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COMPARISON OF ABBOTT REAL TIME SARS-COV-2 ASSAY AND LIFERIVER NOVEL CORONAVIRUS REAL TIME MULTIPLEX KIT FOR THE RT-PCR BASED DETECTION OF SARS-COV-2 FROM NASOPHARYNGEAL SWABS

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SUMMARY – The objective of this study was to compare performance of two authorized tests, the Abbott Real Time SARS–CoV-2 (ACOV) assay (Abbott Molecular Inc., North Chicago, IL, USA) and Liferiver Novel Coronavirus Real Time Multiplex RT-PCR (Liferiver 2019-nCoV) kit (BioVendor Group, Brno, Czech Republic), and to determine whether the selection of targeted genes has an impact on test specificity. We included 105 nasopharyngeal swabs from adult individuals with symptoms or suspected of coronavirus disease 2019 (COVID-19), aged from 26 to 91 years, previously tested by the ACOV and subsequently tested by the Liferiver 2019-nCoV. In this comparative analysis, we found that the ACOV assay detected more cases of COVID-19 infection than the Liferiver 2019-nCoV assay. The Liferiver 2019-nCoV kit showed a high level of agreement with the ACOV assay. The positive percent agreement was 88.89% (95% confidence intervals (95% CI): 77.42%-100.0%), and the kappa coefficient (kappa) was 0.901 (95% CI: 0.817-0.985). The negative percent agreement was 94.12% (95% CI: 89.74%-98.50%), while 4.76% of SARS-CoV-2 cases were false-negative using the Liferiver test. However, due to the possible false-negative results using the Liferiver 2019-nCOV test, we recommend complete testing with the ACOV assay.

Key words: *Molecular diagnostics; Polymerase chain reaction; RNA isolation; Virus detection*

Introduction

Molecular diagnosis of COVID-19 is based on a specific and sensitive detection of viral ribonucleic acid (RNA). It has been considered that methods based on quantitative real-time reverse transcription-polymerase chain reaction (QRT-PCR) assays are most accurate for detecting the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1,2]. There is a variety of available QRT-PCR platforms and we compared performance of two of them, the Abbott Real Time SARS-CoV-2 assay performed on the Abbott m2000 Real Time system (Abbott Molecular Inc., North Chicago, IL, USA) and Liferiver Novel Coronavirus Real Time Multiplex RT-PCR kit (BioVendor Group, Brno,

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Czech Republic) performed on the ExiPrep™16 Dx Fully Automated Nucleic Acid Extraction System (Bioneer Corporation, Daejeon, Republic of Korea), SaCycler-96 (Sacace Biotechnologies S.r.l., Como, Italy) and ABI Prism (R) 7500 instrument (Applied Biosystems, Foster City, CA, USA). QRT-PCR can detect several specific genes that encode viral structural proteins, including the envelope (E), nucleocapsid (N), membrane (M), and spike (S), as well as open reading frames-1 antibodies (ORF1ab), which encode non-structural proteins, enzymes[3-5]. The assembly and release of virions is controlled by E proteins[6]. N proteins play a role in pathogenicity as an interferon (IFN) inhibitor and in packaging the RNA genome and virions[3,7,8]. M proteins are in charge of virion shape[9]. The recognition of host cell receptors is carried out by the envelope glycoprotein S[10]. Orf1ab polygene is a polyprotein region encoding 16 non-structural proteins (NSPs), (NSP1-NSP16), among which are RNA-dependent RNA polymerase (RdRp, NSP12) and 2'-O-ribose-methyltransferase (2'-O-Mtase, NSP16)[4,5,11]. The Orf1ab polyprotein is involved in replication as well, but it is also responsible for the viral mRNA cap methylation that is crucial in immune system evasion[4,5,12]. RdRp is translated after viral RNA release in the host cell and plays a role in the reproduction of viral genetic material inside the cell[12]. The ACOV test detects the structural N gene and the non-structural RdRP/Orf1ab (NSP12) gene, while the Liferiver 2019-nCoV kit detects the structural E and N and non-structural Orf1ab gene[13-15].

The objective of this study was to compare performance of the two authorized tests at different viral loads and to determine whether the selection of targeted genes has an impact on test specificity.

