RESEARCH ARTICLE

Rapid design and implementation of an adaptive pooling workflow for SARS-CoV-2 testing in an NHS diagnostic laboratory: a proof-of-concept study [version 1; peer review: 2 approved]

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Abstract

Background: Diagnostic laboratories are currently required to provide routine testing of asymptomatic staff and patients as a part of their clinical screening for SARS-CoV-2 infection. However, these cohorts display very different disease prevalence from symptomatic individuals and testing capacity for asymptomatic screening is often limited. Group testing is frequently proposed as a possible solution to address this; however, proposals neglect the technical and operational feasibility of implementation in a front-line diagnostic laboratory.

Methods: Between October and December 2020, as a seven-week proof of concept, we took into account scientific, technical and operational feasibility to design and implement an adaptive pooling strategy in an NHS diagnostic laboratory in London (UK). We assessed the impact of pooling on analytical sensitivity and modelled the impact of prevalence on pooling strategy. We then considered the operational constraints to model the potential gains in capacity and the requirements for additional staff and infrastructure. Finally, we...
developed a LIMS-agnostic laboratory automation workflow and software solution and tested the technical feasibility of our adaptive pooling workflow.

**Results:** First, we determined the analytical sensitivity of the implemented SARS-CoV-2 assay to be 250 copies/mL. We then determined that, in a setting with limited analyser capacity, the testing capacity could be increased by two-fold with pooling, however, in a setting with limited reagents, this could rise to a five-fold increase. These capacity increases could be realized with modest additional resource and staffing requirements whilst utilizing up to 76% fewer plastic consumables and 90% fewer reagents. Finally, we successfully implemented a plate-based pooling workflow and tested 920 patient samples using the reagents that would usually be required to process just 222 samples.

**Conclusions:** Adaptive pooled testing is a scientifically, technically and operationally feasible solution to increase testing capacity in frontline NHS diagnostic laboratories.

**Keywords**
laboratory automation, diagnostics, SARS-CoV-2
Introduction
The need for SARS CoV-2 testing as part of the pandemic response has placed significant strain on diagnostic services in terms of testing capacity and the availability of reagents and labware. This has provided multiple challenges to both maintain and, at the same time, increase testing capacity. To address this there has been a recent surge in interest to explore the utility of pooling as a strategy to optimise SARS CoV-2 testing. Initially described during the Second World War for testing soldiers for syphilis, pooling has since been used in multiple settings, from screening, to surveillance and diagnostic testing. One of the simplest strategies, termed Dorfman pooling, involves a 2-step process with initial testing of pool of samples followed by testing individual samples in positive pools. Further rounds of testing can be added to this strategy to reduce the number of individual tests that are required, but these approaches come at the expense of increasing the time to getting and reporting the results. Other proposals of adaptive and non-adaptive pooling promise further increases in efficiency and, although often mathematically elegant, neglect to consider the complexities of required laboratory automation and implementation outside of a single, local laboratory context.

Utilising a pooling strategy for SARS-CoV-2 testing promises the possibility of significantly increasing analyser testing capacity but several limitations have to be considered. These include reduced analytical sensitivity with a possible increase in the reporting of false negative results, a loss in the ability to assess individual sample adequacy, prevalence levels (as pooling is optimal if infection prevalence is low) and the requirement for more complicated laboratory workflows and infrastructure. Due to the scale and rapidity of the SARS-CoV-2 pandemic, many different assays and pooling strategies have been used for SARS CoV-2 testing with some assays having already received regulatory approval. Furthermore, interim guidance for laboratories on the use of pooling has also been developed by the Centers for Disease Control and Prevention (CDC). The CDC describe using pooling to expand SARS CoV-2 nucleic acid diagnostic or screening testing capacity for an FDA approved test and also how to determine prevalence on a local rolling average of the rate of positive tests and communicate the limitations associated with pooled testing.

In the context of the UK National Testing Programme, Public Health England (PHE) has issued a standard operating procedure for pooling to support an increase in testing capacity during a time of low background prevalence, reducing reagent consumption per test and to increase overall total testing capacity in the country. Here, we expand upon this guidance and investigate how pooling could be implemented in the context of an NHS diagnostic laboratory, to understand any potential benefits, while addressing associated technical and operational challenges for implementation in a front-line SARS-CoV-2 testing service.

