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Clinical performance and accuracy of a qPCR-based SARS-CoV-2 mass-screening workflow for healthcare-worker surveillance using pooled self-sampled gargling solutions: A cross-sectional study

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SUMMARY

Introduction: The large number of asymptomatic SARS-CoV-2 infections necessitates general screening of employees. We evaluate the performance of a SARS-CoV-2 screening program in asymptomatic healthcare-workers (HCW), utilizing self-sampled gargling-solution and sample pooling for RT-qPCR.

Methods: We conducted a cross-sectional retrospective study to collect real-life data on the performance of a screening-workflow based on automated-pooling and high-throughput qPCR testing over a 3-month-period at the University Hospital Hamburg.

Results: Matrix validation reveals that lower limit of detection for SARS-CoV-2 RNA in gargling-solution was 180 copies/mL (5-sample-pool). A total of 55,122 self-collected gargle samples (= 7513 HCWs) was analyzed. The median time to result was 8.5 hours (IQR 7.2–10.8). Of 11,192 pools analyzed, 11,041 (98.7%) were negative, 69 (0.6%) were positive and 82 (0.7%) were invalid. Individual testing of pool participants revealed 57 SARS-CoV-2 previously unrecognized infections. All 57 HCWs were either pre-symptomatic or asymptomatic (prevalence 0.76%, CI95%0.58–0.98%). Accuracy based on HCWs with gargle-solution and NP-swab available within 3-day-interval ($N = 521$) was 99.5% (CI95%98.3–99.9%), sensitivity 88.9% (CI95%65.3–98.6%) while specificity 99.8% (CI95%98.9–99.9%).

Conclusion: This workflow was highly effective in identifying SARS-CoV-2 positive HCWs, thereby lowering the potential of inter-HCW and HCW-patient transmissions. Automated-sample-pooling helped to conserve qPCR reagents and represents a promising alternative strategy to antigen testing in mass-screening programs.

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Introduction

Healthcare workers (HCW) are on the frontlines of the current worldwide SARS-CoV-2 pandemic, placing themselves, their families and their patients at further risk of infection. Furthermore, their absence during periods of isolation for either suspected or

proven infections places further strain on an already overburdened healthcare system.

Because of the high number of asymptomatic SARS-CoV-2 infected individuals,¹ the focus of testing in HCWs should shift from a symptom-based approach to continuous screening of all those in contact with patients. However, regular mass screening by RT-qPCR using nasopharyngeal (NP) swabs is currently not feasible due to the scarcity of reagents, turnaround times and lack of trained personnel necessary to perform NP swabs. Similarly, rapid antigen tests have shown detection limits of 10^4 – 10^5 SARS-CoV-2 RNA copies/mL² and lower sensitivity, particularly in asymptomatic individuals.³

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A number of less invasive sampling methods for PCR tests have been explored in recent months. First studies have demonstrated acceptable performance using saliva samples,⁴ which have also been proposed as a candidate for HCW mass screening schemes via sample pooling.^{5,6} However, “real-life” data on the performance of such methods in practice is still lacking at this point. Furthermore, pooling of these kinds of samples entails many technical challenges: e.g., the sample viscosity requires manual processing, which is both time consuming and risks errors and contaminations.

Gargling samples represent another swab-independent sampling method which is both less invasive and offers good performance in detecting SARS-CoV-2 infections.^{7,8}

In this study we report real-life performance data for an RT-qPCR-based mass screening approach in a large cohort of asymptomatic health care workers, utilizing pooled gargling solution as non-invasive sample type.

Methods

Technical and clinical validation of gargling-solution as sample matrix

Gargling-solution is not approved for use with the cobas SARS-CoV-2 PCR test by the manufacturer. To rule out substantial impairment of target detection due to the matrix, pooled SARS-CoV-2 negative gargling-solution was used to prepare a two-fold dilution series of SARS-CoV-2 reference material (Accuplex SARS-CoV-2, Infl-A/B, RSV) and subjected to testing on the system (9 dilution steps, 8 repeats each, 500–2 cp/mL). Analytical LoD was assessed by probit-analysis.