Material and Method

Study design and data analysis

We performed prospective research at a tertiary inpatient health care facility in Novi Sad (University Clinical Center of Vojvodina, UCCV) and Institute of Public Health of Vojvodina in Novi Sad. The inpatients were enrolled by the UCCV Department for Infectious Diseases outpatient clinic ('red zone') and other UCCV hospital units from January 30, 2021 to February 3, 2021. Before commencement of the study, all of the physicians and nurses involved received 10 days of training on proper sampling and sample handling methods[16]. A total of 105 nasopharyngeal swabs (NPS) were collected by previously trained medical staff in 3-mL sterile commercially available tubes containing a specific viral transport medium (VTM) (SANLI Medical Technology Development Co., Liuyang, Hunan, China) with antifungal and antibiotic supplements. Specimens were collected consecutively from adult individuals with suspected COVID-19. One NPS sample was obtained from each patient. Clinical samples were transported in a hand refrigerator (from +2 to +8°C) from the sampling site to the UCCV Virology Laboratory.

For QRT-PCR SARS-CoV-2 laboratory confirmation, samples were held refrigerated at 4°C and tested within 12 hours of collection. In order to reduce the risk of accidental transmission of SARS-CoV-2 to laboratory staff, the 105 NPS samples were heat inactivated in a water–bath, at 56°C for 30 minutes prior to testing[16]. Each NPS specimen was used for both the ACOV reference assay (tested first) and the Liferiver 2019-nCoV test (tested subsequently) for comparison.

The ACOV assay was performed in the UCCV Virology Laboratory. Testing was carried out according to the manufacturer's instructions on two separate Abbott m2000 System instruments, i.e., a sample preparation unit, the Abbott m2000sp (Abbott Molecular Inc., North Chicago, IL, USA) and an amplification and detection unit, the Abbott m2000rt (Abbott Molecular Inc., North Chicago, IL, USA)[13]. Viral RNA was extracted using the Abbott mSample Preparation Systems DNA kit (Abbott Molecular Inc., North Chicago, IL, USA) on the Abbott m2000sp platform. Automated extraction was performed using a sample input volume of 500 µL VTM followed by automated addition of amplification pack reagents and extracts (40 µL volume) used for QRT-PCR amplification and detection. The cycle threshold (Ct) of the fluorophores FAM (6-carboxyfluorescein), (RdRP/Orf1ab), ROX (6-carboxy-X-rhodamine) (N gene), and VIC (50-fluorescein) (internal control (IC)) was determined. Amplification curves were interpreted by the m2000rt system software and reported as detected or not detected. Samples were considered positive when a signal was detected at Ct ≤37 for any gene. A sample was interpreted as negative if the IC was amplified, but not the viral genes. If

there was no amplification of the IC, a specimen was considered invalid.

Liferiver 2019-nCoV testing was performed by the Virology Laboratory, Institute of Public Health of Vojvodina. Liferiver 2019-CoV was performed as *per* manufacturer's instructions and on three separate platforms, i.e., a sample preparation unit, the ExiPrep™16 Dx Fully Automated Nucleic Acid Extraction System, an amplification and detection unit, the SaCycler-96 (Sacace Biotechnologies S.r.l., Como, Italy) and ABI Prism (R) 7500 instrument (Applied Biosystems, Foster City, CA, USA). RNA was extracted using the ExiPrep™ Dx Viral DNA/RNA Kit (Bioneer Corporation, Daejeon, Republic of Korea). The Ct from the fluorophores FAM (ORF1ab), HEX (hexachloro-fluorescein) (N gene), and Cal Red 610 (E gene) and Cy5 (cyanine 5) (IC) was acquired. Samples were considered positive when a signal was detected for any gene. A sample was interpreted as negative if the IC was amplified, but not the viral genes. If there was no amplification of the IC, a specimen was considered invalid[14,15].

Positive percent agreement and 95% CI for the Liferiver 2019-nCoV assay were calculated using ACOV as the reference test. An additional 40 negative specimens were selected to evaluate negative agreement. Cohen's kappa of qualitative results (detected/not detected) between the Liferiver 2019-nCoV test and ACOV was also calculated with 95% CI. The values of Cohen's kappa greater than 0.600 were defined as a moderate level of agreement, and kappa values of 0.80-0.90 were interpreted as high agreement between the two tests[17].

