Swab pooling enables rapid expansion of high-throughput capacity for SARS-CoV-2 community testing

Jamie Fagg a,1, Rupert Beale a,b,1, Matthias E. Futschik c,d,1, Elena Turek e, David Chapman e, Susan Halstead f, Marc Jones g, Joanna Cole-Hamilton e, Rory Gunson h, Malur Sudhanva c,i, Paul E. Klapper e,j, Harper Vansteenhouse f, Sarah Tunkel c, Anna Dominiczak f, Timothy EA Peto k, Tom Fowler c,i,*

a Royal Free London NHS Foundation Trust, London, UK
b University College London, Division of Medicine, Royal Free Hospital, London, UK
c UK Health Security Agency, London, UK
d Faculty of Health, School of Biomedical Sciences, University of Plymouth, Plymouth, UK
e Deloitte, London, UK
f University of Glasgow, Glasgow, UK
g Lighthouse Labs, University of Glasgow, UK
h West of Scotland Specialist Virology Centre, Glasgow, UK
i King’s College Hospital NHS Foundation Trust, London, UK
j University of Manchester, Manchester, UK
k University of Oxford, Oxford, UK
l William Harvey Research Institute, Queen Mary University of London, London, UK

ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Pool testing
Swab pooling
High-throughput
Community testing

ABSTRACT

Background: The challenges of rapid upscaling of testing capacity were a major lesson from the COVID-19 pandemic response. The need for process adjustments in high-throughput testing laboratories made sample pooling a challenging option to implement.

Objective: This study aimed to evaluate whether pooling samples at source (swab pooling) was as effective as qRT-PCR testing of individuals in identifying cases of SARS-CoV-2 in real-world community testing conditions using the same high-throughput pipeline.

Methods: Two cohorts of 10 (Pool10: 1,030 participants and 103 pools) and 6 (Pool6: 1,284 participants and 214 pools) samples per pool were tested for concordance, sensitivity, specificity, and Ct value differences with individual testing as reference.

Results: Swab pooling allowed unmodified application of an existing high-throughput SARS-CoV-2 testing pipeline with only marginal loss of accuracy. For Pool10, concordance was 98.1% (95% Confidence interval: 93.3–99.8%), sensitivity was 95.7% (85.5–99.5%), and specificity was 100.0% (93.6–100.0%). For Pool6, concordance was 97.2% (94.0–99.0%), sensitivity was 97.5% (93.7–99.3%), and specificity was 96.4% (87.7–99.6%). Differences of outcomes measure between pool size were not significant. Most positive individual samples, which were not detected in pools, had very low viral concentration. If only individual samples with a viral concentration > 400 copies/ml (i.e. Ct value < 30) were considered positive, the overall sensitivity of pooling increased to 99.5%.

Conclusion: The study demonstrated high sensitivity and specificity by swab pooling and the immediate capability of high-throughput laboratories to implement this method making it an option in planning of rapid upscaling of laboratory capacity for future pandemics.

* Corresponding author.
E-mail addresses: Tom.Fowler2@ukhsa.gov.uk, tom.fowler@nhs.net (T. Fowler).
1 These authors contributed equally to this work and share first authorship.

https://doi.org/10.1016/j.jcv.2023.105574
Received 25 March 2023; Received in revised form 11 August 2023; Available online 19 August 2023
1386-6532/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
1. Introduction

To reduce the spread of SARS-CoV-2, mass testing can be an effective public health intervention but requires high-throughput of tests and rapid turnaround of results to support targeted public health action [1]. As such, mass testing is operationally challenging and very costly to implement at scale [2,3]. This issue remains to be of critical importance for pandemic preparedness and future contingency planning, as laboratory capacities have been greatly reduced. Public health agencies need to find innovative methods to maximize the capacity of remaining limited testing infrastructure if needed in the advent of new coronavirus strains or other infectious pathogens.

