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Gargle pool PCR testing in a hospital during medium and high SARS-CoV-2 incidence

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SUMMARY

Background: Hospitals need to be protected from SARS-CoV-2 infections to protect vulnerable patients. Thus, a safe, efficient, and cost-effective SARS-CoV-2 testing system for hospitals, in addition to standard hygiene measures and vaccination of staff, is necessary. Here we report on the feasibility and performance of a pool real-time reverse-transcriptase polymerase-chain-reaction (rRT-PCR) test system at, medium and high incidence.

Methods: We implemented a testing concept based on gargling at home and pooling of samples in the hospital before PCR testing in the laboratory. We used two PCR systems (point of care and standard 96-well plate system) to adapt to challenges in the hospital setting and respond to a rising incidence in the Omicron wave.

Findings: During our 10-week study period, we performed 697 pool PCRs (8793 tests in total) and identified 65 asymptomatic staff members by pool PCR and 94 symptomatic staff members by positive individual PCR. Virus loads in those detected by pool testing were significantly lower ($P<0.001$). The test system remained workable even during the peak of the Omicron wave and no outbreaks occurred in any specific area of the hospital during the study period. Unvaccinated individuals were over-represented in the positively tested (37% vs 22% positive tests, $P=0.04$). The test procedure was well accepted by a majority of the hospital staff (84%).

Conclusion: Repeated gargle pool rRT-PCR testing can be implemented quickly in hospitals and is an effective, easily adaptable and well-accepted test system for hospitals, even during phases with very high infection rates.

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Introduction

Hospitals must be safe for patients and staff despite COVID-19, which they were not in the beginning of the pandemic [1,2]. To achieve this goal, vaccination of staff members is a central strategy, but as the occurrence of new virus variants show, vaccination alone is insufficient. In addition, general non-pharmaceutical interventions such as wearing face-masks, keeping social distance, disinfecting hands, and increasing ventilation in rooms are still necessary and useful to contain the spreading of the virus [3]. A sufficient testing regime is thought to be the third pillar in the strategy against the virus.

In the WICOVIR (Where Is the Corona VIRus?) project, we showed previously that a gargle pool real-time reverse-transcriptase polymerase-chain-reaction (rRT-PCR) test system is a safe, efficient, cost-effective, and accurate way to test large numbers of students and teachers in a school setting [1], which can be implemented quickly and easily [4]. We now report on the application of this system to test the staff of a large university pediatric and maternity hospital. Most patients in this setting were still unvaccinated at the end of 2021 and were thus especially vulnerable to nosocomial infection with the Omicron variant. We assessed how the WICOVIR test system can address specific challenges in testing hospital staff. Different to teachers and students, hospital staff work in shifts, cannot be quarantined easily and need results even sooner. Also, we assessed whether pool testing can still be applied efficiently with high numbers of positive results to be expected as was the case during the Omicron wave.

Materials and methods

Study design

The objective of this proof-of-concept study was to explore whether regular gargle pool rRT-PCR testing is safe, efficient and feasible in a hospital environment. The study was approved by the Ethics Committee of the University of Regensburg (file-number: 21-2240-101). Regular and mandatory WICOVIR testing in the hospital started on 20th December 2021, when testing of hospital staff at least twice per week (depending on vaccination status and previous infection history) became mandatory by German law in order to be allowed entry to the hospital premises. The weekly incidence of SARS-CoV-2 infections per 100,000 inhabitants for Bavaria was retrieved from the official website of the Bavarian public health office (Landesamt für Gesundheit und Lebensmittelsicherheit, LGL) [5].

A browser-based software tool developed with MaganaMed GmbH (Regensburg, Germany) for the study was used to keep track of barcoded pools, pool results, pool dissolving (de-pooling) and to allow for automated correspondence of test results and summary statistics of test results as previously described [1]. Immunization data (SARS-CoV-2 vaccination history and past infections) were collected from all staff members. To comply with the prerequisites of the federal infection protection act, test documentation was combined with a database query to match the immunization status of each individual staff member with the necessary test frequency. Additional information on the software is available upon request from the authors or from the company (https://maganamed.com).