Statistical analysis was performed using IBM® SPSS Version 20.0 software (IBM, Armonk, NY, USA).

Results

Our study included 105 NPS specimens (65 (61.9%) positive and 40 (38.1%) negative) previously tested by the ACOV and subsequently tested by the Liferiver 2019-nCoV. All patients were adult individuals with symptoms or suspected of COVID-19, aged from 26 to 91 years. The mean age was 62.3 years for positive samples and 68.9 years for negative samples. In general, the majority of positive results were obtained from male samples (61.5%) and the majority of negative results were obtained from female samples (62.5%) (Table 1).

Testing results provided by the Liferiver 2019-nCoV and Abbott RT SARS-CoV-2 are shown in Table 2. In 60 (57.1%) samples, SARS-CoV-2 gene sequences were detected by both assays, and 40 (38.1%) samples tested negative for SARS-CoV-2 RNA by both assays. Compared to ACOV, the Liferiver 2019-nCoV test correctly identified 60/65 positive samples with SARS-CoV-2 target RNA sequences and 40/40 negative samples, yielding an overall percent agreement of 95.24% (95% CI: 91.17%-99.31%) (Table 2). The positive percent agreement was 88.89% (95% CI: 77.42%-100.0%), while Cohen's kappa coefficient was 0.901 (95% CI: 0.817-0.985) (Table 2), indicating a high level of agreement between these two tests. The negative percent agreement was 94.12% (95% CI: 89.74%-98.50%). The median Ct value of concordant positive samples tested by the ACOV assay was 16.26 (95% CI: 14.61-17.90), ranging from 3.90 to 25.76, with a standard deviation (SD) of 6.87. The median Ct value for concordant samples by the Liferiver 2019-nCoV assay was 27.44 (95% CI: 25.59-29.29), ranging from 15 to 41, with SD of 7.26 for N gene.

The ACOV assay produced 65 (61.9%) positive results (Table 2). The median Ct value of positive samples on the ACOV assay was 17.37 (95% CI: 15.57-19.17), ranging from 3.90 to 31.99, with SD of 7.38. Our study did not find cases in which NPS specimens tested by ACOV were negative in the face of a positive result on Liferiver 2019-nCoV. Five (4.76%) samples that tested positive on ACOV but negative on the Liferiver 2019-nCoV test had a median Ct value of 30.77 (95% CI; 30.11-31.43), ranging from 30.11 to 31.99, with SD of 0.76. The Ct values of the N gene obtained by the Abbott Real Time SARS-CoV-2 assay were compared with those obtained on the Liferiver 2019-nCoV Kit T-test. The Ct values obtained on the Abbott Real Time SARS-CoV-2 assay were significantly lower ($p<0.001$).

Figure 1 shows a comparison of Ct values recorded in samples detected by both the ACOV assay and Liferiver kit with Ct values in samples detected only by the ACOV assay. Median Ct value differences were compared using the Mann-Whitney U-test. The difference was not statistically significant ($p=0.1556$).

Table 1. Demographics of involved patients

Abbott Ct Category	Mean age (years)	Male (%)	Female (%)
Positive	62.3	40 (61.5)	25 (38.5)
Negative	68.9	15 (37.5)	25 (62.5)

Ct = cycle threshold

Table 2. Detection of SARS-CoV-2 RNA by Abbott RT SARS-CoV-2 assay and Liferiver 2019-nCoV

Abbott RT SARS-CoV-2	Liferiver 2019-nCoV		Total	% Positivity	Value of kappa (95% CI)
	Detected	Not detected			
Detected	60	5	65	62	0.901 (0.817- 0.985)
Not detected	0	40	40		
Total samples tested	60	45	105		

Abbott RT SARS-CoV-2 = Abbott RealTime SARS-CoV-2; Liferiver 2019-nCoV = Liferiver Novel Coronavirus Real Time Multiplex RT-PCR; CI = confidence interval

