

Impact of Point-of-Care Laboratory Diagnostics on Emergency Department Processing Times During the COVID-19 Pandemic

Utjecaj laboratorijske dijagnostike uz krevet bolesnika na vrijeme obrade hitnih bolesnika tijekom pandemije COVID-19

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Abstract. Aim: In 2020 Emergency department (ED) in the Clinical hospital centre Rijeka established Point-of-care polymerase chain reaction (POC-PCR) testing for *Severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2). In addition to suspect cases, the ED also regularly tested hospital employees. To reduce the number of samples, employee's swab samples were pooled for testing. This study aimed to analyse the impact of POC-PCR on diagnostic samples' processing times, evaluated the efficacy of the pooling method among hospital personnel, and determined percentage of false-negative rapid antigen tests (RATs) in comparison with POC-PCR. **Materials and Methods:** The study included ED patients, hospitalized patients, and hospital personnel. Patients underwent oropharyngeal and nasopharyngeal swabbing. Personnel were screened using the pooling method, combining up to five swabs per test tube. Real time PCR (RT-PCR) was modified to POC-PCR, with or without automated nucleic acid extraction. High-priority patients were tested using the Multiplex PCR QIAstat-Dx Respiratory SARS-CoV-2 Panel. **Results:** Before the POC-PCR laboratory was established, the median processing times from sample collection to PCR results were 74.29 and 8.35 hours, respectively. Following the establishment of the POC-PCR laboratory in 2021, the median further reduced to 3.25 hours in September 2021, 2.30 hours in September 2022, and eventually reached 1.30 hours in September 2023. Additionally, out of the total 1608 RATs conducted over a 3-month period, 3.4% were false negatives. The pooling method allowed hospital staff samples to be analysed within 2.30 hours, or in the case of a positive pool, within 6 hours of each shift. Multiplex PCR for severe ED patients showed predominant SARS-CoV-2, followed by Rhinovirus/Enterovirus, Influenza, and other Coronaviruses. **Conclusion:** POC-PCR integration in the ED has significantly reduced time to diagnoses and proved the efficacy of the pooling method, easing the burden on ED and paving the way for further POC-PCR laboratory development.

Keywords: emergency medicine; point-of-care systems; polymerase chain reaction; SARS-CoV-2

Sažetak. Cilj: U Objedinjenom hitnom bolničkom prijemu (OHBP) Kliničkog bolničkog centra Rijeka 2020. godine uspostavljeno je molekularno testiranje kraj kreveta bolesnika (engl. *Point-of-care polymerase chain reaction; POC-PCR*) na teški akutni respiratorni sindrom koronavirus 2 (engl. *Severe acute respiratory syndrome coronavirus 2; SARS-CoV-2*). Uz bolesnike sa sumnjom na infekciju virusom SARS-CoV-2 redovito su testirani i zaposlenici. Ova studija analizira utjecaj POC-PCR-a na vrijeme obrade uzoraka, učinkovitost metode zbirnog testiranja te postotak lažno negativnih brzih antigenskih testova (BAT) u usporedbi s POC-PCR-om. **Materijali i metode:** Istraživanje je uključivalo bolesnike OHBP-a, hospitalizirane bolesnike i bolničko osoblje. Bolesnicima su uzeti brisevi orofarinksa i nazofarinksa. Osoblje je testirano zbirnim testiranjem (engl. *"pooling"*) koje uključuje do pet briseva nazofarinksa u istoj testnoj epruveti. PCR u stvarnom vremenu modificiran je u POC-