To test the viability of gargle-samples for SARS-CoV-2 detection, we asked SARS-CoV-2 positive HCWs and patients to self-collect gargle-samples in addition to a diagnostic NP sample performed by a medical doctor. NP flocked swabs were collected via the left or right naris using UTM based collection kits by Copan (Italy, Brescia). The gargle-sample collection procedure is described below in more detail.

Pre-existing screening programs and introduction of gargle testing

Before the introduction of gargle pooling in December 2020, HCWs at the University Hospital of Hamburg were instructed to get tested for SARS-CoV-2 at the occupational doctor's practice for free at their own discretion, in case they experienced any symptoms. Testing was carried out by RT-qPCR using NP swabs.

Following an institutional agreement, all HCWs were invited to self-collect gargle samples weekly or twice weekly, depending on their area of work, for general SARS-CoV-2 screening. This scheme was limited to asymptomatic employees with direct contact with patients; those experiencing symptoms continued to receive standard testing by NP swab.

Study setup

We performed a cross-sectional retrospective study from 1st December 2020 to 28th of February 2021 in a large tertiary care hospital in Germany. We compared the incidence of SARS-CoV-2 infections and proportion of positive tests in an occupational practice for HCWs with a gargling solution-pooling technique. The latter was also compared with the proportion of positive tests in Germany in the same period. The study was performed in compliance with the Helsinki declaration, ethics votum was waived since data were collected anonymously. As a consequence, demographic data and clinical characteristics were not accessible.

Gargling-solution collection procedure

Users were instructed (with flyers and instruction videos) to gargle at home with 5–7 mL of tap water for 30 s and then spit into an empty 10 mL sterile polypropylene container using a straw (step I in the Fig. 1). The tube was then labeled by the user with an individualized barcode tag and sent to the microbiology department for analysis (step II, Fig. 1).

Sample pooling

After reception and registration, 1 mL of cobas PCR Media (< = 40% Guanidine hydrochloride solution in Tris-HCL) was added to each gargling-sample using a Hamilton Starlet IVD liquid handler (step III, Fig. 1). Pools of 5 (300 µL per sample for 1500 µL total volume) were generated using IVD pooling software (Hamilton STARlet IVD, Hamilton company, Reno, Nevada, step IV, Fig. 1) and pooling protocols released to the LIS (laboratory information system) for result allocation (step IV, Fig. 1).

The sample pools were consecutively analyzed using the cobas SARS-CoV-2 IVD test for the cobas6800 system (Roche, Mannheim, Germany). Target-1 (ORF1ab) and Target-2 (E gene) results were analyzed separately, though the entire assay was deemed positive as long as one target returned a positive result (step V Fig. 1).⁹ Pool results were automatically transferred back to the LIS (Swisslab-Nexus, Berlin, Germany, step VI Fig. 1) and allocated to individual orders. If the pool was negative, all 5 participants were automatically set negative; conversely, if positive, all 5 samples were forwarded to individual retesting. Negative results were reported to respective HCWs by SMS to their private cell phone (step VII Fig. 1). If positive, the occupational doctor would inform the respective HCW in person.

Statistical analysis

Sensitivity, specificity and overall accuracy including two-sided 95% Wald confidence intervals (CI95%) were assessed for those patients for whom a gargle sample and NP swab as gold standard were performed within a 3 day-interval. Invalid samples (NP swabs or gargles) were excluded from the analysis. A follow-up of positive gargle solutions was performed in order to assess a confirmation of the positive results by NP swabs or by serology beyond 3 days, although these samples were not included in the accuracy analysis. We calculated the incidence rate per 100,000 days at risk considering the time-at-risk started with 1st of December 2020 and ended the 31th of January 2021. All statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, San Diego, CA) and STATA 15.

Role of the funding source

For this study we received no funding.