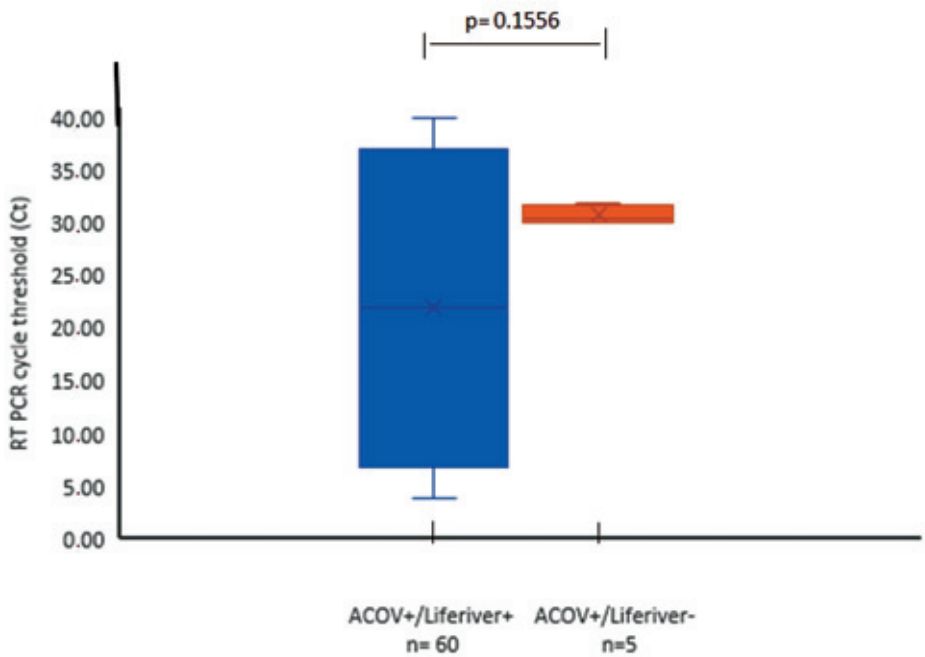

Fig. 1. Comparison of Ct values between samples detected by both assays (ACOV and Liferiver) and Ct values in samples detected by the ACOV assay only.

Discussion

Early, rapid and accurate detection of SARS-CoV-2 is essential for successful control of COVID-19[18]. COVID-19 infections caused by SARS-CoV-2 are primarily diagnosed in the laboratory using nucleic acid amplification tests (NAATs) on respiratory tract samples. Indeed, during the outset of symptoms, upper respiratory tract specimens such as NPS, generally have elevated SARS-CoV-2 virus contents[19]. Many NAATs for SARS-CoV-2 are available because of the high demand for COVID-19 testing across the world. This breakthrough has a significant benefit of making a wide range of diagnostic tests available to health systems, allowing health care clinicians to respond to the pandemic diagnostic demands[20]. The golden standard for molecular diagnosis of SARS-CoV-2 is considered the nucleic acid QRT-PCR test[21]. Differences in SARS-CoV-2 detection are well-known and documented in the literature, and they are the result of a number of factors, including the target gene and the Ct used to designate a positive sample (some methods go beyond 39 Ct, which means a very low viral load)[22].