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PCR, a metode su izvođene s automatskom ekstrakcijom nukleinskih kiselina ili bez ekstrakcije. Visoko prioritetni slučajevi testirani su koristeći multipleks QIAstat-Dx SARS-CoV-2 respiratorni panel. **Rezultati:** Prije integracije POC-PCR laboratorija u OHBP, medijani potrebnog vremena od prikupljanja uzoraka do PCR rezultata iznosili su 74,29 i 8,35 sati. Nakon otvorenja POC-PCR laboratorija 2021. g. medijan se smanjio na 3,25 sati 2021. g., 2,30 sati 2022. g. te konačno dosegno 1,30 sati 2023. g. Također, od ukupno 1608 BAT-ova testiranih tijekom perioda od tri mjeseca, 3,4 % bilo je lažno negativno. Uvođenjem zbirnog testiranja brisevi bolničkog osoblja obrađeni su unutar 2,30 sati, a u slučaju pozitivnog testa, unutar šest sati od početka svake smjene. Najteže kategorije pacijenata testirane su multipleks PCR-om i rezultati pokazuju da SARS-CoV-2 prevladava, a slijede ga rinovirus/enterovirus i virusi influence. **Zaključak:** Integracija POC-PCR dijagnostike u OHBP-u značajno je ubrzala obradu uzoraka i pokazala učinkovitost zbirnog testiranja među bolničkim osobljem, čime se smanjuje pritisak na sustav hitne medicine i potiče daljnji razvitak POC-PCR laboratorija.

ključne riječi: hitna medicina; lančana reakcija polimerazom; pretrage uz bolesnika; SARS-CoV-2

INTRODUCTION

More than 775 million cases of *Corona Virus Disease of 2019* (COVID-19) have been reported since the beginning of the pandemic, according to the World Health Organization (WHO). This pandemic has had a significant impact on the society and the economy worldwide¹. It has posed significant challenges to healthcare systems while also emphasizing the critical need for efficient and fast diagnostic procedures and expertise in employing them, particularly in emergency departments (EDs). As these departments faced exceptional patient loads alongside limited resources², healthcare institutions were forced to reevaluate their operational strategies.

The integration of point-of-care (POC) laboratory diagnostics, performed close to the site of patient care, such as real-time polymerase chain reaction (RT-PCR) method or rapid antigen tests (RATs), emerged as a vital strategy to streamline patient care and mitigate the spread of the virus³. While RATs are easy to implement and require minimal training, its low sensitivity requires RT-PCR as a method of precise clinical validation. Even though RT-PCR has a low turnover time, it has been a gold standard in COVID-19 diagnostics since the start of the pandemics due to its high

specificity and accuracy and relatively short duration⁴. Rapid and efficient diagnosis on-site eliminates the need for sample transportation to centralized laboratories, which is important for the early diagnosis, isolation, and efficient treatment of infected individuals and their contacts⁵. The COVID-19 pandemic led to the implementation of POC-PCR at the Clinical Hospital Rijeka ED shortly after the pandemic began, contributing significantly to pandemic control in this region. This article highlights the transformative impact

This article highlights the transformative impact of POC diagnostics on ED processing times during the COVID-19 pandemic, focusing on September (2020-2023) when EDs started to experience an increased rate of respiratory infections. It demonstrates the superiority of POC-PCR method over RATs.

of POC diagnostics on ED processing times during the COVID-19 pandemic, focusing on September (2020-2023) when EDs started to experience an increased rate of respiratory infections. We demonstrate the efficacy of the sample pooling method^{6,7}, facilitating the expedited screening process of hospital personnel, which is crucial for the prevention of hospital outbreaks. Additionally, implementation of POC diagnostics has had a major impact on quick detection of false-negative RATs. Finally, POC-PCR has enabled testing of high-priority patients using the Multiplex PCR QIAstat-Dx Respiratory SARS-CoV-2 Panel with the ability to detect 21 pathogens (bacteria and viruses including *Severe acute respiratory syndrome coronavirus 2*, SARS-CoV-2).

We highlight the importance of the integration of POC-PCR diagnostics within the emergency setting and aim to highlight its key role in optimizing patient flow and enhancing overall healthcare delivery, by taking COVID-19 pandemic as a proof of concept.

