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COMPARISON OF ABBOTT REAL TIME SARS-COV-2 ASSAY, GENEFINDER™ COVID-19 PLUS REAL*AMP* KIT AND BIOMERIEUX ARGENE® SARS-COV-2 R-GENE® KIT FOR THE RT-PCR BASED DETECTION OF SARS-COV-2 FROM NASOPHARYNGEAL SWABS

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Early, rapid and reliable identification of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is crucial for successful control of coronavirus disease 2019 (COVID-19). The quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) assay is considered the gold standard for molecular diagnosis of SARS-CoV-2. The objective of this study was to compare the clinical performances of the three authorized tests — the Abbott Real Time SARS-CoV-2 (ACOV) assay (Abbott Molecular Inc., North Chicago, IL), GeneFinder™ COVID-19 Plus Real*Amp* (GeneFinder) Kit (OSANG Healthcare Co., Ltd, Dongan-gu Anyang, Korea) and the Biomerieux ARGENE® SARS-COV-2 R-GENE® real-time detection (ARGENE) kit (bioMérieux SA., Marcyl'Étoile, France) and to determine whether the selection of targeted genes has an impact on test's specificity. In this study, we included 155 nasopharyngeal swabs (NPS) from adult individuals with symptoms or suspected of COVID-19, aged from 17 to 91 years, previously tested by the ACOV and subsequently tested by the GeneFinder and the ARGENE. In this comparative analysis, we found that the GeneFinder assay detected the most cases of COVID-19 infection, followed by the ACOV assay, and then by ARGENE. Positive agreement ranged from 74.74% to 95.41%,

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with the strongest agreement observed between the GeneFinder and ACOV assays — 95.41% (95% confidence intervals (CI): 89.37%-98.36%) indicating an excellent agreement between these two tests and the lowest agreement between the GeneFinder and ARGENE assay — 74.74% (95% CI: 65.08%-81.41%). The negative percent agreement was 100% (GeneFinder/ACOV, GeneFinder/ARGENE and ACOV/ARGENE). Only 3.2% of cases were false-negative using the ACOV test, while 18.0% of samples were false-negative using the ARGENE assay to detect SARS-CoV-2. Combined usage of the Abbott SARS-CoV-2 and the GeneFinder assays can be applied to maximize SARS-CoV-2 detection accuracy.

Keywords: COVID-19, SARS-CoV-2; molecular testing, RT-PCR, in vitro diagnostic tests

INTRODUCTION

The first cases of pneumonia of unknown origin were recorded in Wuhan, the capital of China's Hubei Province, in early December 2019. SARS-CoV-2 was named after the causative agent, a newly identified RNA beta-coronavirus that was related to the current SARS-CoV. The new virus matched 85 percent with the SARS-CoV virus, which infects bat, and human angiotensin converting enzyme 2 (ACE2) receptors, allowing it to reach cells (CORMAN *et al*., 2020). As of 20 June 2021, 175,541,600 cases of SARS-CoV-2 infection had been identified worldwide, with 3,798,361 deaths (https://covid19.rs).

In the Republic of Serbia, the first case of COVID-19 was registered on March 6, 2020. and the epidemic is still ongoing. The epidemiological situation is currently positive, with a downward trend in disease incidence in all areas of the world. According to the data of the Ministry of Health of the Republic of Serbia from June 20, 2021, 4,335,783 people have been tested in Serbia so far, of which 715,753 are confirmed cases with 7,001 deaths (https://covid19.rs).

Due to the presence of coronavirus infection in the Republic of Serbia as well as the global epidemic, all evidence from this quickly progressing COVID-19 pandemic point to the relevance of correct molecular diagnosis of coronavirus infection. Laboratory research is important for identifying the disease characteristics and epidemiology of an evolving infectious pathogen like SARS-CoV-2, as well as monitoring its dissemination. The RT-PCR test is the gold standard for laboratory validation of SARS-CoV-2 infection (RISTIĆ *et al*., 2021). Patients with COVID-19 have elevated viral loads in their upper and lower respiratory tracts within 5 to 6 days of onset of symptoms (PAN *et al.,* 2020; LAUER *et al*., 2020). COVID-19 patients must be identified and managed using diagnostic procedures of high sensitivity and specificity. High diagnostic precision testing used early in the disease, in particular, will allow for the early diagnosis of COVID-19 patients and the timely application of intervention measures to limit household and neighborhood spread. At the moment, researchers are working around the world to establish new strategies for detecting novel coronaviruses (TANG *et al*., 2020). There are about 400 commercially available genetic tests at the moment (https://www.ecdc.europa.eu/en/covid-19/latest-evidence/diagnostic-testing).

