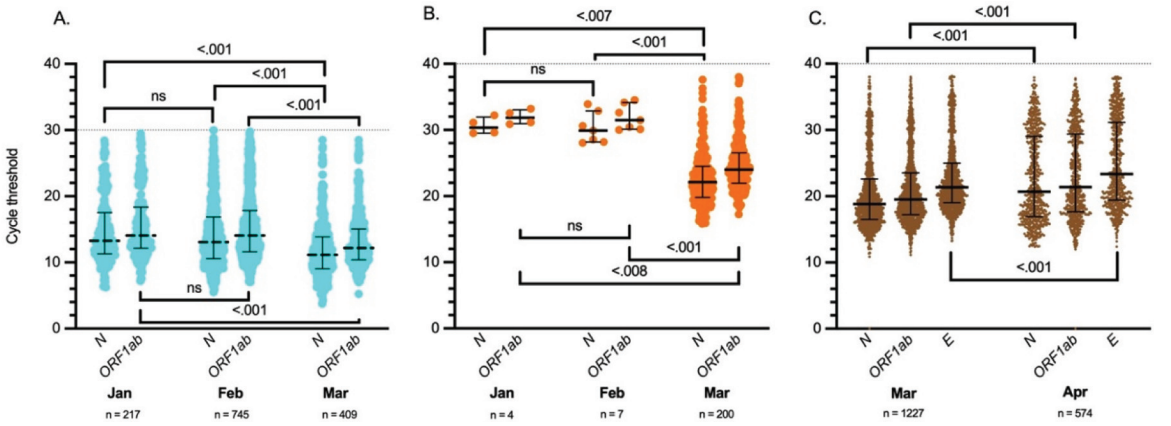

Figure 2. Scatter dot plot demonstrating the Ct value between each target gene in each month. Each color labels different detection kits, as shown in Figure 1. (A) Scatter dot plots showing the Ct values of the *N* and *ORF1ab* genes derived from the Fast DaAn Gene 2019-nCoV kit in January, February, and March. (B) Scatter dot plots showing the Ct values of the *N* and *ORF1ab* genes derived from the regular DaAn Gene 2019-nCoV kit in January, February, and March. (C) Scatter dot plots showing the Ct values of the N, *ORF1ab* and *E* genes derived from the Uni-Medica Real-Time PCR kit in March and April. The thick dashed line and thin dotted line within a scatter dot plot represent the median and interquartile range (IQR).

the *ORF1ab* gene, with a median difference of 1.75 (95% CI 1.480 to 2.100) (Figure 1). The Ct values of the *N* gene were significantly lower than those of the *ORF1ab* gene, with a median difference of 1.89 (95% CI 1.32 to 2.77) ($p<0.001$) for the regular DaAn Gene 2019-nCoV kit and 0.98 (95% CI 0.78 to 1.38) for the Fast DaAn Gene 2019-nCoV kit.

When analyzing each month, there were significant differences in the Ct values within each detection kit. For the Fast DaAn Gene 2019-nCoV kit, the Ct values of the *N* and *ORF1ab* genes from March were significantly lower than those from January and February with median differences of 2.13 (95% CI 1.97 to 3.26) and 1.94 (95% CI 1.67 to 2.65) for the *N* gene and 1.91 (95% CI 1.36 to 2.6) and 1.91 (95% CI 1.24 to 2.16) for the *ORF1ab* gene, and between January and February versus March, respectively (Figure 2A). Similar trends were observed for the regular DaAn Gene 2019-nCoV kit, where the Ct values of the *N* and *ORF1ab* genes from March were significantly lower than those from January and February (Figure 2B).

However, for the Uni-Medica Real-Time PCR kit, the Ct values of the *N*, *ORF1ab*, and *E* genes

from March were significantly lower than those from April. The median difference was 1.85 (95% CI 1.04 to 2.16) for the *N* gene and 1.89 (95% CI 1.11 to 2.2) and 2 (95% CI 1.04 to 2.17) for the *ORF1ab* gene and *E* gene, respectively (Figure 2C).

