Full title: Pooled-sample analysis strategies for COVID-19 mass testing: a simulation study

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Running title: Analysis strategies for COVID-19 mass testing

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DISCLAIMER

This paper was submitted to the Bulletin of the World Health Organization and was posted to the COVID-19 open site, according to the protocol for public health emergencies for international concern as described in Vaseer Moorthy et al. (http://dx.doi.org/10.2471/BLT.20.251561).

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RECOMMENDED CITATION

Abstract

Objectives: To evaluate two different pooled-sample analysis strategies, a “routine high-throughput” heterogeneous group and a “door-to-door” homogeneous group strategy in order to compare the effect of these two testing strategies on the overall required number of tests.

Methods: We perform Monte Carlo simulation analyses with varying prevalence and group sizes. In the routine high-throughput pooled-sample PCR analysis the sample pools are composed randomly. In a first step the samples are divided into pools and then these pools are analysed. In a second step each sample of a positive pool is analysed again. In the novel “door-to-door” pooled-sample PCR analysis groups of similar people of defined size are first formed, and swab tests of all group members undergo pooled PCR analysis. In a second step, all members of the positively tested groups will be analyzed individually.

Findings: Both pooled-sample testing strategies save substantial resources during surge testing and enhanced pandemic surveillance. The “door-to-door” approach bears the greatest potential. This approach requires around 56% to 93% less tests in low to moderate prevalence settings and group sizes up to 25 in a population of 150 000, compared to individual testing. The corresponding range of the routine high-throughput strategy was from 24% to 86% less tests.

Conclusions: Pooled-sample PCR analysis strategies will save substantial resources for COVID-19 mass testing. In particular, the “door-to-door” pooled-sample approach can facilitate mass screening in early stages of COVID-19 outbreaks, especially in low- and middle-income settings, and in containing foreseeable second wave outbreaks worldwide.

Keywords: COVID-19, SARS-COV-2, polymerase chain reaction, testing, outbreak, pandemic, low- and middle-income countries
**Introduction**

The variable incubation period of COVID-19 infections of up to 14 days and an unknown number of asymptomatic carriers capable of transmitting the infection are big challenges for COVID-19 control and mitigation efforts [1, 2, 3, 4, 5, 6]. Although about 80% of COVID-19 cases are reported to be mild [7], the remaining 20% of cases often result in a severe disease with the potential of crushing already overstrained health facilities [8]. Consequently, most countries intend to delay major surges of patients and levelling the demand particularly for intensive care beds for cases with COVID-19 respiratory failure [9]. This has necessitated the adoption of drastic containment measures in the severely affected countries in the Northern hemisphere, including complete lockdowns of regions and countries.

Until recently, there were little or no reported cases in majority of African and Latin American countries. Researchers however predicted that, there was an imminent danger of the importation and spread of COVID-19 in Africa [10, 11]. Although most SSA countries instituted targeted traveler screenings, this has proven to be ineffective due to the natural history of the disease and the potential for spread during the incubation period. Hence, unless swift and collective interventions are instituted, the effects might be rather devastating for countries with fragile health systems [11]. As a consequence, the WHO recommends that especially countries with few first cases of COVID-19 perform active surveillance, including testing, isolating cases and tracing every contact [9].

Furthermore, the elimination of infection transmission in countries with well-established outbreaks in the coming months is highly unlikely. What will dominate is a controlled epidemic, leading to stepwise withdrawal of the restrictions, albeit with localized flare-ups, which have the potential to bring back strict containment measures. Here, comprehensive, rapid and cost-effective localized mass testing strategies may be required to identify both symptomatic and asymptomatic cases in order to prevent further spread.

Both scenarios, active surveillance in settings with few cases and second wave outbreaks, require the identification of all cases (both symptomatic and asymptomatic) within a short period. Mass testing is important for a wide-range of further COVID-19 control strategies [12]. However, confirmation of infection even in asymptomatic persons largely relies on real-time PCR (RT-PCR) tests [4]. Although RT-PCR has been used in epidemiological studies [13, 14], it is laborious and costly. With some high-income countries struggling to meet early testing needs, it is evident that most resource-constrained settings will face even greater challenges with enhanced testing activities. A longer-term lockdown may not be an option for many low- and middle-income countries (LMICs) where the economic necessity does not allow for self-isolation.