Gargle, pooling and de-pooling procedures

The general feasibility of gargling (throat washings) for SARS-CoV-2 detection [6] and the specific WICOVIR procedure have been described previously [1]. Even though the diagnostic sensitivity is slightly lower when compared with nasopharyngeal swabs, the absence of invasiveness of gargling is a decisive advantage for the acceptance of repetitive testing. In brief, all participants gargled with approximately 6 mL of tap water at home twice or three times per week for approximately 30–60 s to achieve maximal recovery of virus from throat rinsing. A video providing exact guidance and documentation of the gargling procedure is available online at www.we-care.de/WICOVIR. Gargle recovery fluid was collected by the participant in a screw-cap tube and divided into a second screw-cap tube in to approximately equal amounts (2–3 mL each). Both tubes were brought into the hospital in a zip-lock bag. One was for pooling and the other (back-up) was retrieved from staff members and tested only in case of a positive pool result.

In the hospital, one tube was emptied by the participant into a pooling container in a pooling station. The maximum number of participants accepted for one pool was 20 (later reduced to 10) consecutive staff members were attending the pooling station as they entered the hospital. Pooling was supervised by an individual who linked the barcode of the staff member to the pool barcode in our COVID hospital COVIDA software (MaganaMed GmbH, Regensburg). A video documenting the pooling procedure in general is available at www.we-care.de/WICOVIR.

All test procedures were handled by a 50% laboratory worker, a 50% student for support sample handling, and a 50% medical assistant for organizing pools and recall of backup samples. In the event of a positive pool, the COVIDA software immediately generated a list of participants in the positive pool and provided contact details. Pool participants were contacted by the test team and the backup tube with gargle fluid was retrieved usually within 10–20 min from each participant. Individual testing of participants in positive pools was performed immediately. Thus, de-pooling was achieved within 3–4 h after a positive pool was detected. Results from negative pools could be retrieved online using the barcode of the respective pool, which was known to the participants of such a pool.

SARS-CoV-2 pool rRT-PCR testing

We used two set-ups to process gargle pool samples for rRT-PCR: (i) the point of care (PoC) GX-VI-4 module of the GeneXpert instrument (Cepheid, Sunnyvale, CA, USA) as previously described [7] and (ii) a combination of RNA isolation by the Auto-Pure96 Nucleic Acid Purification System (Hangzhou Allsheng Instruments, Shanghai, China) and subsequent PCR on a Bio-Rad real-time PCR system (CFX96; Bio-Rad, Hercules, CA, USA) as previously described [1,4]. Briefly, the GX-VI-4 module of the GeneXpert instrument allows the use of four cartridges of predefined mastermix concomitantly detecting SARS-CoV-2 E and N2 genes. Feasibility for pooling has been shown elsewhere [3]. The Allshang/Bio-Rad system has a capacity of 96 samples per run. Briefly, RNA is extracted from both single and pool samples using the MagnifiQ™ RNA buffer kit (A&A Biotechnology, Gdansk, Poland) on the Auto-Pure96.
Nucleic Acid Purification System according to the manufacturer protocol. RT-PCR-based SARS-CoV-2 RNA detection was performed on a Bio-Rad real-time PCR system using the single-well dual target (ORF1b and N2 gene). We ensured that both systems detected both the Delta and the Omicron variants with high specificity and sensitivity using RNA from sequenced samples as references.

**Online survey on acceptance of test regime**

To assess the acceptance of the WICOVIR gargle pool rRT-PCR by hospital staff, we designed an anonymous online survey applying our previously reported "qnome" database and questionnaire system (www.qnome.eu). The questionnaire consisted of seven questions and is available upon request.

**Statistical analyses**

Data from the gargle pool tests are presented using descriptive statistics. Normally distributed data are presented as mean with standard deviation (SD) and non-parametric data are presented as the median and interquartile range (IQR). Uncensored data were compared using a Wilcoxon test, and in case of censored values, a generalized Wilcoxon test was applied using the ‘survival’ package in R statistics. Permutation tests were performed to calculate differences in infection rates between SARS-CoV-2-naïve and immunized staff by using the ‘coin’ package in R statistics, version 4.1.2. A P-value <0.05 was considered statistically significant.