The goal of this study was to compare the clinical performance of 3 commercial SARS-CoV-2 RNA viral detection kits — the Abbott Real Time SARS-CoV-2 assay, GeneFinder™

COVID-19 Plus Real*Amp* and the Biomerieux ARGENE® SARS-COV-2 R-GENE® real-time detection kit.

MATERIALS AND METHODS

Design of the Study and Data Analysis

We have performed a prospective research at the Clinical Centre (CC) of Vojvodina in Novi Sad, in the period between March 29th 2021 and April 4th 2021.

The research was approved by the Ethics Committee of the CC Vojvodina on March 26th, 2021 (Decision No.00-57).

A total of 155 nasopharyngeal swab specimens (NPS) collected consecutively in 3-ml disposable viral transport medium (VTM) (SANLI Medical Technology development Co., Liuyang, Hunan, China) from symptomatic (cough or fever or shortness of breath) COVID-19 adult individuals and tested within 24 h of collection. If all processing could not be done on the same day, residual NPS specimens in transport media were kept refrigerated at 4°C. To reduce the risk of SARS-CoV-2 transmission to laboratory personnel, all samples in VTM were heat inactivated for 35 minutes at 56°C prior to testing. For comparison, three tests were conducted on each NPS specimen: the ACOV reference assay (tested first), the GeneFinder, and the ARGENE test (tested subsequently).

Assay Descriptions

GeneFinder™ COVID-19 Plus RealAmp Kit

The GeneFinder testing was performed as per manufacturer's instructions (https://www.fda.gov/media/137116/download). Viral RNA was extracted using Viral DNA and RNA Extraction Kit *(Xi'an Tianlong Science and Technology Co., Ltd., Xi'an City,* China) (https://www.fda.gov/media/137742/download) for the *Rotary Nucleic Acid Extraction* System *(GeneRotex 96L) (Xi'an Tianlong Science and Technology Co., Ltd., Xi'an City,* China*).* Amplification and detection were performed on the Gentier Real-time Quantitative PCR (Gentier 96E) *(Xi'an Tianlong Science and Technology Co., Ltd., Xi'an City,* China*).* A sample input volume of 200-µl VTM was used for automated extraction. For RT-PCR amplification and detection, the overall reaction volume was 15 µl of master mix (primer/probe mix), nuclease-free water, and 5 µl of isolated RNA sample. The original GeneFinder protocol's assay run parameters were used (https://www.fda.gov/media/137116/download). The cycle thresholds (Cts) from the fluorophore FAM (fluorescein) (RdRp gene), HEX (hexachloro-fluorescein) (N gene), Texas Red (sulforhodamine 101 acid chloride) (E gene) and Cy5 (cyanine 5) (internal control (IC)) were acquired. Samples were considered positive for SARS-CoV-2 if there is a sigmoidal amplification curve in the FAM, HEX, and/or Texas Red channel, with Ct values ≤40 (Table 1). The IC should be positive in all samples, with Ct values no higher than 35. If the IC was amplified but not the viral genes, the sample was considered negative. A specimen was found invalid if the IC was not amplified. When only the E gene was detected in the sample, it was retested and the result was interpreted as positive in case the E gene was detected again (ONG *et al*., 2020).

Table 1. Comparison of RT-PCR assays dedicated to SARS-CoV-2 gene target points

Name of the commercial kit	Gene target	Fluorophore	Supplier recommended C_t cut off
GeneFinder COVID-19 Plus RealAmp Kit	RdRp	FAM	≤40
	N	HEX	
	E	Texas Red	
Biomerieux ARGENE® SARS-COV-2 R-GENE® real-time detection kit	RdRp	Cy5	
	N	FAM	≤40
Abbott Molecular RealTime SARS-CoV-2 assay	RdRp		
	N	FAM	≤37