Discussion

In the present laboratory-based study, the authors found a high PPC as high as 96.08% between rapid antigen detection tests (RADTs) and rRT-PCR during the Omicron variant pandemic in Thailand. The overall results showed that PPC ranged from 80.71% to 96.08%. These proportions were concordant with 83.3% to 99.4% and 95.9% to 98.7% PPA among symptomatic COVID-19 from Brihn et al, and Leber et al, respectively, and 82.3% to 99.4% PPA from Pekosz et al, comparing RADTs versus viral culture[8-10]. However, these studies included mostly non-Omicron variants. According to the literatures conducted during the Omicron wave, the PPA was 97.87% to 98.48%, concordant to the present study results[11,12]. To the authors' knowledge, the present study is the largest to examine the PPC between RADTs and rRT-PCR during the Omicron wave. Nevertheless, there was a report that a specific batch of RADT might result in false positive up to 42%[3]. The present study results confirmed the currently used RADTs had high PPC compared to rRT-PCR during both BA.1 and BA.2 Omicron variant periods.

In the present study, the authors found 19.29% (95% CI 14.66 to 23.91) discordance, where samples were positive for RADTs but negative for rRT-PCR during January 2022. The PPC subsequently increased between March and April 2022, irrespective of the PCR detection kits used. Between January and February, most isolates were BA.1 sublineages, while BA.2 sublineage proportions surged to 90% in March and near 100% in April according to the unpublished reports from the Center of Excellence in Clinical Virology, Chulalongkorn University and random samples from the authors' molecular laboratory sent for whole genome sequencing at the Department of Medical Sciences. The PPC between RADTs and rRT-PCR was significantly higher during the BA.2-dominant Omicron variant period (March to April) than during the BA.1-dominant Omicron variant period (January to February). Whether the RADTs used during the BA.2-dominant period cause fewer false positive results using rRT-PCR as the confirmation test is still questionable. The authors postulated that a high prevalence of COVID-19 infection in the community during the BA.2 period may cause fewer false-positive RADTs. Nevertheless, the present study findings are reassuring for patients and health care personnel that the available RADTs can effectively detect true COVID-19 infection.

In the Ct value analysis, the authors found most positive RADTs corresponded to Ct values between 10 and 29.99 for most target genes, where the cut-off Ct value for the PCR detection kit was 40. During the BA.2-dominant period, all PCR detection kits showed significantly lower Ct values for all target genes. These findings reflect higher viral loads from the respiratory samples of BA.2-infected individuals compared to BA.1[13]. Additionally, 8.5% to 19% of RADT-positive samples had Ct values of more than 30, proportions similar to results from Scheiblauer et al, and Chu et al[14,15]. The authors found the *N* gene was reliably detected at lower Ct values than other gene targets during both the BA.1- and BA.2-dominant periods[13]. These findings support the use of PCR detection kits that include at least one *N* gene target during the Omicron variants pandemic.

There are limitations in the present study. First, the authors could not demonstrate a specific PPA for each manufacturer's kit due to a lack of data. Second, clinical, type of samples, same day testing between RADTs and rRT-PCR and genotyping data were not available. The clinical data regarding symptom onset affected the Ct values, with trends toward lower Ct values when testing at early onset[8,10,15]. Additionally, same day RADTs and rRT-PCR comparison may provide a better PPC[15]. Third, the authors could not ascertain whether the discordance results were a true negative or false negative rRT-PCR due to a lack of clinical correlation. And lastly, VTMs were chosen depending on each hospital's availability, which a chemical composition might differ. These has been shown to affect the rate of nucleic acid detection and may result in an increase in discordant rates[16]. Temperature control during transportation is another important issue for a detection of nucleic acid, in which the authors were not able to gather this information. However, temperature during transportation have little effect on the stability of nucleic acid when performing test within a day[16,17]. Additionally, the Department of Medical Sciences of Thailand suggest a temperature of 2 to 8 degrees during the specimen transportation and all hospitals were required to pass this quality check.

Conclusion

The PPC between RADTs used in the authors' community and rRT-PCR was very high. False positive

RADTs when using rRT-PCR as a confirmation test were low, especially during the BA.2-dominant period. Evaluation of the clinical performance of RADTs during the Omicron variant wave, including sensitivity, specificity, positive predictive value, and negative predictive value, requires further study.

What is already known about this topic?

Discordant positive results between rapid antigen tests and rRT-PCR have been reported up to 42%.

What this study adds?

Positive results from rapid antigen tests used in Thailand highly correlate with the positive rRT-PCR results with PPC 80.71% to 96.08% during the Omicron wave. BA.2-dominant period provided a higher PPC with rRT-PCR than during the BA.1-dominant period.

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Authors' contributions

SP: first draft, writing, study design, data collection, statistical analysis, interpretation of data, critical revision; PR: study design, data collection, interpretation of data. All authors read and approved the final manuscript.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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