**Results**

The study was performed at the St. Hedwig’s hospital which houses the KUNO University Children’s Hospital and the University Maternity Hospital, totaling approximately 650 regular staff members (and 70 students) over 10 weeks between December 2021 and March 2022 (Figure 1). During a pre-test phase in the autumn of 2021, we implemented a Cepheid PoC rRT-PCR system to allow for rapid diagnosis of influenza, respiratory syncytial virus (RSV) and COVID-19 cases by multiplex PCR in our large emergency department at the hospital. Subsequently, we explored the possibility of using that system for pool PCR testing of our staff members. From October we offered a free and voluntary gargle pool PCR test service to our hospital staff, symptomatic or asymptomatic. In December of 2021, regular testing became mandatory for all hospital staff to be allowed to enter hospital premises by federal law. Detailed regulation on who was to be tested, how often and by which test system (antigen tests or PCR) were officially published (Supplementary Table S1). In brief, all staff members had to be tested at least twice a week, and test strategies had to be documented. We used the WICOVIR software for the documentation of all testing procedures and combined it with the COVIDA software which held all information (e.g., SARS-CoV-2 vaccination status and infection history) needed to regulate hospital entry and to determine necessary test frequencies by algorithm. All staff members received a personalized barcode linked to that software to enter the hospital through a gate with a barcode scanner. The same barcode was used to link the test samples to sample pools and PCR results. That way, participants of a positive pool could be identified immediately and called back to provide their back-up sample for de-pooling and single PCR testing.

During the medium-incidence phase of the project (incidence of 200 positive PCR tests per 100,000 inhabitants in Bavaria in the last week of December 2021) the size of the gargle test pool was set at 20 staff members and all PCR tests were conducted with the PoC system, which has the capacity to analyze four samples in parallel in 45 min. In case of a positive pool result, backup samples were retrieved immediately, which took approximately 10–20 min and the pool of 20 was dissolved into four pools of five which ran on a PoC system again. That way, 15 of 20 staff members knew that they were negative within 45 min after the positive pool was detected and could continue to work without any restrictions, while the last five samples from the positive pool in the second run were now tested individually. Thus, it took approximately 2 h to identify the positive sample. This set-up was feasible as long as no more than two positive pools occurred per test day.

When incidence rose to 1522 positive PCR tests per 100,000 inhabitants in Bavaria in week 4 of 2022 due to the Omicron wave (high incidence), leading to three or more positive pools per day in our hospital, we reduced the pool size to N = 10 participants per pool and increased the test interval to three tests per week and activated the Allshang/Bio-Rad system in addition to the Cepheid test system. Thus, we could combine the flexibility of testing with increased capacity. All pools until 8:00 a.m. were now tested with Cepheid (early tests) while the Allshang/Bio-Rad system was used to handle the large number of staff members who entered the hospital at regular work times between 7:30 and 8:45 a.m. During this second round of pool tests, all positive pool tests from the early test round were de-pooled running single samples individually. Results were ready by 10.45 a.m. and a second run for dissolving positive pools from the second round and additional pools of latecomers were run at 12:00 p.m., with results available at 13:45 p.m. latest. PCRs for pools and de-pooling in the afternoon were performed on the PoC Cepheid system again. Thus, the time for receiving results increased to maximum of 5 h while the average was less than 3 h.

Overall, we performed 8793 systematic tests during the study period translating to 697 pool PCR runs. Of these, five pools were false positive (0.7%). Additionally, 852 PCR runs were necessary for de-pooling. During the study period of 10 weeks, we identified 65 asymptomatic staff members to be positive by pool testing and 97 staff members became symptomatic and were tested positive by single/individual PCR tests (Figure 1). In general, Ct values of staff members identified by regular pool testing were significantly higher compared with individual PCR tests of symptomatic staff members (median (IQR): 31.5 (26.4—33.6) vs 26.3 (22.1—30.2); P<0.001). In a great majority of cases, these values were beyond the detection limit of antigen tests (Figure 2). Of note, gargle pool tests could not be performed for one week due to an Omicron infection of laboratory personnel (week 9–10 of the study). During that time, virus loads of tests performed when individuals became symptomatic increased by two PCR cycles (Ct values were representing two exponential steps difference). During the study period, we neither observed an outbreak in a specific section of the hospital nor an increase in nosocomial infections in patients but many random infections in the staff. The small group of unvaccinated staff members were over-represented in the positively tested (37.1% positive tests in 2021, regular testing became mandatory for all hospital staff to be allowed to enter hospital premises by federal law. Detailed regulation on who was to be tested, how often and by which test system (antigen tests or PCR) were officially published (Supplementary Table S1). In brief, all staff members had to be tested at least twice a week, and test strategies had to be documented. We used the WICOVIR software for the documentation of all testing procedures and combined it with the COVIDA software which held all information (e.g., SARS-CoV-2 vaccination status and infection history) needed to regulate hospital entry and to determine necessary test frequencies by algorithm. All staff members received a personalized barcode linked to that software to enter the hospital through a gate with a barcode scanner. The same barcode was used to link the test samples to sample pools and PCR results. That way, participants of a positive pool could be identified immediately and
SARS-CoV-2-naive staff vs 21.9% in staff with at least one vaccination or infection; $P=0.04$; Figure 3).