Biomerieux ARGENE® SARS-COV-2 R-GENE® real-time detection kit

The second assay introduced was the ARGENE (bioMérieux SA., Marcyl'Étoile, France). Assay was developed to detect SARS-CoV-2 RdRp, N, and E genes in nasopharyngeal swabs. Nucleic acids were purified and extracted using the *Rotary Nucleic Acid Extraction* System *(GeneRotex 96L)* from VTM using an input sample volume of 200 µl into 825 µl of lysis buffer with the specific protocol to which a final eluted volume of purified nucleic acids was 80 µl. Amplification and real-time detection were performed on the the Gentier Real-time Quantitative PCR (Gentier 96E) *(Xi'an Tianlong)*. For RT-PCR amplification and detection, the overall volume per reaction was 15 µl of master mix (primer/probe mix), nuclease-free water, and 10 µl of isolated RNA sample. Assay run parameters were as described in the original ARGENE® protocol (https://www.fda.gov/media/137742/download). Samples were considered positive for SARS-CoV-2 if there is a sigmoidal amplification curve in the FAM (N gene), Cy5 (RdRp) and/or HEX (IC), channel, with Ct values not higher or equal than 40 (Table 1). The IC should be positive in all clinical samples, with Ct values no higher than 35. If the internal control was amplified but not the viral genes, the sample was considered negative. If there was no amplification of the internal control, a specimen was considered invalid.

Abbott Molecular RealTime SARS-CoV-2 assay

ACOV testing was carried out according to the manufacturer's instructions on the Abbott m2000 System consisting of a sample preparation unit, the Abbott m2000sp (Abbott Molecular Inc., North Chicago, IL), and an amplification/detection unit, the Abbott m2000rt (Abbott Molecular Inc., North Chicago, IL) (https://www.molecular.abbott/int/en/products/infectious-disease/realtime-sars-cov-2-assay). Viral RNA was extracted using Abbott mSample Preparation Systems DNA kit (Abbott Molecular Inc., North Chicago, IL) on Abbott m2000sp instrument. With a sample input volume of 500 µl VTM, automated extraction was conducted, followed by automated addition of amplification package reagents and extracts (40 µl of each) for RT-PCR amplification and detection. The target sequences for the Abbott RealTime SARS-CoV-2 assay are RdRp and N genes of the SARS-CoV-2 genome. To show that the sample preparation procedure was performed correctly with each specimen and control, the IC is added into each specimen at the start of the process. The two SARS-CoV-2-specific probes are labeled with the

same fluorophore (FAM) and the IC-specific probe is labeled with a different fluorophore (VIC), thus allowing for simultaneous detection of both SARS-CoV-2 and IC amplified products in the same reaction well (Table 1). The m2000rt system software analyzed the amplification curves and the result was reported as detected or not detected. Samples were considered positive when a signal was detected at Ct $\leq$ 37 for any gene. A sample was interpreted negative if the internal control was amplified, but not the viral genes. If there was no amplification of the internal control, a specimen was considered invalid (MOORE *et al*., 2020).

Statistical analysis

Statistical analysis was performed using the IBM® SPSS (version 20.0, IBM SPSS Inc., Armonk, NY, USA). To evaluate assay performance at varying viral concentrations, 104 positive specimens were selected to represent the full range of observed Ct values on the Abbott assay, ranging from 5–30 cycles. Additional 51 negative specimens were selected to evaluate negative agreement. Overall percent agreement, positive percent agreement, negative percent agreement, and associated 95% CI for the GeneFinder™ and ARGENE assays were calculated using ACOV as the reference test (LANDIS *et al*., 1977).

RESULTS

Our study included 155 NPS specimens (104 (67.1%) positive, 51 (32.9%) negative) tested by the ACOV and subsequently by the GeneFinder and ARGENE. All patients were adult individuals with symptoms or suspected COVID-19, aged from 17 to 91 years. The average age was 57.9 years for positive samples and 57.2 years for negative samples. The majority of positive and negative results were obtained from female samples (56.7%) and (58.8%) individually (Table 2).

Table 2. Demographics of involved patients.

Abbott C_t Category	Average Age (years)	Male (%)	Female (%)	Total no. of patients
Positive	57.9	45 (43.3%)	59 (56.7%)	104
Negative	57.2	21 (41.2%)	30 (58.8%)	51
Total no. of patients		66	89	155

Data are given in absolute number (percentage)

Testing results provided by GeneFinder, ARGENE, and ACOV are shown in Table 3. In 80 (51.6%) samples SARS-CoV-2 gene sequences were detected by all three assays and 46 (29.7%) samples were tested negative for SARS-CoV-2 RNA by all assays. Two-way positive and negative agreements between results are shown in Table 4. Positive agreement ranged from 74.74% to 95.41%, with the biggest agreement observed between the GeneFinder and ACOV assays, and the lowest agreement between the GeneFinder and ARGENE assays. Negative agreement was 100% (GeneFinder/ACOV, GeneFinder/ARGENE and ACOV/ARGENE).