In this study, the Abbott Real Time SARS-CoV-2 assay and the Liferiver Novel Coronavirus Real Time Multiplex RT-PCR kit were compared for clinical performance. In this comparative analysis, we found the ACOV assay to detect more cases of COVID-19 infection than the Liferiver 2019-nCoV assay. Also, we found that the Liferiver 2019-nCoV kit demonstrated a high level of agreement with the ACOV assay[17]. The positive percent agreement was 88.89% (95% CI: 77.42%-100.0%), while Cohen's kappa was 0.901 (95% CI: 0.817-0.985) between these two tests. Our results comparing the ACOV and Liferiver 2019-nCoV assays are concordant with those recorded by Harrington *et al.*, who have reported increased detection of SARS-CoV-2 RNA gene sequences by ACOV compared to the ID Now COVID-19 (IDNCOV) assay (Abbott). Overall agreement was 75% positive agreement (95% CI: 67.74%-80.67%) and 99% negative agreement (95% CI: 97.64; 99.89%) between ACOV and IDNCOV[23]. Moore *et al.* report on increased detection of SARS-CoV-2 RNA gene sequences by ACOV compared to IDNCOV and a laboratory-developed CDC 2019-nCOV reverse transcriptase PCR (CDC COV) assay[24]. Positive agreement ranged from 75.2% to 100%, with the lowest agreement observed between the ACOV and IDNCOV assay and the biggest between ACOV and CDC COV assays. Negative agreement ranged from 92.4% (ACOV/CDC COV) to 100% (ACOV/IDNCOV). Igloi *et al.* have published results of their research stating that the efficiency of the Liferiver 2019–nCoV test is >90%, and analytical sensitivity of the test is 3.3 to 33 RNA copies for the N and ORF1ab genes, respectively[15]. However, according to Zhou *et al.*, the maximal specificity confirmation target gene ORF1ab is considered to be less sensitive than other targets in clinical use[25]. Furthermore, the performance of our assays was very good. There were no 'unconvincing findings', possible 'false negative' or 'invalid'. The IC is a powerful element of both of our assays. The Abbott assay IC target sequence is obtained from the hydroxypyruvate reductase gene of the pumpkin plant, *Cucurbita pepo*, and is delivered in an Armored RNA® particle diluted in negative human plasma, and its detection is important to demonstrate the sampling procedure reliability[13]. IC in the Liferiver 2019-nCoV assay is a plasmid containing non-target RNA fragment that will be added into the specimen before RNA extraction procedure to evaluate RNA extraction efficiency and identify possible PCR inhibitors[14]. Also, in reality, proper NPS collection has been shown to be a critical feature of the preanalytical phase that greatly influences NAAT findings, being one of the most common and likely causes of false-negative results and therefore of a late diagnosis[20]. In addition, we did heat inactivate specimens at 56°C for 30 min prior to testing because they provide adequate protection for operators against aerosol and droplet exposure. Thermal inactivation may degrade the single-stranded RNA target and cause false negatives in QRT-PCRs, so our results may differ from laboratories that choose to do so[26].

The median Ct value of concordant positive samples by the ACOV assay was 16.26 (95% CI: 14.61-17.90), ranging from 3.90 to 25.76, with SD of 6.87 for N gene. The median Ct value for concordant samples by the Liferiver 2019-nCoV assay was 27.44 (95% CI: 25.59-29.29), ranging from 15 to 41, with SD of 7.26 for N gene. We found similar data in the research by Moore *et al.*, where median Ct for concordant positive samples by the ACOV assay was 15.34 (95% CI: 11.27-18.13)[24].

Our study did not find cases in which NPS specimens tested by ACOV were negative in the face of a positive result on Liferiver 2019-nCoV. We found

similar results in the research by Moore *et al*[24]. None of the 200 NPS samples tested for SARS-CoV-2 was negative on ACOV and positive on the other two commercial QRT-PCR kits.

Five (4.76%) samples that tested positive on ACOV but negative on the Liferiver 2019-nCoV test had a median Ct value of 30.77 (95% CI: 30.11-31.43), ranging from 30.11 to 31.99, with SD of 0.76, and were consistent with lower viral loads. Ct is the number of replication cycles required to produce a fluorescent signal, with lower Ct values representing higher viral RNA loads[26,27]. In a study by Moore *et al.*, eight discordant samples (of a total of 200 tested for COVID-19) were not detected or gave inconclusive results by the CDC COV assay but were detected by the ACOV assay. The median Ct value for these samples by the ACOV assay was 27.73 (95% CI: 27.37-28.40)[24]. Discrepant results were observed almost exclusively in samples with higher Ct values, i.e., with lower viral concentrations. Higher viral loads are inversely related to Ct values[25,27]. These findings point to differences in the test lower limit of detection (LOD). The specified LOD in the published instructions for ACOV usage[13,29] is 100 copies/mL and 1,000 copies/mL for Liferiver 2019-nCoV[14,15]. False-negative results on Liferiver 2019-nCoV are most likely due to the higher LOD on Liferiver 2019-nCoV, and there is also the possibility of a preanalytical sampling error[23,25,27]. However, a 'positive' PCR result reflects only detection of viral RNA and does not necessarily indicate the presence of a viable virus[26,30]. The negative results obtained by using the Liferiver 2019-nCoV assay can be partially explained by the smaller input volumes used for the extraction (200 μL) and amplification (5 μL) compared to extraction volumes of 500 μL and amplification volumes of 40 μL in the ACOV test. However, since the targets for amplification and detection in the ACOV assay are easier to reach, the ACOV gave more positive SARS-CoV-2 results, suggesting that the same samples were false-negative using Liferiver 2019-nCoV kit[24,31].