MATERIALS AND METHODS

Sample collection and handling

Several groups were included in our study: a) patients presenting at the ED, with or without clini-

cal symptoms indicative of SARS-CoV-2 infection, b) hospitalized individuals diagnosed with COVID-19 and receiving care within the clinical setting, c) healthcare personnel operating within the hospital environment; d) and high priority patients. For sample collection, patients were swabbed with oropharyngeal (OPS) and nasopharyngeal (NPS) synthetic swabs (Copan). Swabs were then placed within RNA and RNase free 15 mL conical tubes (Falcon) containing either 2 mL of molecular grade (MG) water (RNase, DNA, and DNase-free water), 2 mL of Universal Transport Medium (UTM) (Copan) or 3 mL of specimen transport medium (Xi'an tianlong science and technology, Xi'an, China). To procure MG water, demineralized water underwent purification using an OmniaPure UV/UF water purifier (StakPure GmbH, Niederahr, Germany). A special sample category entailed "pooled samples," combining five oropharyngeal swabs within the same sample tube containing 2 mL of MG water. These samples were collected at the beginning of the shift from healthcare personnel. All collected samples were stored at +4°C until processing, for a maximum of 5 hours. In instances where transportation to alternate locations such as other clinics or facilities was necessary, swabs were dispatched on ice packs within thermo-bags or Styrofoam boxes. If samples could not be processed within the specified 5-hour window, they were stored at -80°C.

RNA isolation and RT-qPCR

A portion of the swab samples was placed into UTM for RT-qPCR, facilitating the subsequent isolation of RNA utilizing the NucleoSpin RNA Virus isolation kit (Macherey Nagel), according to manufacturer's instructions. For detection of SARS-CoV-2 without RNA isolation (direct method), the Seegene Allplex SARS-CoV-2 Assay (Seegene, Seoul, Korea) was modified into a POC direct quantitative polymerase chain reaction (POC-dqPCR) method employing premixed aliquots as described previously⁷. Post-2022, RNA isolation was conducted using the PANA 9600 S automatic nucleic acid extractor (Xi'an tianlong science and technology, Xi'an, China), employing the 1 copy COVID-19 qPCR 4plex Kit (Clinomics Inc./1 drop

Inc, Republic of Korea) and the 1 copy COVID-19/FluA/FluB/RSV qPCR Kit according to manufacturer's instructions. All PCR analyses were performed using the CFX96Dx instrument (Bio-Rad, Hercules, California, USA). Samples from patients with the highest priority according to clinicians opinion underwent multiplex analysis for COVID-19 and other respiratory pathogens using the QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAGEN, Hilden, Germany) on the QIAstat-Dx Analyzer 1.0 (QIAGEN, Hilden, Germany), following the manufacturer's instructions.

Statistics

Data are presented (SD) or medians (interquartile range, IQR) based on normality testing by Shapiro–Wilk test. To compare nonparametric data, Mann–Whitney U Test and Kruskal–Wallis test (with Dunn's multiple comparison post-test) were used. The percentage of false negative RATs was calculated by dividing the number of false negative RAT results by the total number of samples that underwent both PCR and RAT testing. GraphPad Prism software (Version 8.0.1., La Jolla, CA, USA) was used.

RESULTS

The duration from sampling to final diagnosis of SARS-CoV-2 infection before and after POC-PCR establishment

The period from sample collection to the result of PCR testing was compared across the years before and after the opening of a POC-PCR laboratory during the COVID-19 pandemic. Prior to the establishment of the POC-PCR laboratory, the median time in March 2020 was 74.29 hours [IQR 40.08-89.49], which decreased to 8.35 hours [IQR 6.22-20.00] by September 2020 (Fig. 1, Table 1) when the samples have been analysed using RT-PCR but at a distinct location from the ED. Following the opening of the POC-PCR laboratory in 2021, the median time was shortened to 3.25 hours [IQR 2.55-12.40], 2.30 hours [IQR 2.15-3.00], and 1.30 hours [IQR 1.20-2.00] in September 2021, 2022, and 2023, respectively. March 2020 and September 2020 were compared to each of September 2021, 2022, and 2023, with all comparisons showing statistically significant