For the ACOV assay, SARS-CoV-2 target RNA sequences were detected in 104 (67.1%) samples (Table 3). The median Ct value for positive samples on ACOV assay was 19.07 (95% CI: 17.10-20.89), ranging from 4.48 to 29.72, with a standard deviation of 6.11 for both N and RdRp genes (Table 5).

Table 3. Detection of SARS-CoV-2 RNA by GeneFinder assay, ARGENE, and ACOV assay.

Total no. of samples tested (n = 155)	GeneFinder	ARGENE	ACOV
80 (51.6%)	Detected	Detected	Detected
46 (29.7%)	Not detected	Not detected	Not detected
23 (14.8%)	Detected	Not detected	Detected
1 (0.7%)	Detected	Invalid[a]	Detected
5 (3.2%)	Detected	Not detected	Not detected

[a]A sample that yielded neither a positive nor a negative test is considered invalid.

[b]Categories of no samples are not displayed.

Table 4. Performance agreement for detection of SARS-CoV-2 RNA GeneFinder, ARGENE, and ACOV assay.

Assay comparison	Positive percent agreement (95% CI)	Negative percent agreement (95% CI)	Overall rate of agreement (95% CI)
GeneFinder/ ARGENE	74.74% (65.08%-81.41%)	100% (92.30%-100%)	81.82% (74.98%-87.11%)
GeneFinder/ ACOV	95.41% (89.37%-98.36%)	100% (92.30%-100%)	96.77% (93.92%-97.37%)
ACOV/ ARGENE	78.64% (69.0%- 84.79%)	100% (93.0%-100%)	85.71% (81.28%-92.83%)

Data are presented in absolute number (percentage)

Table 5. COVID-19 positive samples are tested for gene characteristics.

	GeneFinder	ARGENE	ACOV
Number positive for RdRp gene	71 (65%)	66 (78%)	104 (100%)
Median C_t-value for RdRp gene	30.2	30.05	19.07
Number positive for N gene	109 (100%)	80 (94%)	104 (100%)
Median C_t-value for N gene	32.71	32.61	19.07
Number positive for E gene	50 (46%)	CANNOT BE APPLIED	CANNOT BE APPLIED
Median C_t-value for E gene	28.53	CANNOT BE APPLIED	CANNOT BE APPLIED

Data are presented in absolute number (percentage)

The GeneFinder assay yielded 109 (70.3%) positive results and no invalid results (Table 3). The median Ct value for positive samples on GeneFinder assay was 32.32 (95% CI: 30.07-35.15), ranging from 17.01 to 39.69, with a standard deviation of 6.05 for N gene, 30.2 (95% CI: 29.29-32.27), ranging from 17.85 to 39.90, with a standard deviation of 5.73 for RdRp gene, and 28.53 (95% CI: 25.51-31.55), ranging from 18.20 to 39.40, with a standard deviation of 5.67 for E gene. In 109 positive samples, the GeneFinder assay detected all three genes in 50 (45.9%) samples, RdRp gene and N gene in 21 (19.2%), and N gene only in 38 (34.9%).

Five samples (3.2%) that were tested positive on GeneFinder but negative on the ACOV and ARGENE test had a median Ct value of 39.15 (95% CI: 37.0-39.69), ranging from 37.0 to 39.69, with a standard deviation of 1.07 for N gene.

The ARGENE assay yielded 85 (54.8%) positive results (Table 3). The median Ct value for positive samples on ARGENE assay was 32.61 (95% CI: 30.07-35.23), ranging from 18.3 to 39.89, with a standard deviation of 6.01 for N and 29.89 (95% CI: 28.70-30,88), ranging from 18.02 to 39.25, with a standard deviation of 6.46 for RdRp gene (Table 5). In 80 positive samples, the ARGENE assay detected both RdRp gene and N gene genes in 66 (82.5%) samples, and N gene only in 14 (17.5%).

Twenty-three samples (14.8%) were not detected by ARGENE but were detected by the GeneFinder and ACOV assay (Table 3) and had a median Ct value of 37.35 (95% CI: 36.88-37.91), ranging from 33.46 to 39.50, with a standard deviation of 1.81 for N gene and were consistent with lower viral loads.

Statistical analysis was performed using SPSS Version 20.0 software. A Kruskal-Wallis H test showed that there was a statistically significant difference in both RdRp and N gene Ct values between the different assays ($p<0.001$). Dunn-Bonferroni *post hoc* test was performed afterwards to detect which specific assay results are significantly different from the other. For both RdRp and N gene, the difference in obtained Ct values between the GeneFinder and ARGENE assays was not significant ($p=1.00$ and $p=0.836$, respectively), while ACOV assay yielded significantly more positive results than either the GeneFinder or ARGENE assay ($p<0.001$).