Methods
Determination of analytical sensitivity
Previously characterised and quantified MS2 VLPs containing the N-gene (accession number: NC_045512) of SARS-CoV-2 were transferred using an Echo 525 acoustic liquid handler (Labcyte) to create a standard curve from 100 000 to 50 copies/mL for RNA extraction. A sample of 200 μl was used for RNA extraction using the Maxwell HT Viral TNA kit (Promega) on the CyBio FeliX liquid handler (Analytik Jena) with an elution volume of 50 μl. Subsequent RT-qPCR was performed using the TaqPath™ COVID-19 CE-IVD RT-PCR Kit (ThermoFisher Scientific) according to the manufacturer’s instructions and thermocycled on a qTower3 (Analytik Jena).

Impact of pooling on capacity
The following assumptions were used for modelling the impact of pooling on capacity. These assumptions were based on timing various steps as well as the time taken during the technical feasibility experiment.

Capacity benefit of the integrated pooling workflow:

\[ \text{Increase in capacity} = \frac{\text{Total Number of Samples}}{\text{Number of tests used for testing} + \text{Number of tests used for retesting}} \] (1)

Technical feasibility of pooled testing
North West London Pathology’s Sunquest LIMS system was used to store patient information and associated final positive/negative/inconclusive results. A partitioned test environment of Sunquest was created to allow for feasibility testing of the complete workflow while performing the pilot. A cloud-based ‘command centre’ web app was engineered specifically for SARS-CoV-2 plate-based pooling diagnostics and utilised the Riffyn Nexus® software platform as a backend data architecture.

In bulk, patient samples were booked into the Sunquest LIMS system. Swabs were removed and samples were then neutralised in Copan™ tubes with 2.5 mL of MagBead Viral RNA Lysis Buffer (BioServUK) in a Class 2 Microbiological Safety Cabinet.

Patient samples were then plated in batches of 92, accommodating four wells that are required for control samples. Using a handheld barcode scanner, a barcode on a KingFisher 96 deep well plate (ThermoFisher Scientific) and anonymized barcodes (container IDs) on each neutralised sample were registered in the command centre. Neutralized samples were placed into custom 3D printed SBS format racks for the Biomek i5 liquid handling robot (Beckman Coulter). A volume of 500 μl was transferred from sample tubes to the registered 96 well plate using a Biomek i5 Span 8 liquid handler (Beckman Coulter).

Pooling and analysis were initiated by registering the plate in which pooled samples would be contained. The registered barcodes on multiple sample plates were scanned along with a new KingFisher 96 deep well plate (ThermoFisher Scientific). Subsequently, 50 μl of sample from each sample plate was transferred to the pooling plate using a CyBio FeliX liquid handler (Analytik Jena). A total pooled sample of 500 μl was used for RNA extraction using the Maxwell HT Viral TNA kit (Promega) on the CyBio FeliX liquid handler (Analytik Jena) with an elution volume of 50 μl using a custom extraction protocol. Subsequent RT-qPCR was performed using the
Results from RT-qPCR were processed using the pooling command centre analysis capabilities, resulting in two exportable outputs of deconvoluted data on a per-sample basis: All negative patient results (in a LIMS-compatible format) and a picklist of positives with instructions (in a Biomek i5-compatible format) for rearraying samples from their original 96 well plates. A volume of 360 μL was transferred from all positive wells to a KingFisher 96 deep well plate (ThermoFisher Scientific, pre-registered in the command centre) using a Biomek i5 Span 8 liquid handler (Beckman Coulter). The retest plate was used for RNA extraction using the Maxwell HT Viral TNA kit (Promega) on the CyBio FeliX liquid handler (Analytik Jena) with an elution volume of 50 μl using a custom protocol. Subsequent RT-qPCR was performed using the TaqPath™ COVID-19 CE-IVD RT-PCR Kit (ThermoFisher Scientific) according to the manufacturer’s instructions and thermal cycled on a qTower3 (Analytik Jena). Final results were processed in the command centre and exported in a LIMS compatible format for reporting.

Impact of pooling on analytical sensitivity

In order to address the impact of pooling on analytical sensitivity, we used our previously characterised MS2 synthetic viral-like particles (sVLPs) containing the SARS-CoV-2 N gene. By performing end-to-end RNA extraction-PCR analyses of different sVLP concentrations, we were able to analyse the lower limit of detection (LLOD) (Figure 1). Using the N gene primer set (VIC channel) of the ThermoFisher Scientific COVID-19 assay, we showed that for our sVLP reference material, the LLOD was approximately 250 viral copies/mL. We also observed a linear response between 100 000 and 250 copies/mL (Figure 1) and the deviation from the expected Ct (Table 1) was always less than 1 Ct cycle.