By comparing the mean value of Ct among the samples detected by both the ACOV test and the Liferiver set with the values of Ct among the samples detected only by the ACOV, no statistically significant difference was found (p=0.1556). The Ct values obtained by the Abbott RealTime SARS-CoV-2 assay were significantly lower (p<0.001) in comparison with those obtained by the Liferiver 2019-nCoV kit. Overall, the Liferiver 2019-nCoV assay compared to the Abbott RealTime SARS-CoV-2 assay demonstrated no significantly different performance characteristics.

It should also be mentioned that the ACOV has longer runtime than the Liferiver 2019-nCoV assay, approximately 3 h for one full run of 94 patient samples, but detects more cases of COVID-19 than the Liferiver 2019-nCoV test. Total testing time for the Liferiver 2019-nCoV kit is shorter. The run should take approximately 1 hour and 20 minutes to complete. The Liferiver 2019-nCoV test was easier to perform and provided a result in a shorter period of time, but detected fewer cases of SARS-CoV-2[14,24].

An important powerful aspect of the Liferiver 2019-nCoV kit is that it is intended to be open, either for the nucleic acid extraction step or in the QRT-PCR assay that will be performed on several instruments (in this study, we tested two of them). Therefore, the Liferiver 2019-nCoV assay can be used in any molecular biology laboratory. Additionally, Liferiver 2019-nCoV kit is free from the industrial production logic of 'closed systems' such as ACOV and, conversely, is hypothetically available for distribution in large quantities. This aspect, at a time of great demand for tests and the well-known shortcomings of commercial kits, can be a significant strength in facilitating their introduction into microbiological laboratories[32].

Conclusion

In conclusion, we found that the ACOV assay detected more cases of COVID-19 infection than the Liferiver 2019-nCoV assay. As only 4.76% of cases were false-negative using the Liferiver test to detect SARS-CoV-2, there was high agreement between the Abbott SARS-CoV-2 and Liferiver 2019-nCoV assays. However, due to possible false-negative results using the Liferiver 2019-nCOV test, we recommend complete testing with the ACOV test.

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Sažetak

USPOREDBA TESTOVA ABBOTT REAL TIME SARS-COV-2 I LIFERIVER NOVEL CORONAVIRUS REAL TIME MULTIPLEX ZA RT-PCR DETEKCIJU SARS-COV-2 IZ NAZOFARINGEALNIH BRISEVA

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Cilj ove studije bio je usporediti učinkovitost dvaju odobrenih testova, Abbott Real Time SARS-CoV-2 (ACOV) testa (Abbott Molecular Inc., North Chicago, IL, SAD) i Liferiver Novel Coronavirus Real Time Multiplex RT-PCR (Liferiver 2019-nCoV) testa (BioVendor Group, Brno, Češka Republika) i utvrditi utječe li odabir ciljanih gena na specifičnost testa. Uključili smo 105 nazofaringealnih briseva odraslih osoba sa simptomima ili sumnjom na koronavirusnu bolest 2019 (COVID-19) u dobi od 26 do 91 godine, koji su prvobitno testirani primjenom testa ACOV, a potom testa Liferiver 2019-nCoV. U ovoj usporednoj analizi otkrili smo da je test ACOV otkrio više slučajeva infekcije COVID-19 od testa Liferiver 2019-nCoV. Liferiver 2019-nCoV test pokazao je visoku razinu slaganja s testom ACOV. Pozitivni postotak slaganja bio je 88,89% (95% interval pouzdanosti (95% CI): 77,42%-100,0%) i Cohenov kappa koeficijent 0,901 (95% CI: 0,817-0,985). Negativni postotak slaganja bio je 94,12% (95% CI: 89,74%-98,50%), dok je 4,76% SARS-CoV-2 slučajeva bilo lažno negativno pomoću Liferiver testa. Međutim, zbog mogućih lažno negativnih rezultata pomoću testa Liferiver 2019-nCOV preporučamo kompletno testiranje testom ACOV.

Ključne riječi: *Molekularna dijagnostika; PCR; Izolacija RNK; Otkrivanje virusa*