DISCUSSION

Early, rapid and reliable identification of SARS-CoV-2 is crucial for successful COVID-19 disease control. The nucleic acid RT-PCR assay is considered the gold standard for molecular diagnosis of SARS-CoV-2.

In this comparative analysis, the ACOV assay demonstrated the highest level of agreement with the GeneFinder assay. Overall agreement between the ACOV and GeneFinder assay was 96.77% (95% CI: 93.92%-97.37%). The positive percent agreement was 95.41% (95% CI: 89.37%-98.36%) indicating an excellent agreement between these two tests. The negative percent agreement was 100% (95% CI: 92.30%-100%). The results of our study were in correlation with those published by Moore *et al*. Positive percent agreement between the ACOV and a laboratory-developed modified CDC 2019-nCOV reverse transcriptase PCR assay was 100% (95% CI: 96.9%-100%) while negative percent agreement was 92.4% (95% CI: 84.2%-97.2%) (MOORE *et al*., 2020).

The GeneFinder assay detected the most cases of COVID-19 infection, followed by the ACOV assay, and then by ARGENE. Samples (3.2%) that were tested positive on GeneFinder but negative on the ACOV and ARGENE test had a median Ct value of 39.15 (95% CI: 37.0-39.69), ranging from 37.0 to 39.69, with a standard deviation of 1.07 for N gene. Discrepant results were observed almost exclusively in samples with higher Ct values, i.e., with lower viral titer. These results indicate differences in the lower limit of detection (LOD) of the tests. The specified LOD in the published instructions for ACOV application is 100 copies/ml and 0.5 copies/µl for GeneFinder (https://www.fda.gov/media/137116/download;https://www.molecular.abbott/int/en/products/inf

ectious-disease/realtime-sars-cov-2-assay; DEGLI-ANGELI *et al*., 2020). However, a "positive" PCR result reflects only the detection of viral RNA and does not necessarily indicate presence of viable virus (SETHURAMAN *et al*., 2020; WÖLFEL *et al*., 2020). This study shows that the sensitivity of the GeneFinder assay was excellent as all positive samples according to the reference ACOV method were also identified as positive by this platform. Our findings strongly indicate that using the N gene as an additional gene target can improve sensitivity of SARS-CoV-2 detection (CHENG *et al*., 2020).

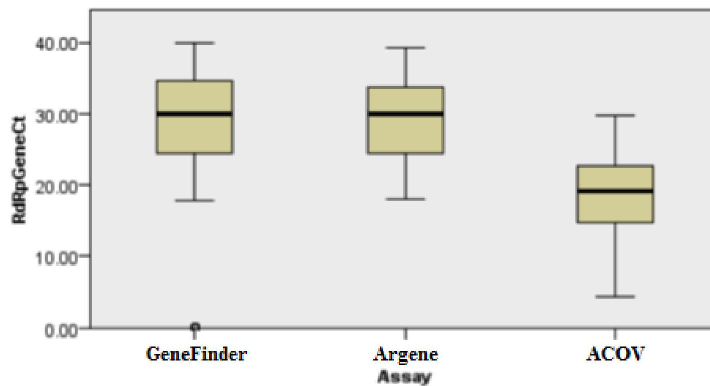

Figure 1. Comparison of RdRp gene Ct values between samples detected by GeneFinder, Argene, and Abbott ACOV assay.

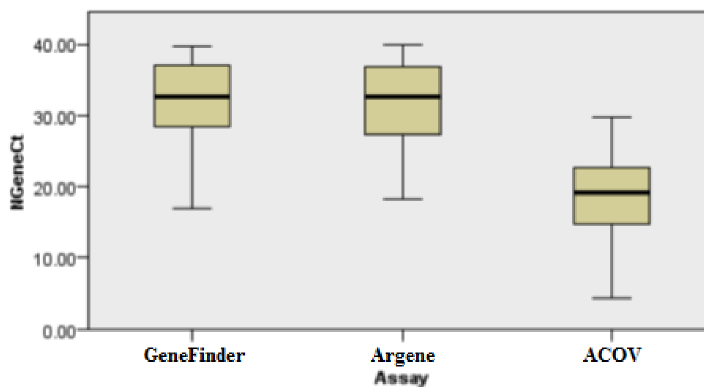

Figure 2. Comparison of N gene Ct values between samples detected by GeneFinder, Argene, and Abbott ACOV assay.