Evidence of the effectiveness of mass testing of an entire local population has been reported in a small Italian town, Vò Vecchio, with around 3000 inhabitants [15]. After isolating around 3% (N=89) of the population who were infected in a first round of testing without showing symptoms, there was no further transmission with only six individuals remaining infected after 14 days. In larger populations however, such an enhanced outbreak surveillance may be impracticable and too costly. Furthermore, test workloads may rapidly outstrip testing capacity and resources [16]. An established way to conserve resources during surge testing is by conducting pooled-sample analysis [16, 17, 18, 19, 20, 21].
Based on this idea, this study describes applications of this resource-conserving testing algorithm in fighting the current COVID-19 pandemic. We evaluate two different pooled-sample analysis strategies, a “routine high-throughput” heterogeneous group and a “door-to-door” homogeneous group strategy, with the potential of considerably reducing the number of PCR tests required. In the routine high-throughput two-step pooled-sample PCR analysis (hereafter referred to as “routine high-throughput” approach) the sample pools are composed randomly. In a first step the samples are divided into pools and then these pools are analysed. In a second step each sample of a positive pool is analysed again [16, 17, 18, 19, 20, 21, 22]. However, during an outbreak of COVID-19, there is a high likelihood, that other members of homogeneous groups such as families, neighbours, etc. are also negative or positive depending on the status of an exposed member. Outbreak response teams that are carrying out contact tracing and other outbreak activities could use this opportunity and already form homogenous groups in the field for subsequent pooled-sample analysis, which we call the novel “door-to-door” two-step pooled-sample PCR analysis (hereafter referred to as “door-to-door” approach). Here, groups of similar people of defined size are first formed, and swab tests of all group members undergo pooled PCR analysis. In a second step, all members of the positively tested groups will be analyzed individually. This approach could offer several advantages for mass testing for COVID-19, including further reduction of number of tests required, thus, less testing manpower and costs implications.

**Methods**

Computer simulations are common tools for the assessment of the cost-effectiveness of PCR pooled samples screening [23, 24, 25, 26]. Owing to the wide range of uncertain parameters in the current COVID-19 pandemic, we applied Monte Carlo simulation techniques to enable the comparison of the effect of the two mass testing strategies on the required number of tests [27, 28].

**Simulation of the “routine high-throughput” approach with heterogenous groups**

We investigated different scenarios of a given COVID-19 infection prevalence in two populations with fixed sizes of 150 000 and 15 000 people respectively. The overall infection prevalence varied from 0.5% to 20% in incremental steps of 0.5%. The group size varied from 2 to 100, thus the number of groups from 75 000 to 1500 in a population of 150 000. Other parameters were calculated depending on this frame.

In order to simulate the dispersion of the infection, we first formed the groups and then applied the Binomial distribution (parameters: overall prevalence, group size). The number of required tests was the sum of the number of groups (first step: all groups get tested) plus the number of positive groups times the group size (second step: all members of the positive groups get tested). The simulation results were transferred to a 3-dimensional graph. Owing to the application of stochastic variables, the surface of the plot contained some small-scale ripples; hence, a spline smoothing function was applied. Additionally, for better visibility the figure is restricted to group sizes up to 50. All simulations were conducted in SAS 9.4 TS level 1M4.

**Simulation of a “door-to-door” approach with homogenous sample pools**

We repeated the simulation as described above, but now with homogeneous sample pools. Under condition of homogeneous groups, it is meaningful to assume that the “within-group” variation of members’ resemblance is lower than the “between-group” variation. Hence, if one member of a group is infected with
COVID-19, there is a high likelihood, that other group members are also infected. Additionally, we assume that the “within-group” prevalence of infection decreases non-linearly with increasing group size, since the groups’ composition become more diverse:

$$p_{group} = p_{all} + \frac{0.5}{e^{\frac{s_{group}}{200}}}$$

($p_{group}$: “within-group” prevalence, $p_{all}$: overall prevalence, $s_{group}$: group size). The corresponding curve of the “within-group” prevalence shown in figure 1 is exemplary for an overall prevalence of 5%. We then kept the overall prevalence fixed and calculated how many positive groups must exist in each scenario, given the “within-group” prevalence.

Furthermore, we performed a Bernoulli experiment for each group (parameter: probability for positive groups). Subsequently, we applied the Binomial distribution (parameters: “within-group” prevalence, group size) in the positive groups in order to get the number of positive people within each positive group. As a control measure, we calculated the post-simulation overall prevalence, which differed negligibly from the assumed overall prevalence (see supplementary table S1). Further steps remained the same as described above.

**Comparison of both approaches**

We selected two infection prevalence scenarios and plotted the percentage and factorial reduction of number of tests required against the group sizes. Here, we did not apply a smoothing function.