Results

Technical and clinical validation of gargling solution as sample matrix

Analytical LoD for the cobas SARS-CoV-2 IVD test in gargling solution was determined as 36 copies/mL (CI95% 27.78–58.81 copies/mL; see also Appendix Fig. 1). This corresponds to an LoD of 180 copies/mL (CI95% 138.9–294.05 copies/mL) for one clinical sample in a pool of five. To compare SARS-CoV-2 RNA detection in self-sampled gargling solution and professionally performed NP swab, we analyzed 21 sample pairs obtained from SARS-CoV-2 positive HCWs ($n = 7$) and patients ($n = 14$). Viral RNA ranged from 6.76×10^2 – 3.98×10^8 copies/mL and 5.37×10^2 – 6.17×10^6

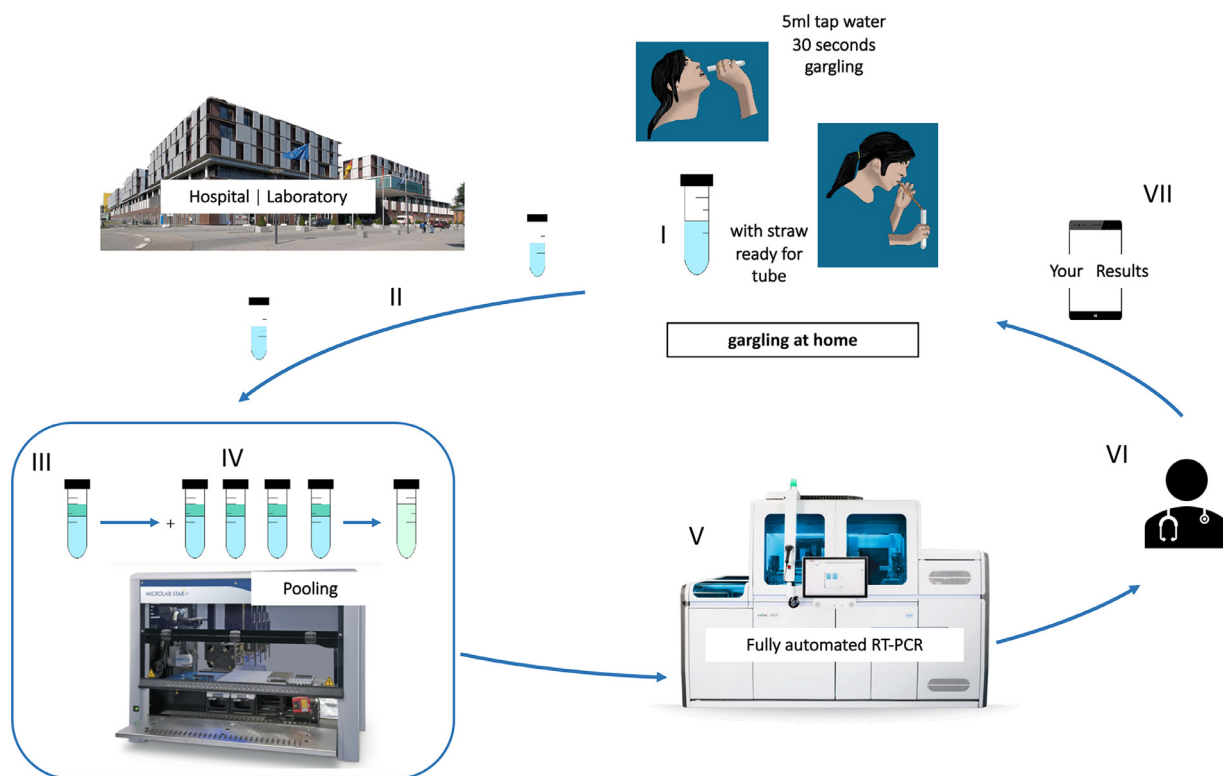


Fig. 1. Flow-chart of the pooling workflow: gargle sample collection at home, step I; transport to the laboratory, step II; Hamilton STARlet IVD processing: addition of 1 mL of cobas PCR Media ($\leq 40\%$ Guanidine hydrochloride solution in Tris-HCL) to each gargling-sample, step III, and pools of 5 gargle samples (300 μ L per sample for 1500 μ L total volume, step IV); pooled samples tested by a fully automated RT-PCR, step V; results validation, step VI; transmission of results by phone message, if negative, or by the occupational doctor, if positive, step VII.

copies/mL in NP swab and gargling solution respectively. Overall qualitative agreement was 21/21 (100%) while viral loads were on average 1.43 log₁₀ copies/mL lower in self-collected gargling solution (see Appendix Fig. 2). Interestingly, quantitative difference between NP swab and gargling was smaller in HCW indicating that correct gargling procedure and the ability to hold breath for 30s, particularly difficult for critical ill patients, is important for successful self-sampling.