Furthermore, solitary N gene positive cases were linked to a longer period between the onset of symptoms and the timing of nucleic acid amplification testing, suggesting that the N gene has a wider detection window than other target genes. While nucleic acid amplification tests have a high accuracy, their susceptibility is affected by the timing of disease presentation, the nature and location of sampling, and the intensity of the illness (ZOU *et al*., 2020). Since subgenomic N gene messenger RNAs are more abundant than other targets, the N gene should theoretically be the most sensitive target for SARS-CoV-2 detection (ONG *et al*., 2020).

Our results, comparing the ACOV and ARGENE assays are concordant with those of Harrington et al., who reported increased detection of SARS-CoV-2 RNA gene sequences by ACOV compared to ID NOW COVID-19 assay (Abbott). Overall agreement was 78.64% positive agreement (95% CI: 69.0%-84.79%) and 100% negative agreement (95% CI: 93.0%-100%) between ACOV and ARGENE. Overall agreement was 75% positive agreement (95% CI: 67.74%-80.67%) and 99% negative agreement (95% CI: 97.64%-99.89%) between IDNCOV and ACOV (HARRINGTON *et al*., 2020).

Similar research results are shown by Moore et al. Positive percent agreement between the ACOV and IDNOW assay was 75.2% (95% CI: 66.7%-82.5%) while negative percent agreement was 100% (95% CI: 95.4%-100%). The specified LOD in the published instructions for ARGENE usage is 380 copies/ml (https://www.fda.gov/media/137742/download). Twenty-three samples (14.8%) were not detected by ARGENE but were detected by the GeneFinder and ACOV assay and had a median Ct value of 37.35 (95% CI: 36.88-37.91), ranging from 33.46 to 39.50, with a standard deviation of 1.81 for N gene and were consistent with lower viral loads. The ARGENE assay's negative results can be interpreted in part by the lower input volumes used for extraction (200-µl) and amplification (25-µl) relative to the ACOV test's extraction volumes of 500-µl and amplification volumes of 40-µl. Since the targets for amplification and detection in the ACOV assay are easier to achieve, the ACOV produced more positive SARS-CoV-2 data, implying that the same samples were false-negative using the ARGENE package (MOORE *et al*., 2020; HOGAN *et al*., 2020).

In comparison to the Abbott and ARGENE, the GeneFinder test was easier to perform and provided results in the shorter period of time, and also detected the most cases of SARS-CoV-2. Total testing time for the GeneFinder assay is approximately 3 hours. The ACOV assay has the longest runtime, approximately 4 hours for extraction and preparation of samples for amplification and 3 h for amplification and detection of PCR products for one full run of 94 patient samples, but detected less cases of COVID-19 than the GeneFinder test. Using the ARGENE assay, the results were delivered in 4 to 5 hours for a full series of 94 patient samples, but the fewest cases of COVID-19 were detected (ONG *et al*., 2020).

There was a statistically significant difference in both RdRp and N gene Ct values between the different assays ($p<0.001$). For both RdRp and N gene, the difference in obtained results between the GeneFinder and ARGENE assays was not significant ($p=1.00$ and $p=0.836$, respectively), while ACOV assay yielded significantly more positive results than either the GeneFinder or ARGENE assay ($p<0.001$).

The potential influence of the input VTM sample volume used for extraction could explain the differences in Ct values between the ACOV, GeneFinder, and ARGENE assays. The extraction volumes of the ACOV test of 500 µl allow the targets for amplification and detection

to be easier to achieve than the extraction volumes of the Gene Finder and ARGENE tests of 200 µl. Ct is used to calculate the initial DNA copy number because the Ct value is inversely related to the starting amount of target. For example, in comparing real-time PCR results from samples containing different amounts of target, a sample containing twice the starting amount will yield a Ct one cycle earlier than a sample that contained half as many copies of the target prior to amplification (https://www.gene-quantification.de/real-time-pcr-handbook-life-technologies-update-flr.pdf)

In addition, different real-time instruments may have different C_t values. The two PCR technologies differ in several aspects, including the software, light sources and the approach to the acquisition of fluorescence data, so they employ different reagents. Therefore, we performed analyses using the reagent mix which is proprietary for each system and we used the same set of samples. Two systems may amplify at different efficiencies, i.e., cycle numbers may represent different levels of amplification, though the same primers were used. We took notice of the differences in ramp rate settings between Abbott, OSANG Healthcare, and Biomerieux ARGENE. This could be significant, because theoretically, a fast ramp rate (Biomerieux ARGENE) may not allow sufficient time for DNA denaturing or for primer annealing, which could have dramatic effects on PCR performance, even causing reaction failures. The ramp rate of a thermal cycler indicates the change in temperature from one PCR step to another over time and is usually expressed in degrees Celsius per second (°C/sec) (IGLÓI *et al*., 2020).