**Results**

**“Routine high-throughput” approach**

As expected, the results of the analysis for the “routine high-throughput” approach show an increasing number of tests required with increasing infection [26]. In the two selected scenarios shown in figure 2, group sizes from 3 up to 25 yield around 24% to 80% less tests compared to individual sample analysis (see also supplementary table S2). Given low prevalence, a significant reduction can be achieved within a wide window of group sizes. For example, a window of group sizes between 5 and 50 in a prevalence scenario of 1% guarantees at least 58% less tests. In a high prevalence scenario of 10%, there is still a considerable reduction of number of tests of around 40% achievable (not shown in the figure), yet, within a narrower window of group sizes around 3. Furthermore, in high-prevalence settings and large groups sizes, the number of tests required slightly exceeds the number of tests required in individual testing.

The full picture of the “routine high-throughput” approach simulation is depicted in figure 3. Here, the global minimum number of tests required (N=20 388) for a population of 150 000 is achieved in a scenario of 0.5% prevalence and a group size of 14. For this there is a reduction in the number of tests required by factor 7.4 (86% less tests). For a population of 15 000, the shape of the surface plot is similar, with the y-axis scaled by the factor 0.1 (not shown).
“Door-to-door” approach

Similarly, the result of the analysis for the “door-to-door” approach shows an increase in the required number of tests with growing overall infection prevalence. In the two selected scenarios shown in figure 2, group sizes from 3 up to 25 considerably lowered the number of tests (around 56% to 89% less tests) (see also supplementary table S1). Given a low prevalence, a significant reduction can be achieved within a wide window of group sizes. For example, a window of group sizes between 5 and 50 in a prevalence scenario of 1% guarantees at least 76% less tests. In a high prevalence scenario of 10%, there is still a considerable reduction of number of tests of around 65% achievable (not shown in the figure), yet, within a narrower window of group sizes around 10.

The full picture of the “door-to-door” approach simulation is depicted in figure 4. Here, the global minimum number of tests required (N=10 875) for a population of 150 000 is achieved in a scenario of 0.5% infection prevalence and a group size of 27. For this there is a reduction in the number of tests required by factor 13.8 (93% less tests)

Discussion

This simulation study aimed to evaluate two different pooled-sample analysis strategies: a “routine high-throughput” heterogeneous group strategy and a “door-to-door” homogeneous group strategy, and to compare their effects on the overall required number of tests in COVID-19 outbreaks. Both strategies will save substantial resources during surge testing and enhanced pandemic surveillance. However, the “door-to-door” approach with homogeneous sample pools offers a greater potential for a considerable reduction in the number of tests required, compared to the “routine high-throughput” approach, which has already been proven to be cost-effective [22].

In our simulation of the conditions reflective of a start of a general outbreak or a second wave outbreak in a localized area with low overall prevalence, for example 0.5%, the number of tests required in the “door-to-door” approach varied only slightly in an optimal window of group sizes between 8 and 50, with a minimum reduction of around 4 times (76%) and a maximum reduction of around 14 times (93%) less tests required compared to individual testing. Even a group size of 5 would still require 5 times (78%) less tests. This flexibility in the choice of effective group sizes makes this approach well suited for outbreak investigation in a real-world setting. Here, field teams may form homogenous groups of different sizes based on field conditions. It is noteworthy that even in a high epidemic area with a prevalence of around 20% there is still a considerable reduction in number of tests required (up to 50% less tests than individual testing) for group sizes around 10.

As seen for the “door-to-door” approach, the “routine high-throughput” approach provides wide windows of effective group sizes for “start of outbreak” scenarios. However, the number of tests required is always higher than in the “door-to-door” approach with homogeneous groups. In particular, as shown in figure 3, with increasing prevalence and group sizes a plateau is reached much faster where the number of tests equals or even exceeds the number required for individual testing. Comparing the optimal group sizes for both scenarios we find, that they are similar for low prevalence but differ in high prevalence settings.
Applying the “routine high-throughput” approach to the example of Vò Vecchio in Italy [15], the complete testing of this community in groups of size 5 or 10 would have required 1050 and 1110 respectively. With the suggested “door-to-door” approach the number of tests required would have been 795 or 540 tests respectively.

In order to effectively curb COVID-19 outbreaks, the prompt identification and isolation of infected individuals within a short period of time is a necessity, as demonstrated in Vò Vecchio and recommended by WHO [15, 9]. This recommendation is not only relevant for high-income countries, but even more so in LMIC settings, where each lost case can be the seed of a major outbreak which will threaten the already weak health systems. The RT-PCR analysis ensures the best results in terms of case detection, however, because of its resource requirements, individual testing will most likely not be an affordable option for most LMICs. Especially during surge testing and enhanced epidemic surveillance, pooled-sample analysis strategies could be a better option. The coordinated conduct of a “door-to-door” approach seems to bear the greatest saving potential, in particular considering the probability of much higher than expected COVID-19 infection prevalence at the start of an outbreak. For example, in Vò Vecchio the actual overall prevalence was already 3% on 6th of March 2020 [17], although the number of detected cases in Italy was only 4636 [27]. However, the “door-to-door” approach would require the allocation of individual samples to groups in the field.