Using the MS2 sVLP standard curve and LLOD, we were able to predict the effects of various pooling strategies on sensitivity (Table 2). For three different sVLP concentrations, and 6 different simulated pool sizes from 1-IN-2 (equivalent to having two samples in the same pool) to 1-IN-10, we demonstrate how the analytical sensitivity translates into a practical pooling strategy. For example, with an individual sample with 1000 copies/ml, we were not able to detect the N-gene in 1-IN-5, 1-IN-8 and 1-IN-10 simulated pools (Table 2). These values were selected because they are the most practical pool sizes that could be implemented in a diagnostic laboratory context, given turnaround time constraints and availability of liquid handlers.

Impact of prevalence on pooling size

The prevalence of SARS CoV-2 in the population to be tested using pooling, has a significant impact on the pooling strategy. With increasing prevalence, the number of positive pools increases, reducing the efficiency of the pooling process. Local prevalence will vary and the decision on pool size will need to be a dynamic one, informed not only by SARS CoV-2 prevalence but also assay sensitivity and specific local laboratory constraints. A suggested approach is to determine prevalence based on a local rolling average of the preceding 7–10 days with PHE suggesting consideration of pooling when the prevalence is less than 10%.

Analysis (incorporating the model described by Regen et al.) was undertaken to estimate the optimal pool size based on prevalence in a conventional modular extraction/qPCR plate system. Turnaround requirements are such that tested pools should

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**Figure 1.** Standard curve of end-to-end RNA extraction and RT-qPCR with concentrations from 100 000 copies/mL to 50 copies/mL of MS2 sVLP containing the N gene. Each concentration has 3 technical replicates. Dotted line shows the cycle limit of detection. Line of best fit is fit to the Ct values from 250 copies/mL to 100 000 copies/mL. Error is the 95% confidence interval.
positive pools, allowing calculation of the expected number of samples that would require retest (see Table 3). Whilst pools of 20 and 15 samples are unlikely due to loss in analytical sensitivity, they were included for completeness.

Impact of pooling on capacity
Pooling offers the possibility of increasing overall testing capacity by increasing analyser capacity with the introduction of some extra pooling specific steps to the existing laboratory process. For significant increases in testing capacity to be realised, the entire workflow needs to be optimised. While pooling addresses an analyser capacity bottleneck it will not provide capacity benefits if the limiting factor sits elsewhere in the process (e.g. limited safety cabinets for sample neutralisation). Each laboratory will have its own particular set of challenges to overcome and potential capacity improvements will be variable based on the local context.

Two different pooling workflow scenarios were considered, namely an independent pooling workflow and an integrated pooling workflow. Both provide distinct advantages depending on the local analyser infrastructure.

(1) Independent pooling workflow - In this workflow all requirements for pooling, namely testing of pooled and retest samples, are done as part of an independent workflow (i.e. not feeding into the routine testing workflow). As such both steps are completed before the cycle can be repeated. In order to estimate the potential capacity impact achievable by implementation of an independent pooling workflow, the local testing process was modelled based on the following assumptions: (i) prevalence levels of 0.9%; (ii) adequate resources were available to address other bottlenecks in the sample processing; (iii) a single analyser platform was available (Analytik Jena FeliX for extraction and Analytik Jena qTower for amplification) and that both the pooled plate and retest plate were run on the same analyser. A time analysis of the steps in the process is shown in the Methods (Methods Table 1). Although this simple model is limited, as it only considers a single platform for analytical

| copies/mL | mean Ct | expected Ct | expected Ct - mean Ct |
|-----------|---------|-------------|-----------------------|
| 100017    | 26.99   | 26.99       | 0                     |
| 50008     | 27.95   | 27.99       | 0.04                  |
| 33292     | 28.77   | 28.58       | -0.19                 |
| 24933     | 28.86   | 28.99       | 0.13                  |
| 19975     | 28.92   | 29.31       | 0.39                  |
| 12467     | 29.32   | 29.99       | 0.67                  |
| 10058     | 29.83   | 30.3        | 0.47                  |
| 4958      | 30.98   | 31.32       | 0.34                  |
| 3400      | 31.42   | 31.87       | 0.45                  |
| 2550      | 31.92   | 32.28       | 0.36                  |
| 1983      | 32.21   | 32.65       | 0.44                  |
| 1275      | 33.46   | 33.28       | -0.18                 |
| 1000      | 33.73   | 33.63       | -0.1                  |
| 500       | 34.21   | 34.63       | 0.42                  |
| 333       | 35.6    | 35.22       | -0.38                 |
| 250       | 34.86   | 35.63       | 0.77                  |