We compared the incidence of staff members identified to be SARS-CoV-2 positive by our test regime to the weekly incidence for the general Bavarian population in the age range (18–60 years) most similar to our hospital staff as provided by the Bavarian public health office (Supplementary Table S2; Figure 1). For every week, the incidence in our hospital staff surpassed the incidence in the general population by an average factor of 1.5- to 2-fold.

At the end of the study period, we asked hospital staff to answer an anonymous online questionnaire about their opinion...
on the implementation, safety and convenience of the gargle pool rRT-PCR test system. Approximately 1/3 of the staff members (202/650) from all areas of the hospital (doctors \( N = 43 \), nursing staff \( N = 96 \), administration and scientific offices \( N = 33 \) and midwives and supportive services \( N = 30 \)) participated in the questionnaire. Overall, 75% rated the implementation as ‘good’ or ‘very good’ (22% ‘fair’ or ‘sufficient’, 3% ‘insufficient’) and only a minority (13%) experienced waiting times (mean: 3 min). An overwhelming majority rated the gargle pool PCR system superior in safety for staff and patients when compared with antigen-based tests (90% vs 10%) and when asked for the preference of a test system, 84% selected the gargle pool PCR system over any antigen-based test system.

**Discussion**

Repeated gargle pool rRT-PCR testing can be implemented quickly and with high acceptance in hospitals and adapted easily even to massive increases in incidence. Due to the high

![Figure 2. Ct values and median value of individuals positively tested for SARS CoV-2 by pool testing (asymptomatic) and single PCR (symptomatic).](image-url)
sensitivity of the PCR test system, positive staff members could be removed from hospital service early enough to avoid infection chains in the hospital.

Our proof-of-concept study aimed to describe the technical, digital and logistical set-up of a gargle pool rRT-PCR testing system in a medium-sized hospital during the onset of the Omicron wave in Germany, and we were able to show the feasibility and acceptance of such an approach. Furthermore, it gave a detailed and accurate picture of Omicron infection-dynamics in hospital staff during that time.

The aim of any testing in a hospital setting is to avoid infection of patients and other staff members. Ideally and theoretically, nobody with a potential infection should work in the hospital. Realistically, this cannot be achieved without major interference with hospital services and the availability of staff. A regular hospital testing scheme in addition to high vaccination/immunization rates is therefore a more feasible approach to that end. The specific challenge of testing in a hospital environment is the need for very high accuracy (which can only be achieved by PCR testing) and the need for transmitting many test results very fast (which is difficult to achieve by PCR testing). Gargle pool PCR test systems can help to overcome limitations in PCR testing rates [1,8,9] and with intelligent software, data transmission of results can be speeded up as we have shown in the WICOVIR project for schools [1].

Our WICOVIR test system, as described in detail elsewhere [1], is based on gargling at home and pooling samples on entering the institution, in this case the hospital. Thus, pooling logistics in the laboratory are not necessary, pre-analytic sample handling is dramatically reduced and time is gained, which is key to successful hospital testing. When a pool was found positive, samples were retrieved immediately from all staff members pertaining to that pool. This was facilitated by the software COVIDA which was based largely on pre-developed software from WICOVIR [1] but adapted and expanded specifically for the hospital test set-up. During the time of de-pooling, members of such a pool were asked to follow strict hygiene measures and to avoid direct patient contact wherever possible. As de-pooling was so fast, this never disrupted hospital service. Importantly, members of single departments and units were not tested in pools clustering the respective department, unit or ward but by random order. Thus, if a pool was tested positive, no single department, unit or ward had to shut down completely. This was a fundamental change in strategy from school testing, where school classes are recommended to be tested together due to logistics [4].

The limitations of such a gargle pool test system are the machines and consumables needed and, as a key factor, experienced staff to run the tests. While machines can be ordered in advance and represent an investment of approximately €100,000, consumables were a limiting factor throughout the pandemic. Shortages in supply chains especially in the PoC test system threatened to shut down operations and forced us to adapt procedures. However, it was the combination of a fast and individualizable PoC system (Cepheid) and a high-throughput ‘workhorse’ system (Allshang/Bio-Rad) which proved ideal for the challenges of the hospital setting. Conversely, the technical expertise required to run the Allshang/Bio-Rad system is substantial. Furthermore, infection of technical personnel needs to be considered and thus, a backup test system with antigen-tests was put in place and had to be activated in week 9 of the project, when lab workers were not available due to Omicron infection. During that time, staff members had to perform self-tests at least twice a week for regular screening testing without symptoms and only one staff member went to PCR testing without symptoms due to a positive antigen test. Interestingly, during that same time more
symptomatic infections were recorded, and Ct-values of the symptomatic tests decreased substantially, indicating a higher virus load at the time infections were detected. Our interpretation of this observation is that the antigen test was not sensitive enough to detect positive cases in the time interval between infection and symptoms. Therefore, more staff members remained undetected while already positive and potentially infectious. This is also reflected by the higher virus loads found when staff members were finally tested when they were symptomatic. While this is not surprising, our study is one of the few that provides actual (but limited) data for that observation.