Table 1. Statistical analysis of RT-PCR duration (in hours) at the beginning of COVID-19 pandemic and in September 2020-2023

	March 2020	September 2020	September 2021	September 2022	September 2023
Number of Values	30	1130	2880	2755	1545
Minimum	20.55	2.20	1.55	1.00	0.30
25% Percentile	40.08	8.22	2.55	2.15	1.20
Median	74.29	8.35	3.25	2.30	1.30
75% Percentile	89.49	20.00	4.15	3.00	2.00
Maximum	187.30	44.30	12.40	10.00	60.00
Range	187.10	42.10	10.85	9.00	60.00

differences ($p < 0.001$). The number of values, minimum, maximum and range are provided in Table 1. In summary, the implementation of POC diagnostics at the ED has reduced the time from sampling to final diagnosis by a factor of almost 57 times.

Percentage of false negative RAT

As the number of COVID-19 patients increased, the Croatian Ministry of Health provided RATs to all hospitals for POC testing of suspected COVID-19 cases. RATs do not require any specialized equipment or additional testing; however, since they detect antigen without additional amplification steps, they can also display lower sensitivity⁸. Having already an established POC-PCR laboratory, we set out to compare the accuracy of qualitative RAT to POC-PCR diagnostics. Over a 3-month period in 2022, 1608 patients were tested at the ED using RAT and POC-PCR, of which 54 were confirmed as false negatives by PCR, accounting for 3.4% of falsely negative tested samples or 96.6% sensitivity (Fig. 2). This underscores the necessity of PCR validation before proceeding with management, especially in clinical settings with sensitive patients.

Hospital staff "pooling" screening method

In response to the high risks faced by hospital staff in acquiring and potentially spreading infections before the onset of first symptoms, a POC-PCR laboratory introduced fast screening of the staff at the start of each shift. This pooling method analyzed swab pools from groups of five individuals, enabling testing of the whole clinic staff within a timeframe of 2.30 hours. Individuals

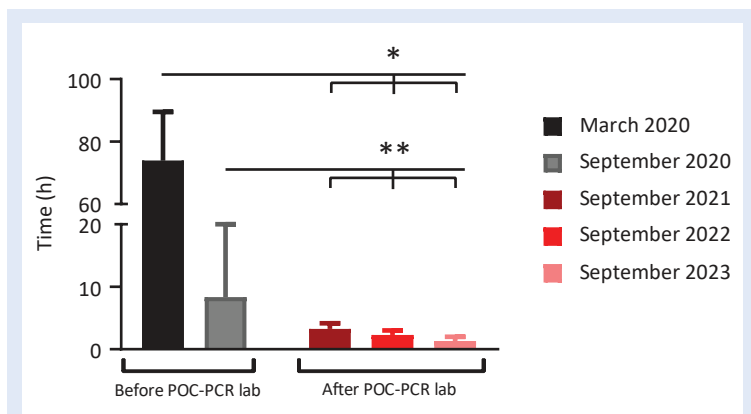


Figure 1. The average time of COVID-19 PCR diagnostics before and after POC-PCR laboratory establishment at the emergency department. The period (in hours) from sample collection to result of a PCR test has been quantified for each patient ($n=8457$). Data are presented as median (interquartile range [IQR]). Kruskal–Wallis test (with Dunn’s multiple comparison post-test) was used to compare March 2020 and September 2020 with September 2021, 2022, and 2023, respectively. (* $p < 0.001$, ** $p < 0.001$)