Table 1. The mean Ct is the mean of three technical replicates. Expected Ct assumes that halving starting concentration should increase the Ct value by 1.

Table 2. Mean Ct values from the VLP standard curve used to determine the sensitivity impact depending on the proposed pooling strategy. Copies/mL is the concentration of the positive sample in the simulated pool. Neat shows the Ct value without dilution and the following columns show the effect of a serial dilution (simulating a positive sample in a pool of negatives). Below 250 copies/mL there is no longer reliable detection of the target (not detected in all technical replicates).

| copies/mL | Neat | 1-IN-2 | 1-IN-3 | 1-IN-4 | 1-IN-5 | 1-IN-8 | 1-IN-10 |
|-----------|------|--------|--------|--------|--------|--------|---------|
| 100017    | 26.99| 27.95  | 28.77  | 28.86  | 28.92  | 29.32  | 29.83   |
|           | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| 10058     | 29.83| 30.98  | 31.42  | 31.92  | 32.21  | 33.46  | 33.73   |
|           | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| 1000      | 33.73| 34.21  | 35.6   | 34.86  | Not Reliably Detected | Not Reliably Detected | Not Reliably Detected |
|           | Detected | Detected | Detected | Detected | Not Reliably Detected | Not Reliably Detected | Not Reliably Detected |
processing, it is useful to illustrate the potential capacity impact of pooling in the following scenarios:

Scenario A - Analyser capacity is the limiting factor. In this scenario, any pre-analytical bottlenecks have been resolved with a single extraction / amplification workflow testing both the pool and retest plates (with no reagent supply constraints). For a pool size of 10, we estimate that the projected upper limit of capacity for routine testing increases twofold (Figure 2).

Scenario B - Analyser capacity is constrained. In this scenario the assumptions are similar to scenario A except that the number of runs on a single analyser are limited (for example as a result of reagent supply issues). Using a pool size of 10, we estimate that the projected upper limit of capacity increase for routine testing is fivefold (Figure 3).

(B) Integrated pooling workflow. In this workflow, only the pooled samples are tested through the pooling workflow with retesting of individual samples feeding into the standard testing workflow. This allows more rapid cycling through the testing of pooled plates but requires sufficient capacity in the standard workflow to test samples from the positive pools. We would anticipate that the majority of laboratories would adopt this approach. In order therefore to estimate the capacity benefit of this model we used an equation where the increase in capacity is equal to the total number of samples divided by the sum of the number of tests used to tests pools plus the number of tests for retesting (Methods, Equation 1).

The prevalence and pool size will be key determinants on any capacity increase. For example, in the setting where there are no positive pools, the magnitude of the potential capacity increase will be the pool size multiplied by the number of tests available for pooling (i.e. the theoretical maximum capacity benefit). As prevalence increases the capacity benefit will decrease. Assuming a scenario where 100 tests are available for pooling, the capacity increase by pool size and prevalence is provided in Table 4.

Reagent and labware utilisation
In order to explore the potential reduction in testing resources for pooling, we calculated usage of plasticware, tips and testing reagents using different pool sizes using our modular plate-based workflow. We found significant savings in all resource requirements with up to 45% reduction in tips, 76% reduction in plates and 90% reduction in both RNA extraction and qPCR reagents (Table 5). These significant reductions in resource requirements are particularly pertinent for diagnostics laboratories in national testing programs, where acute shortages of reagents and plastics during the pandemic have caused decreases in testing capacities.