During a COVID-19 pandemic, fast, easy-to-access, and accurate test results are essential, and each of the three tests tested in this analysis would provide significant clinical details (SMITHGALL *et al*., 2020). The availability of various platforms allows for diversity in meeting the research needs of various communities and health care environments (NICOLA *et al*., 2020).

CONCLUSION

In conclusion, we found that the GeneFinder assay detects more cases of COVID-19 infection than the ACOV and ARGENE assay. Only 3.2% of cases were false-negative using the ACOV and 14.8% using the ARGENE test to detect SARS-CoV-2. There is an excellent agreement between the Abbott SARS-CoV-2 and the GeneFinder assays, and their combined usage can be applied to maximize the accuracy of the SARS-CoV-2 testing.

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REFERENCES

CHENG, M.P., J., PAPENBURG, M., DESJARDINS, S., KANJILAL, C., QUACH, M., LIBMAN, S., DITTRICH, C.P., YANSOUNI (2020): Diagnostic testing for severe acute respiratory syndrome-related coronavirus 2. Ann. Internal Med., *172*(11):726-734.

CORMAN, V.M., O., LANDT, M., KAISER, R., MOLENKAMP, A., MEIJER, D.K.W., CHU, T., BLEICKER, *et al*. (2020): Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveillance, *25*(3):1-8.

DEGLI-ANGELI, E., J., DRAGAVON, M.L., HUANG, D., LUCIC, G., CLOHERTY, K.R.,JEROME, A.L., GRENINGER, R.W., COOMBS (2020): Validation and verification of the Abbott RealTime SARS-CoV-2 assay analytical and clinical performance. J. Clinical Virology, *129*:18-20.

HARRINGTON, A., B., COX, J., SNOWDON, J., BAKST, E., LEY, P., GRAJALES, J., MAGGIORE, S., KAHN (2020): Comparison of Abbott ID Now and Abbott m2000 methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from symptomatic patients. J. Clinical Microbiol., *58*(8):e00798-20.

HOGAN, C.A., N., GARAMANI, A.S., LEE, J.K., TUNG, M.K., SAHOO, C.H., HUANG, B., STEVENS, J., ZEHNDER, B.A., PINSKY (2020): Comparison of the Accula SARS-CoV-2 test with a laboratory-developed assay for detection of SARS-CoV-2 RNA in clinical nasopharyngeal specimens. J. Clinical Microb., *58*(8):e01072-20.

IGLÓI, Z., M., LEVEN, Z., ABDEL-KAREM ABOU-NOUAR, B., WELLER, V., MATHEEUSSEN, J., COPPENS, M., KOOPMANS, R., MOLENKAMP (2020): Comparison of commercial realtime reverse transcription PCR assays for the detection of SARS-CoV-2. J. Clinical Virol., *129:* 4-6.

LANDIS, J.R., G.G., KOCH (1977): The measurement of observer agreement for categorical data. Biometrics, *33*(1):159–174.

LAUER, S.A., K.H., GRANTZ, Q., BI, F.K., JONES, Q., ZHENG, H.R., MEREDITH, A.S., AZMAN, N.G., REICH, J., LESSLER (2020): The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Ann. Internal Med., *172*(9):577-582.

MILLER, T.E., W.F., GARCIA BELTRAN, A.Z., BARD, T., GOGAKOS, M.N., ANAHTAR, M.G., ASTUDILLO, *et al*. (2020): Clinical sensitivity and interpretation of PCR and serological COVID-19 diagnostics for patients presenting to the hospital. The FASEB Journal, *34*(10):13877-13884.

MOORE, N.M., H., LI, D., SCHEJBAL, J., LINDSLEY, M.K., HAYDEN (2020): Comparison of two commercial molecular tests and a laboratory-developed modification of the CDC 2019-nCoV reverse transcriptase PCR assay for the detection of SARS-CoV-2. J. Clinical Microb., *58*(8):e00938-20.

NICOLA, M., N., O'NEILL, C., SOHRABI, M., KHAN, M., AGHA, R., AGHA (2020): Evidence based management guideline for the COVID-19 pandemic. Int. J. Surgery, *77*:206-216.