Implementation of the screening program

Clinical departments were added successively to the general screening program as outlined above. As a result, sample numbers increased approximately 10-fold over the course of the observation period (Appendix Fig. 3). A total of 55,122 self-collected gargle-samples were processed, representing 7513 individual asymptomatic healthcare-workers (Fig. 2). The median number of samples submitted per HCW was 4 (range 1–18). Of the submitted gargle-samples, 2.8% (1,554/55,122) had to be rejected due to improper preparation (e. g. not closed properly, too full, etc.). The remaining 52,715 samples were used to generate a total of 11,192 pools, which were consecutively analyzed by PCR. The median time to result was 8.5 h (IQR 7.2–10.8).

Results of general screening and testing of symptomatic HCWs

Of the tested 11,192 sample pools, 11,041 were negative, 82 returned an invalid result and 69 were positive. Individual testing of the positive pools revealed 51 previously unknown SARS-CoV-2 infections in employees. In addition, 6 positives were residues of known previous infections. In a total of 12 positive pools (55 in-

dividual samples) no positive individual sample was found, thus requiring individual retesting of all HCWs involved.

The overall prevalence of SARS-CoV-2 infections in asymptomatic HCWs was 0.76% (CI95 0.58–0.98%). Incidence rates per 100,000 days at risk over the trial period can be seen in Appendix Table 1, as reported by.¹⁰ The median concentration of viral RNA per positive gargle-sample (in individual testing) was 1.45×10^3 (IQR: $< 5 \times 10^2$ - 3.09×10^4) copies/mL.

Parallel to the screening of asymptomatic HCWs, a total of 4301 symptomatic HCWs were tested by NP swab at the occupational doctor's practice during the trial period. Of these, 150 returned positive for SARS-CoV-2, representing a prevalence of 3.5% (CI95 2.7-4.1%)

The overall prevalence of SARS-CoV-2 infection among HCWs across the study period (symptomatic and asymptomatic) was 2.3% (201/ 8,640, CI95: 2–2.7%).

Comparing results of gargle samples with NP swab

The accuracy of pooled gargle samples was approximated by comparing gargle test results with NP swab results if performed within a timeframe of 3 days. 521 such sample pairs were available, 504 of which were negative and 17 positive in the gargle test. In two cases, SARS-CoV-2 RNA was detectable by NP swab following a negative gargle-test within 3 days. Overall accuracy was determined as 99.4% (CI95 98.3–99.9%), sensitivity as 88.9% (CI95 65.3–98.6%) and specificity as 99.8% (CI95 98.9–99.9%). Results of available follow-up of confirmation by serology or by NP swabs of all 51 positive gargle solutions is available in the Appendix Table 2. In total from 40/51 cases confirmatory test were available and 39/40 positive gargling tests could be confirmed.