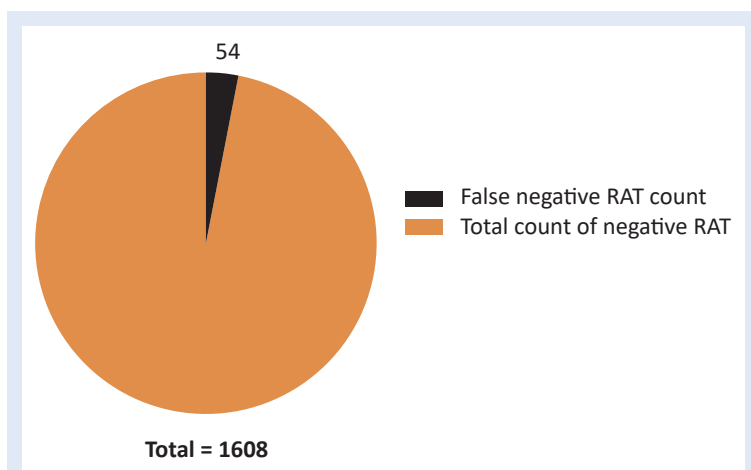


Figure 2. Number of false-negative RAT tests ($n=54$) compared to a total count of negative RATs ($n=1608$) confirmed by the RT-PCR analysis over a period of 3 months (2022)