During second wave outbreaks, pooled-sample analysis could help to quickly test an entire localised community. Especially in high-income settings with functional civil registration systems, the “door-to-door” approach could be easily implemented. Here, the allocation of members to homogenous groups can be prepared before field work. Time restricted local lockdowns during the time of testing might facilitate the complete identification of both symptomatic and asymptomatic cases. Only the test positives have to be isolated. This may prevent the re-implementation of drastic measures on a general population level.

The complete identification of all cases by PCR related approaches is limited by the PCR sensitivity and specificity. Both quality measures could be lowered if samples are analysed in a pooled manner. However, studies on infectious diseases report preserved high PCR sensitivities and specificities for pooled analysis [21, 29, 16].

In technically well-equipped countries with the possibility to perform high-throughput pooled PCR analysis a multiple-steps pooled PCR analysis could be an option in larger communities and cities. Such an extended testing algorithm would follow a tree structure; starting with a few large groups which then are subsequently narrowed down. This could further reduce the number of tests required, albeit requiring more complex processes.

**Strength and limitation**

The strength of this simulation is that the model can be easily adjusted once more accurate estimates regarding local COVID-19 prevalence are available. The same holds true for the “within-group” prevalence assumption, which might not yet fully mirror the dependency on group size. Furthermore, our approach of first performing Bernoulli experiments in the “door-to-door” approach followed by the application of the Binomial distribution within the positive groups reaches its limits for group sizes smaller than three. Other
distributions such as the Poisson distribution might be more suitable. Nevertheless, the results of the simulation of the “door-to-door” approach (see supplementary table S1), indicates a good model of the course of an outbreak. For example, in the scenario of a relatively low overall COVID-19 prevalence of 1% (start of an outbreak), we would expect few positive groups but with a higher “within-group” prevalence in homogenous groups. This is well-reflected in the model where the “within-group” prevalence for a small group size of 10 is around 30 times higher than the overall prevalence. Likewise, as expected in a later stage of an outbreak with an overall prevalence of 10%, for instance, the “within-group” prevalence in groups of size 10 is only around 4 times higher.

The simulation results suggest a group size for pooled samples of around 10 to be optimal for most of the scenarios for the “door-to-door” approach. However, it is not yet clear, whether this optimal group size is also the technically optimal group size for the best performance of the PCR analysis. Another limitation is the simplified nature of the simulation, the transfer to reality may require the implementation of complex processes and structures.

**Policy implications**

A long-term state of the world may be that we constantly need to contain small new outbreaks – the starting point for these containments is mass testing. Countries should adopt the “door-to-door” approach, in particular LMICs, but also high-income countries for long-term epidemic control strategies that might require constant ongoing testing efforts unless a vaccine is developed or a cheap and effective treatment is discovered.

**Conclusions**

The simulation of our two-step “door-to-door” approach reveals a huge theoretical potential for conserving resources during COVID-19 mass testing. This could help effectively curb local COVID-19 outbreaks in early stages, in particular in low-income settings, and in containing second wave outbreaks. We highly recommend to feed the parameters of these models with better estimates based on more accurate data from the field and to test its feasibility in reality. Furthermore, investigations regarding an effect of the group sizes on the quality measures of the COVID-19 PCR analysis are required.

**Ethics committee approval**

This is a simulation study; no patient data were involved. Hence, ethical approval was not required.

**Competing interests**

The authors have no conflicts of interest.

**Acknowledgement**

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**Authors’ contribution**

AD and NK developed the idea of the two step “door-to-door” pooled-sample PCR analysis strategy for homogeneous groups and conceived and designed the study. AD wrote the software code, run the simulation
and performed the analysis, NK and TB checked the results. AD and NK drafted the manuscript, TB revised the manuscript. All authors were responsible for the final content. Both authors have read and approved the final manuscript.

References


Figure 1: “Within-group” prevalence depending on group size for a fixed overall prevalence of exemplary 5% (“door-to-door” approach)
Figure 2: Comparison of the “routine high-throughput” and the “door-to-door” pooled-sample PCR strategies for selected simulation scenarios
Figure 3: “Routine high-throughput” pooled-sample PCR testing: Simulation results
Figure 4: “Door-to-door” pooled sample PCR testing: Simulation results
Table S1: COVID-19 two-step “door-to-door” pooled sample testing: selected simulation results

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¹ population 150 000
² population 15 000
Table S2: COVID-19 two-step standard high-throughput pooled sample testing: selected simulation results

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\(^1\) population 150 000  
\(^2\) population 15 000  
\(^3\) calculated after simulation