When the infection numbers rose to unprecedented heights in week 4 of January 2022, due to the Omicron wave, we were not sure whether the pooling system would withstand and allow us to handle such a high number of positive pools to be processed in time. This was always the major argument of opponents to PCR pooling tests. However, with two adjustments to our system, namely adding two runs of the Alshang/Bio-Rad system and decreasing the pool size from 20 to 10 participants when the (true) incidence was beyond 3000 infections per 100,000 individuals, the turn-around time for results was still very acceptable within a 4-h frame. To optimize pool size for our set-up, we developed a pool size calculator: expected incidence, plate- and laboratory personal capacity, as well as requested turn-around time of results were taken into account.

For a good performance of the testing procedure, its acceptance by the hospital staff was imperative. To investigate this, we invited all hospital staff to participate in an online survey. A participation rate of approximately 30% was achieved and can be considered representative, also according to the distribution of participants over employment groups. Interestingly, gargle pool PCR was not only viewed as superior in safety over antigen-testing by the staff, but staff members also preferred the gargle pool testing over self-tests by nasal swabs at home. The reason for this may be that swabbing the nose every two to three days is indeed unpleasant in the long run and more invasive than gargling. This should be considered for the acceptance of future test strategies for hospitals and nursing homes for the elderly, where testing regimes are considered to be needed for the future.

Applying PCR-based tests allowed very precise detection of SARS-CoV-2 infections in our staff. Patients were tested routinely on hospital referral and on a regular basis while they were in-patients. This allowed for a comprehensive picture of infection dynamics in our hospital during the study period which coincided with the beginning of the Omicron wave in Germany. We also compared numbers of positive tests in our staff with officially reported infection rates (Supplementary Table S2). We had full information on the vaccination status and infection history of our staff to plot against infections. While vaccination rates of our staff were much higher than in the general population, the number of detected infections in our hospital were much higher than reported for the general population. This might be explained partly by the fact that detection of SARS-CoV-2 for the wider population is now primarily based on antigen PoC tests, which are inferior to PCR in terms of diagnostic validity, and because a concept of closely knitted, sensitive testing in a defined cohort and setting such as medical staff in a hospital can detect cases more effectively. Conversely, the incidence on the population level might have been underestimated due to delays in reporting because of the high case numbers. Furthermore, the vaccine efficacy in terms of protection against infection (estimated by the Farrington method) might be overestimated due to misclassification concerning vaccination status. Therefore, we conclude that publicly reported infection rates underestimate the true number of infections by approximately a factor of 2. In our cohort, the relative risk of getting infected by Omicron was higher in unvaccinated staff members. However, these numbers need to be interpreted with caution, as we only studied a small cohort. Interestingly, outbreaks and nosocomial infections may be avoided in a hospital setting, even in times of high infection rates, when non-pharmaceutical interventions are complemented with vaccination and a truly functional test regime. Our analysis of infection chains revealed that the vast majority of infections of our staff members occurred in the private setting or during the private contact of staff members (e.g., during breaks).

We conclude that repeated gargle pool rRT-PCR testing can be implemented quickly in hospitals and is an effective, easily adaptable and well-accepted test system for hospitals, withstanding even very high infection rates. Our data show that with a proper testing concept in place, hospitals can be a safe place for patients and staff members even during a pandemic.

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Author Contributions

P.K., M.K. and A.A. contributed to conception and design of the study. P.S. established and supervised test procedures, which were performed by P.K., P.S., E.F. and M.K. A.A. supervised the labwork. B.M.J.L. analysed public health measurements. J.N. and P.P. provided IT support and developed the software for the project. V.D.G. performed the statistical analysis. M.K. and P.K. wrote the first draft of the manuscript. V.D.G. provided specific section of the text. M.K. wrote the final version of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Conflict of interest statement

Maganamed GmbH is a commercial software company and Jakob Niggel and Philip Pagel are employed by Maganamed GmbH. All other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

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