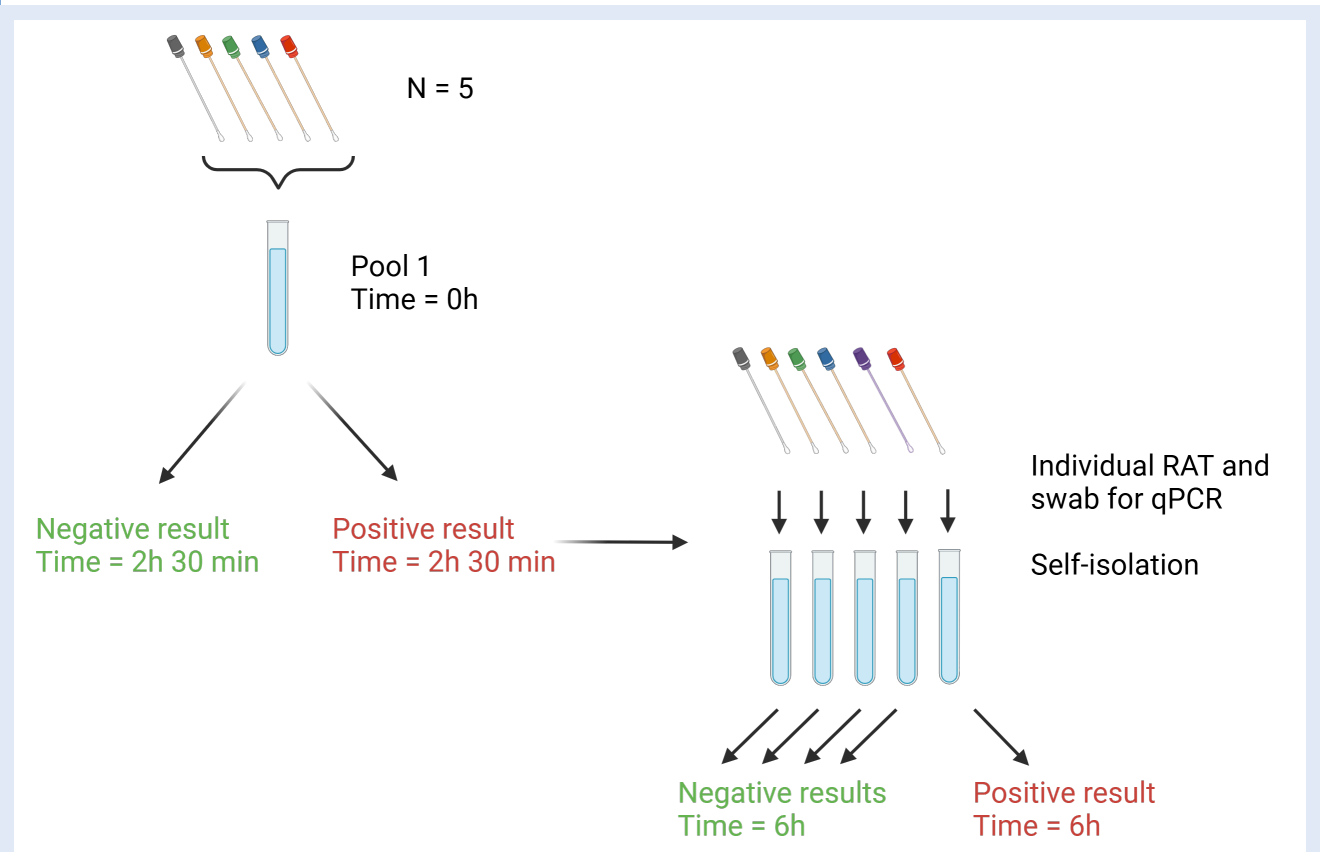


Figure 3. Timeline scheme of staff COVID-19 screening Hospital staff members were tested at the beginning of a shift using the pooling method for fast screening of COVID-19 positive individuals. Results were finished within six hours.

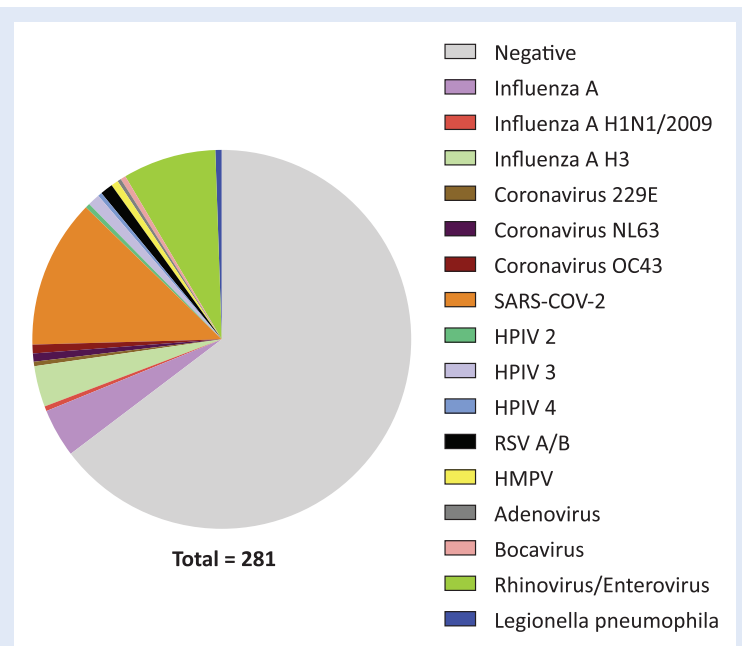


Figure 4. Appearance of other respiratory pathogens during the COVID-19 pandemic using the respiratory panel on the rapid QIASTAT Analyzer. (HPIV-Human parainfluenza viruses; RSV-Respiratory syncytial virus; HMPV-Human metapneumovirus)

from positive pools underwent further testing using both RAT and POC-PCR and were isolated until results were available (Fig. 3). Within 6 hours of starting each shift, positive individuals among hospital staff were detected and isolated, helping to prevent outbreaks.

QIASTAT respiratory panel

In patients classified under the most severe triage categories, requiring rapid intervention and exhibiting symptoms indicative of COVID-19 or other potential respiratory infection, QIAstat-Dx Respiratory SARS-CoV-2 Panel was performed. The data presented are from the years 2021 and 2022. Coronaviruses had the highest positive rate at 15,41%, among which SARS-CoV-2 had a rate at 13.53%. Rhinovirus/Enterovirus was the next common at 8.65%, along with Influenza A and its subtypes also at 8.65%. Parainfluenza viruses were 1.89%, while Respiratory Syncytial Virus A/B was 1.13%. Other microorganisms were found in less than 1% of cases (Fig. 4, Table 2).