Sample pooling was proposed as a method to scale highly sensitive and specific testing by quantitative reverse transcription Polymerase Chain Reaction (qRT-PCR), whilst reducing resource use and costs [1, 4-6]. The most widespread form of sample pooling, known as Dorfman’s method [7], combines multiple patient samples and tests them all in one reaction. In negative pools, all individual samples in the pool are presumed negative and require no further testing. In positive pools, all individuals in the pool are retested to detect the infected individual(s).

Conventional sample pooling approach involves transporting individual samples to the laboratory where pooling of these samples prior to processing takes place. Residual of individual samples are kept for retest if the pool is positive. Disadvantages of this method are the potential dilution of samples reducing test sensitivity and increased risk of cross-contamination of original samples [8,9]. Furthermore, substantial adjustments of laboratory workflows are required for sample pooling and processing [10]. This is particularly challenging in high-throughput settings such as the UK ‘Lighthouse Laboratories’, which were established to scale the SARS-CoV-2 testing response [11]. These labs were designed to carry out between 1000 and 100,000 tests in a single day, which made the storage, retrieval and re-testing of individual samples unfeasible.

An alternative is swab pooling whereby swabs from different subjects are all collected into a single vial which is processed the same way as an individual sample. Swab pooling avoids the dilution and laboratory workflow issues linked to conventional pooling [9,12,13] making this pooling method particular attractive for high-throughput laboratories to increase testing capacity [12,14].

This evaluation study was set up to study the accuracy and effectiveness of swab pooling for high-throughput testing by comparing the qRT-PCR results for pooled samples to those that would be expected based on testing of individuals.

2. Methods

2.1. Pool size

A cross-sectional study was conducted for pool sizes of 10 and 6 swabs. The pool size of 10 was determined by the operational lab, Glasgow Lighthouse Laboratory (GLL), as the maximum number of older and agree to provide duplicate swabs for pooled and individual swabs. The pool size of 10 was determined by the operational lab, 2.1. Pool size

2.2. Sample collection

Swabs were collected at six regional test sites (RTS) across the northwest of England between 28/8 and 24/9/2020 for Pool10 and between 28/9 and 22/10/2020 for Pool6 cohort, respectively. During this period, wild-type SARS-CoV-2 (lineage B) was dominant with potential low level presence of B.1.1.7 (Alpha variant). Participants were individuals who had booked PCR tests at the RTS, and were invited to participate as they arrived. To be eligible, they had to be aged 16 or older and agree to provide duplicate swabs for pooled and individual samples. Subjects were informed that the result they received would be based on their individual PCR test only and that the additional sample would be used for lab validation of new processes. Combined throat and nose (dual anterior nares) swabbing (using the same swab) was performed by trained (non-healthcare) staff. The first swab was for the individual qRT-PCR test placed into a vial containing 2 ml of viral transport medium. The second swab was for the pooled sample collected in the same way and placed into a single vial containing 2 ml viral transport medium together with the other swabs from the pool. For swabbing, flocked swabs (Miraclean MSC-9600) were used.

2.3. Laboratory processing

Vials with pooled samples did not require additional laboratory preparation compared to individual tests and were tested following the same standard Lighthouse Lab protocols and workflow. As such, pooled samples were processed without making alterations to the existing high-throughput pipeline and therefore the turn-around-time for pooled specimens was the same as for individual samples. Details of the qRT-PCR assay can be found in the Supplementary materials. RNA viral concentrations (copies per ml) were estimated from Ct values based on calibration measurement at the GLL [15,16].

2.4. Statistical analysis

Concordance of pooled and individual samples was attained when the pool result matched its constituent individual results. For positively concordant result, at least one positive individual sample was detected in the positive pool, while for a negatively concordant results all the individual samples were negative in a negative pool. Definitions of performance metrics can be found in the Supplementary Materials.