ONG, D.S.Y., E.C.J., CLAAS, S., BREIJER, N., VAESSEN (2020): Comparison of the GeneFinderTM COVID-19 Plus RealAmp Kit on the sample-to-result Platform ELITe InGenius to the national reference method: An added value of N gene target detection? J. Clinical Virology, *132*:104632.

PAN, Y., D., ZHANG, P., YANG, L.L.M., POON, Q., WANG (2020): Viral load of SARS-CoV-2 in clinical samples. The Lancet Infectious Diseases, *20*(4):411-412.

RISTIĆ, M., N., NIKOLIĆ, V., ČABARKAPA, V., TURKULOV, V., PETROVIĆ (2021): Validation of the STANDARD Q COVID-19 antigen test in Vojvodina, Serbia. *PLoS ONE*, *16*(2):1-13.

SETHURAMAN, N., S.S., JEREMIAH, A., RYO (2020): Interpreting diagnostic tests for SARS-CoV-2. JAMA – J. American Medical Association, *323*(22):2249-2251.

SMITHGALL, M.C., I., SCHERBERKOVA, S., WHITTIER, D.A., GREEN (2020): Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche cobas for the rapid detection of SARS-CoV-2. J. Clinical Virology, *128*:104428.

TANG, Y.W., J.E., SCHMITZ, D.H., PERSING, C.W., STRATTON (2020): Laboratory diagnosis of COVID-19: Current issues and challenges. J. Clinical Microb., *58*(6):e00512-20.

WÖLFEL, R., V.M., CORMAN, W., GUGGEMOS, *et al*. (2020): Virological assessment of hospitalized patients with COVID-2019. Nature, *581*(7809):465-469.

ZOU, L., F., RUAN, M., HUANG, L., LIANG, H., HUANG, Z., HONG, *et al*. (2020): SARS-CoV-2 viral load in upper respiratory specimens of infected patients. The New England J. of Med., *382*:1177–1179.

POREĐENJE ABBOTT REAL TIME SARS-COV-2, GENEFINDER™ COVID-19 PLUS REAL*AMP* AND BIOMERIEUX ARGENE® SARS-COV-2 R-GENE® TESTA ZA RT-PCR DETEKCIJU SARS-COV-2 IZ NAZOFARINGEALNIH BRISEVA

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Izvod

Rano, brzo i pouzdano identifikovanje teškog akutnog respiratornog sindroma Koronavirus-2 (SARS-CoV-2) je presudno za uspešnu kontrolu koronavirusne bolesti 2019 (COVID-19). Kvantitativni test lančanom reakcijom polimeraze nakon reverzne transkripcije (RT-PCR) smatra se zlatnim standardom za molekularnu dijagnozu SARS-CoV-2. Cilj ove studije bio je poređenje kliničkih performansi tri odobrena testa - Abbott SARS-CoV-2 (ACOV) (Abbott Molecular Inc., Severni Čikago, IL), GeneFinder™ COVID-19 Plus Real*Amp* (GeneFinder) (OSANG Healthcare Co., Ltd, Dongan-gu Anyang, Korea) i Biomerieux ARGENE® SARS-COV-2 R-GENE® real-time detection (ARGENE) (bioMérieux SA., Marcyl'Étoile, France) testa i utvrđivanje uticaja odabira ciljanih gena na specifičnost testa. Uključili smo 155 nazofaringealnih briseva (NPS) odraslih osoba sa simptomima ili sumnjama na COVID-19, starosti od 17 do 91 godine, koji su prvobitno testirani primenom ACOV testa, a potom GeneFinder i ARGENE testom. U ovoj uporednoj analizi pronašli smo da je GeneFinder test otkrio najviše slučajeva infekcije COVID-19, zatim ACOV, a potom ARGENE test. Pozitivno slaganje kretalo se od 74,74% do 95,41%, pri čemu je najveće slaganje primećeno između GeneFinder i ACOV testa - 95,41% (95% intervali poverenja (CI), 89,37% -98,36%) što ukazuje na izvrsno slaganje između ova dva testa i najniže sporazum između testova GeneFinder i ARGENE - 74,74% (95% CI, 65,08%-81,41%). Negativni postotak slaganja bio je 100% (GeneFinder/ACOV, GeneFinder/ARGENE i ACOV/ARGENE). Samo 3,2% slučaja bilo je lažno negativno upotrebom ACOV testa, dok je 18,0% uzoraka bilo lažno negativno primenom ARGENE testa za otkrivanje SARS-CoV-2. Kombinovana upotreba testova ACOV i GeneFinder može se primeniti kako bi se maksimizirala tačnost detekcije SARS-CoV-2.

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