Table 2. Number and percentages of all detected respiratory pathogens during the COVID-19 pandemic using QIASTAT Analyzer.

Total Count	266	%
Negative	181	68.05
Influenza A	12	4.51
Influenza A H1N1/2009	1	0.38
Influenza A H1	0	0.00
Influenza A H3	10	3.76
Influenza B	0	0.00
Coronavirus 229E	1	0.38
Coronavirus HKU1	0	0.00
Coronavirus NL63	2	0.75
Coronavirus OC43	2	0.75
SARS-COV-2	36	13.53
Parainfluenza 1	0	0.00
Parainfluenza 2	1	0.38
Parainfluenza 3	3	1.13
Parainfluenza 4	1	0.38
Respiratory Syncytial Virus A/B	3	1.13
Human Metapneumovirus	2	0.75
Adenovirus	1	0.38
Bocavirus	1	0.38
Rhinovirus/Enterovirus	23	8.65
Legionella Pneumophila	1	0.38
Bordetella Pertussis	0	0.00

DISCUSSION

The WHO declared the COVID-19 pandemic in March 2020⁹. Healthcare systems urgently required quick, efficient measures for diagnosing COVID-19, implementing patient segregation, contact tracing, and reducing infection rates to prevent and minimize intra-hospital infections, which could further burden the healthcare system¹. Initially, a small portion of laboratories had the know-how and the capacity to perform COVID-19 diagnostics, leading to overburdened facilities and prolonged waiting times due to the high volume of samples.

At the very beginning of the pandemic, RT-PCR diagnostics were carried out at another facility out of Rijeka, leading to a waiting period of 24 to a maximum of 72 hours. Subsequently, local laboratory facilities such as at the Department of Public Health of Primorsko-goranska County and the Center for Proteomics, Faculty of Medicine, Rijeka established COVID-19 diagnostics, resulting in a re-

markable reduction in waiting times within the region, ranging approximately from 6 to 20 hours. However, these procedures still required managing extensive patient lists and transporting infectious materials to other institutions. Even 6 hours wait time, although significantly shortened from the initial, presented problems in patient management, contributing to an overload in EDs, which were responsible for patient intake, segregation and isolation, highlighting the urgent need to establish local diagnostic capacities.

In response to these challenges, a POC Laboratory for rapid molecular diagnostics was established within the ED of Clinical Hospital Rijeka in 2021, the first of its kind in Croatia. This initiative markedly reduced waiting times to an average of approximately 3.30 hours, a reduction of 22-fold, with a variation between 2 to 4 hours only. This specialized facility provided rapid and precise testing directly within healthcare settings, thereby optimizing patient care management, reducing transmission risk and the burden on healthcare personnel. Method optimization and technology development have even further accelerated the time for PCR protocol, varying from 1.20 to 3 h in 2022 and 2023 (Fig. 1, Table 1). Besides saving time of clinical decision-making, POC-PCR implementation also reduces operational costs. Numerous studies have analysed the cost-effectiveness of the POC methods, resulting in increased profitability when compared to conventional methods, further supporting on-site patient testing and diagnosis¹⁰⁻¹³.

RATs serve as the primary POC diagnostics for COVID-19, typically providing results within minutes. While RATs are valuable for rapid screening due to their speed and simplicity, they may exhibit lower sensitivity compared to RT-PCR tests, particularly in asymptomatic or early-stage infections, as observed in similar studies¹⁴. Furthermore, factors such as sample quality, viral loads and timing of sample collection can reproduce false results¹⁵. Considering that various factors impact the results of RATs, studies have shown variable findings. For instance, a study investigating asymptomatic patients showed that 17% of RATs are false negative¹⁴, while other research, including university students, resulted in 3.7% false negative tests¹⁶.