Statistical significance of differences in concordance, sensitivity, specificity, positive predictive value (PPV) or negative predictive value (NPV) were assessed using $\chi^2$ tests with Yates’ continuity correction. Ninety-five percent confidence intervals (CI) method were calculated using Clopper-Pearson method. McNemar’s $\chi^2$ tests for paired outcomes was applied to compare the results of pooled swabs versus the expected results from individual swabs. For estimation of Ct values for pooled samples based on Ct values of individual samples, the sum was taken after exponentiation of individual Ct values i.e. $C_{\text{predicted}} = \log_2(2^{Ct_1}+...+2^{Ct_k})$ in the case of k non-zero Ct values $Ct_1$ to $Ct_k$. Pearson correlation was calculated to compare Ct values for target genes in pooled and individual samples. Differences in Ct values were assessed using the (paired) Student’s $t$-test. Logistic regression of the outcome of pooled tests was performed with respect to Ct values derived from individual tests and pool sizes. Probabilities of positive pool were predicted based on the fitted regression model. Data analysis was conducted using R version 4.0.3.

3. Results

3.1. Participants and high throughput testing

1587 participants were recruited to the Pool10 cohort, of whom 1030 (65%) had valid and complete Ct data for their individual and pool samples (Fig. 1). The Pool6 cohort was comprised of 1456 subjects of whom 1284 (88%) had complete Ct data. Symptomatic cases dominated with over 60% showing symptoms in Pool10 and over 80% in the Pool6 cohort (Table 1). Most pools included both participants with and without symptoms (Supplementary Fig. S1). The frequency of individual positive results within pools tend to follow the expected binomial distributions (Supplementary Fig. S2). Standard high-throughput workflow (racking of tubes for automatic processing, liquid handling, RNA processing, 96-well qPCR) was applied to both individual and pooled samples and no adjustments to process the pooled samples were carried out. Significantly lower Ct values were obtained for individual samples.
from symptomatic participants compared to those from asymptomatic participants (Supplementary Fig. S3).

### 3.2. Concordance of results from individual and pooled swabs

Results for pooled swabs generally agreed with the results expected based on the individual swabs (Table 2). In the Pool10 cohort, concordance of 98.1% (101/103) was obtained, compared to 97.2% (208/214) in the Pool6 cohort. Sensitivity exceeded 95% for both cohorts and was marginally higher for Pool6 (97.5%) than for Pool10 (95.7%). No false positives were detected for Pool10 resulting in a specificity and PPV of 100%, while in Pool6, the occurrence of two FP led to a lower specificity of 96.5% and PPV of 98.7%. Differences in concordance, sensitivity or specificity, between the two cohorts were not statistically significant. Application of McNemar’s test showed no significant difference between the results for pooled samples and those expected based on individual results for either Pool10 (p = 0.48) or Pool6 (p = 0.68). To minimize confounding due to different number of positive individual samples in pools, statistical outcome measures were also calculated for pools with
only one positive individual sample (Supplementary Table S1). Concordance and sensitivity were lower for this case but no statistically significant differences between the two pool sizes were found.

3.3. Ct values of discordant results

All of the false negative pooled samples had only one positive individual sample associated, and no pooled sample was falsely detected as negative when multiple positive individual samples were included in the pool. To characterise further the discordant results, their Ct values averaged across the three target genes were examined. For the four false negative results in the Pool6, the mean Ct values of associated individual samples were all larger than 30 (i.e. 33.8, 32.1, 32.4 and 30.5). In the Pool10 cohort, there were two false negative pooled samples with corresponding positive individual samples, which had Ct values of 33.2 and 18.3. For Pool6, 2 positive pools with average Ct values of 32.2 and 32.6 were found for pools with negative individual result. In the Pool10 cohort, there were no such cases. To assess the impact of samples with low viral burden on the diagnostic measures, an alternative Ct threshold was used and only individual samples with a Ct <30 were regarded as positive. This led to increased sensitivity of 97.8% for Pool10 and 100% for Pool6 cohorts, or 99.5% if both pools were combined (Supplementary Table S2).