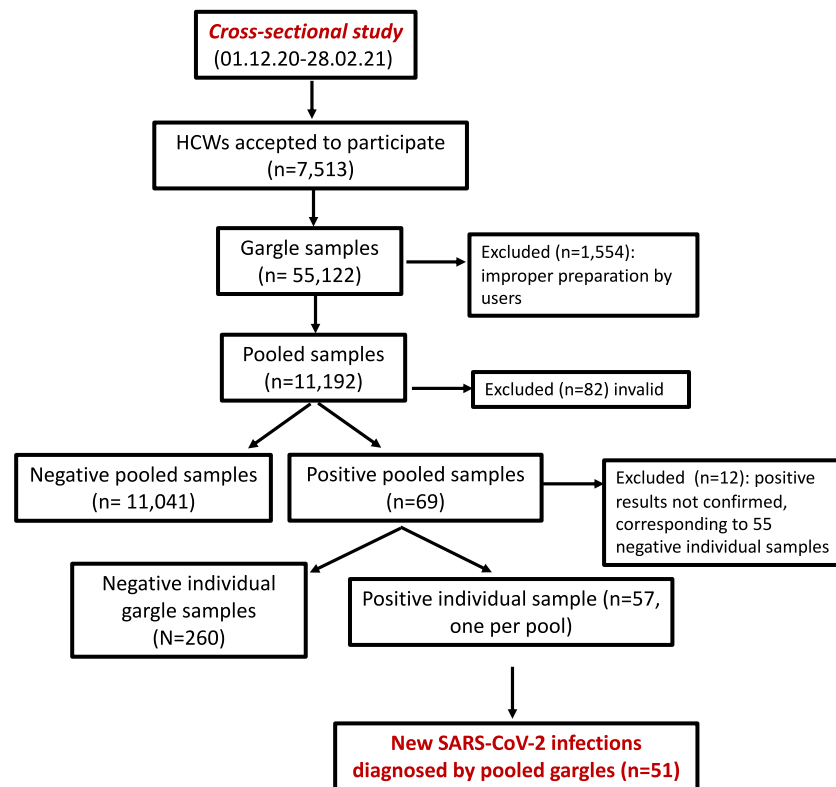


Fig. 2. Diagram of HCW participants and samples obtained through to the pooling workflow from 1st December 2020 to 28th February 2021.

Discussion

In this study we report real-life data of a large-scale screening program in asymptomatic healthcare workers using self-collected gargling solution as sampling method with pooled RT-qPCR for SARS-CoV-2 detection. Preliminary validation showed that expected viral concentrations are systematically lower in gargling solution but without a substantial loss in overall positive agreement.⁴

As part of the program, a total of 55,122 individual tests were performed for over 7000 eligible employees over a 3-month period. 51 asymptomatic healthcare workers were identified as SARS-CoV-2 positive and sent into quarantine. There were only two cases in which HCWs tested positive by conventional NP swab after receiving a negative result by gargling solution testing within a 3-day interval. During the time when general screening was in effect, no transmissions were detected originating from HCWs who participated in the program.

Limitation of this study was the absence of confirmation of positive and negative gargle solution by NP within 3-day-interval for all samples. Furthermore, this workflow of gargling-solution tested via pooled qPCR were not compared with other screening strategies, e. g. by rapid antigen test.

SARS-CoV-2 outbreaks in healthcare facilities have been a major concern during the ongoing pandemic, as these institutions concentrate some of the most vulnerable members of the population. In an environment of broad community spread, infections may be carried into hospitals in many different ways, including infected patients, healthcare workers, visitors and others.¹¹ Given the wide spectrum of potential courses of the disease, infected individuals may actively spread the virus before becoming symptomatic or even without ever developing any symptoms that would help identify the risk.¹² As a result, general SARS-CoV-2 screening, regardless of symptoms, is necessary to control transmission within hospitals. Rapid antigen tests have been proposed for such screening schemes, as they provide fast results and are

widely available in large quantities. However, their accuracy is imperfect, particularly in asymptomatic patients,¹³ thus carrying the risk to miss cases, some of which would then lead to outbreaks. Using mathematical modeling approaches, there have been multiple studies suggesting that increased testing frequency can mitigate lower sensitivity in individual tests, however, there is currently no real-life data available to support this concept in practice.¹⁴ This would also exacerbate the impact of sporadic false positives.

There have been recent reports of screening approaches using pooled RT-qPCR,^{15,16} sacrificing some sensitivity to vastly increase capacity for PCR testing. These have shown success in practice, but still rely on conventional nasopharyngeal swabs as material. Nasopharyngeal swabs have become the mainstay for SARS-CoV-2 detection as they were shown to yield the most amount of virus compared to other modes of sampling.¹⁷ Notably, rapid antigen tests often rely on them for optimal performance. However, NP swabs have inherent disadvantages as they are fairly invasive and uncomfortable to perform and usually require a dedicated operator for collection. A number of alternative sampling methods have been proposed in recent months, including saliva and gargling solution.^{4,5,18}

Our data shows that screening by self-sampled gargling-solution, tested via pooled qPCR was highly effective for identifying SARS-CoV-2 RNA positive HCWs. Expanding the described screening scheme to two-times per week would, in all likelihood, effectively eliminate the potential for large outbreaks with a reasonable margin of safety. This is feasible due to the high accuracy of the RT-qPCR based approach and the unmatched resource efficiency of sample pooling strategies. It thus represents a promising alternative to rapid antigen testing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.08.047.

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