Despite the lower-than-anticipated false negative RATs in our context, validation remains crucial to avoid the viral transmission, with POC-PCR playing a major role in this regard as well. However, since not all RAT results were documented, the actual percentage of false negatives could differ. Additionally, a comparison with the percentage of false-negative PCR results would be valuable, but we currently lack the necessary data for such an analysis.

In response to the high risks faced by hospital staff in acquiring and potentially spreading infections before the onset of first symptoms, a POC-PCR laboratory introduced fast screening of the staff at the start of each shift.

Surveillance testing strategies were crucial for managing the spread of COVID-19 within the healthcare system, requiring systematic and regular testing of the employees dealing with COVID-19 patients and suspected COVID-19 patients^{17,18}. To optimize efficiency and resources, the use of pooled samples has been proposed for the testing of multiple samples in a short period of time^{19,20}. Through routine surveillance testing, units minimized intrahospital transmission by detecting infectious personnel in the early stages of infection, prior to the onset of symptoms²⁰. This was particularly important for the ED which provided care for both COVID-19 patients and individuals with other urgent medical needs^{21,22}. Despite the clear benefits of surveillance testing, its implementation presented logistical challenges, including resource management and workflow disruptions²³. While the individual RT-PCR testing of samples would allow for the rapid detection of positive staff members; it would be cost-ineffective due to the greater number of hospital personnel tested at the beginning of each shift. Additionally, at that time, the POC-PCR machines could handle only 192 samples per batch (2 machines x 96 samples each), meaning that testing the hospital staff would have taken over 3 hours. Therefore, the pooling method proved essential, allowing all tested staff to be screened in a single round and enabling the hospital to continue its operations with minimal disruption. Nevertheless,

the outcomes demonstrated the value of proactive testing in safeguarding healthcare workers and patients against COVID-19 transmission within hospital environments.

In addition to SARS-CoV-2 testing, POC-PCR laboratory has introduced more comprehensive PCR analysis into the ED. The QIAstat-Dx Respiratory SARS-CoV-2 Panel is a multiplex PCR assay that is a closed, fully automated, and detects SARS-CoV-2 in addition to 21 other pathogens that cause respiratory disease in less than 2 hours (Fig. 4). For patients experiencing severe respiratory symptoms, it facilitated rapid screening for other diseases similar to COVID-19 to prevent incorrect patient segregation and further disease transmission, thereby enabling timely initiation of treatment²⁴⁻²⁶. Although the primary focus of the paper is COVID-19, as a proof of concept, the inclusion of the QIASTAT panel shows an example of various possibilities of POC laboratories and the benefits of advanced diagnostic tools that can test for multiple pathogens simultaneously. While facilitating on-site multi-screening, the method faced limitations associated with high costs of resources and materials, making it impractical for routine use or widespread applicability across all patients.

CONCLUSION

POC-PCR diagnostics has significantly accelerated the diagnosis of COVID-19 and other respiratory infections among patients and hospital staff in the ED of Clinical Hospital Rijeka. The rapid diagnosis facilitated a timely initiation of suitable and targeted therapy, as well as the transfer of patients to the appropriate hospital departments, thereby relieving the burden on the emergency medical system. This was crucial for the pandemic control within this region, benefiting both patients and personnel within the clinic. Additionally, it promotes close ongoing cooperation between the medical staff with the laboratory personnel, ensuring effective diagnostic readout and fostering common scientific advancements in the field of healthcare. The time of the pandemic brought many challenges but also advantages for the progress of molecular technologies and methods and thus the improvement of the speed of obtaining findings. Based on these foun-

dations, the newly opened Laboratory for POC molecular diagnostics in emergency medicine can further develop methods for other necessary diagnostic procedures that would contribute to faster diagnostics, better therapeutic approaches and greater patient satisfaction. The future of POC-PCR labs holds promise for ongoing technological advancements, broader testing capabilities, improved healthcare accessibility, and the implementation of personalized medical approaches.

Conflicts of Interest: Authors declare no conflicts of interest.

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