3.4. Differences between CT values of pooled and individual sample

Mean Ct values of the three target genes were lower for pooled samples than for individual samples by 2.0 cycles (95%CI: 0.8 - 3.2, \( p = 0.001 \)) for Pool10, and by 1.5 cycles (1.1 – 1.9, \( p < 0.001 \)) for Pool6 (Fig. 2). The observed shift towards lower Ct values for pooled swabs reflects the accumulative effect of viral RNA from multiple positive individual swabs within pools, as a comparison of Ct values of pooled swabs with a single positive individual swab and the corresponding individual swab showed (Fig. 3A–C). For all three target genes, a high correlation of Ct values (ORF1a: \( r = 0.91 \), N gene: \( r = 0.91 \), S gene: \( r = 0.85 \)) was observed but also lower Ct values for individual samples compared to their corresponding pooled samples (mean Ct difference between pairs for ORF1a: –0.7, N gene: –0.7, S gene: –0.5) when only pools with one positive individual swab are considered. This finding is supported by the comparison of measured pool Ct values and the Ct values that would be expected based on the Ct values of constituting individual swabs (Fig. 3D). Here, a mean difference of 0.53 or 1.46 between measured and predicted Ct values was found for pools of size 6 or 10, respectively. This is considerably less than what would be expected for sample media pooling, which is 2.5 or 3.3 for pools of size 6 or 10, respectively.

3.5. Impact of viral concentration and pool size on sensitivity of pooled testing

To assess statistical dependencies of sensitivity of pooled testing on viral concentration and pool size, we performed logistic regression of the outcome of pooled tests. We included all pools with positive individual samples and estimated their Ct value based on the Ct values of individual samples. Positive test outcome of pooled samples was highly significantly associated with Ct value (Fig. 4A and Supplementary Table S3, \( p < 0.001 \)). The probabilities are insignificantly higher for a pool size of 6 than for pool size 10 (\( p = 0.6 \)). A very similar result was observed if we included only pools with a single positive individual sample and used its Ct value for the logistic regression (Fig. 4B and Supplementary Table S4).

4. Discussion

Pooling of SARS-CoV-2 samples for nucleic acid amplification testing has been indicated as a promising avenue to increase the efficiency of COVID mass testing [1,5,6]. Swab pooling is especially attractive as it avoids the burden of additional handling in the laboratory. The few
conducted lab and cohort studies indicated a high concordance of the results for swab pools with results that would be expected based on individual testing, especially if the individual samples showed high viral load [12,17–20]. This study confirms these previous reports and demonstrates that high concordance can also be achieved in routine community testing settings and using high-throughput labs without adjustment of their workflow.

A high overall sensitivity of 96.9% for pooling was observed with a marginally higher sensitivity for pools of 6 compared to pools of size 10. The higher sensitivity for Pool6 might be consequence of the smaller increase (0.53) of Ct values compared to Pool10 (1.46) due to pooling procedure. However, difference in performance between the usage of pools of size 10 and 6 was statistically not significant, which is in line with a recent study showing a noticeable decrease in sensitivity only for pools of more than 20 swabs [20]. Thus, the choice between these pool sizes can be flexibly made on practical circumstances. In this study, the pool size of 10 was the maximum number of swabs within tube that can be processed by the automated liquid handling systems. The alternative pool size of 6 was chosen since 99.4% of the households in the UK have six or less members [21] and thus would be most relevant if pooled testing is applied to households.

The recorded concordance between tests results for individual and pooled samples was high, especially when considering that specimens were from two different swabs of the same individuals. Thus, different results could simply reflect differences in uptake of the viral material in the repeated swabbing procedure. Nevertheless, only few false negative pooling results were found. With the exception of one case, all of the pools failing to give an expected positive result included a single positive subject whose individual sample had a high Ct value (>30) which is equivalent to viral concentration of less than 400 copies per ml. Considering only those individual samples with viral concentrations >400 copies per ml, the overall sensitivity of pooling increased to 99.5%. The exception in this study was a pool, for which a corresponding individual sample had a Ct value of 18, which equates to a high viral concentration of approximately 1 million copies per ml. In this case, a low pool Ct value (Fig. 3D) and reliable detection would be expected.

**Fig. 3.** Scatterplots of Ct values for (A) ORF1ab, (B) N gene and (C) S gene comparing values from individual and pooled samples, which included one positive sample. (D) Scatterplot comparing estimated and measured average Ct values for pooled samples. Missing Ct values were set to 40 for visualisation purpose, but not included in the calculation of the Pearson correlation coefficient $r$. Diagonal lines are shown to indicate equality of Ct values. The larger number of dots above the diagonal reflects a shift towards higher pool Ct values (A-C) or measured Ct values compared to individual or predicted Ct values, respectively.
Such deviation could be caused by an inaccurately performed second swab for the pool and thus might more reflect variability between swabs than an effect by pooling. Notably, multiple positive individual samples always resulted in a positive pool result independent of their Ct values. This suggests that a sufficient accumulation of RNA from multiple individuals even with low viral burden can be achieved for reliable detection in the pooled sample. Finally, two false positives with high Ct values for the Pool6 cohort were identified. They might result from variability between individual and pooled swab, or from accumulation of viral RNA from multiple swabs that individually were below the detection limit. For low viral burden, other experimental variables such as the type of swab, their uptake or release properties, as well as the volume of viral transport medium might play a more influential role on performance [22]. Further optimisation of such variables could lead to an increased sensitivity of pooling.

A drawback of swab pooling is that subjects in a positive pool must take another test to identify the positive individuals since there are no individual samples. However, in practice, when pools contain samples of close contacts, the public health action (such as self-isolation) might be the same for all subjects included in a positive pool to limit onward infection beyond this group. Such action for close contact groups will be of particular importance to contain highly infectious future variants with features currently presented by the Omicron VOC: high secondary attack rate (up to 80% for Omicron [23,24]), large percentage of pre-symptomatic transmissions (estimated to be 50% within households [25]) together with a decreased protection by vaccination [23,24].

Fig. 4. Predicted probability of a positive pooled test based on logistic regression for different pool size and a range of Ct values of individual samples. (A) Prediction based on logistic regression that included all pools with positive individual samples and used estimated Ct values. (B) Prediction based on logistic regression that included only pools with a single positive individual samples and used the measured Ct value of this individual sample. 95% CI for the predicted probabilities are indicated by the red (Pool10) or cyan (Pool6) shaded bands.

In summary, this evaluation showed that swab pooling is feasible in a routine community testing and high throughput setting with trained non-healthcare staff. It shows a high concordance rate with individual tests, avoids adjustment in the process workflow of laboratories and can reduce the number of tests (see Supplementary Text for estimates). The latter points remain important even if there is sufficient supply of testing materials, as laboratory staff are and will become a limiting factor in testing capacities [26]. This study provides evidence that a switch from individual to pooled testing is possible without reconfiguration of high-throughput laboratory workflows and substantial loss of accuracy. Pooled testing of communities can be applied therefore rapidly if demand outstrips capacities and replaced by individual testing again if demand drops or additional testing capacities have been built up. This selective approach to pooling of samples can also improve the efficiency of testing, as the positive individuals tend to cluster in pools [6]. In these settings, entire households or other close contact groups receive a single result and effective public health action can be initiated immediately. Such applications of swab pooling have been reported for SARS-Cov-2 screening programmes of school children [27] and university students [28]. Importantly, swab pooling appears most reliable for cases with low Ct who tend to be the most infectious [15]. These findings will help to inform short- and long-term COVID-19 contingency planning and the practical implementation of pooling as part of an adaptive testing regime if the need should arise.

Ethical statement

Within the context of the pandemic public health response and roll out of testing interventions, after review using the Health Research Authority (HRA) tool and after further discussions with HRA it was determined that this evaluation would not require HRA research ethics approval. All study participants received routine care through receipt of an individual diagnostic swab test and result.

CRediT authorship contribution statement

Jamie Fagg: Investigation, Writing – original draft, Writing – review
Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data availability

The datasets will be made available after de-identification of participants from the corresponding author on reasonable request.

Funding statement

NHS Test and Trace (originally part of the UK Department of Health & Social Care and subsequently moved to form part of UK Health Security Agency on 1/10/2021) funded this work as part of the UK testing programme response to COVID-19 Pandemic.

Supplementary materials


References