Staffing and infrastructure requirements
While specific requirements for the implementation of pooling will differ between laboratories, generally three different high throughput methods for SARS-CoV-2 testing are employed:

(1) Separate extraction followed by qPCR setup and qPCR measurement performed on 96 well plates (plate-based)

(2) End to end sample in result out system using batches of samples for parallel testing (e.g. Roche 6800, Abbott M2000)

(3) End to end sample in result out system with samples being tested randomly (e.g. Panther, Alinity M)

Here we will only address workflows for 1 and 2 (which were available in the local context) and assess how pooling fits within these workflows, and how the existing infrastructure can

### Table 3. Estimated optimal pool size based on prevalence.

| Prevalence range | Number of samples per pool | Number of samples per pooled plate | Average number of positive samples that will need retest per pool | Average number of positive samples that will need retest per plate |
|------------------|---------------------------|-----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| 0.1-0.2%         | 20                        | 1880                              | 2-4                                                           | 40-80                                                          |
| 0.3-0.4%         | 15                        | 1410                              | 5-6                                                           | 75-90                                                          |
| 0.5-0.9%         | 10                        | 940                               | 5-9                                                           | 50-90                                                          |
| 1.0-1.4%         | 8                         | 752                               | 7-11                                                          | 56-88                                                          |
| 1.5-2.6%         | 6                         | 564                               | 9-15                                                          | 54-90                                                          |
| 2.7-3.8%         | 5                         | 470                               | 13-18                                                         | 65-90                                                          |
| 3.9-6.1%         | 4                         | 376                               | 15-23                                                         | 60-92                                                          |
| 6.2-10.9%        | 3                         | 282                               | 18-31                                                         | 54-93                                                          |
| 11-24.4%         | 2                         | 188                               | 21-46                                                         | 42-92                                                          |

Wellcome Open Research 2021, 6:268 Last updated: 11 Nov 2021
be augmented to maximise available resources, and address capacity and consumable constraints. In Table 6, we describe the additional staff and infrastructure required to double a labs capacity of 2500 samples a day for both workflows. This detail is based on the precise mapping of the entire process (available on Zenodo). Most of the additional staff are required for the pre-analytical sample processing steps which still remains the major bottleneck for scaling testing. Further staff time is required for pooling the primary samples into suitable formats for each testing workflows. After this step, we envisage no further staff time requirements above the normal levels required for testing. Additional infrastructure is required by both workflows for pre-analytic sample processing (bench space and class-I cabinets) and primary pooling of samples (automated liquid handling robots). For plate pooling an additional 96 tip head robot is required (Table 6). We have also identified a key need for sample tracking software that would link the primary tube barcode to the pooled result and allow retesting of those samples in a positive pool.

Development of a deployable software platform to enable pooling
Deconvoluting and re-arraying positive pools required a novel software solution, as traditional LIMS-based and
spreadsheet-based approaches are insufficient in terms of core capabilities and data integrity, respectively. To manage these requirements, a custom cloud-based web application was developed using the Riffyn Nexus™ software platform as its core, which enabled usage of Riffyn’s patented data integration algorithm.

The comprehensive solution was engineered to integrate with laboratory operations to capture and share data (Figure 4). Anonymised container IDs (generated within the laboratory LIMS system) and plate barcodes were registered via simple barcode scans of plated individual and pooled samples. Results from qPCR analyses were processed after a simple

| Prevalence | Pool Size | Tests available | Number of samples tested | Maximum Number of positive pools | Number of initial negative samples reported | Number of samples retested | Capacity increase (number of extra samples tested) | Capacity increase (as multiple of baseline) |
|------------|-----------|----------------|--------------------------|----------------------------------|-------------------------------------------|--------------------------|-----------------------------------------------|---------------------------------------------|
| 10%        | 2         | 100            | 200                      | 20                               | 160                                       | 40                       | 60                                            | 1.43                                        |
|            | 3         | 100            | 300                      | 30                               | 210                                       | 90                       | 110                                           | 1.58                                        |
|            | 4         | 100            | 400                      | 40                               | 240                                       | 160                      | 140                                           | 1.54                                        |
|            | 5         | 100            | 500                      | 50                               | 250                                       | 250                      | 150                                           | 1.43                                        |
|            | 6         | 100            | 600                      | 60                               | 240                                       | 360                      | 140                                           | 1.30                                        |
|            | 7         | 100            | 700                      | 70                               | 210                                       | 490                      | 110                                           | 1.19                                        |
|            | 8         | 100            | 800                      | 80                               | 160                                       | 640                      | 60                                            | 1.08                                        |
|            | 9         | 100            | 900                      | 90                               | 90                                        | 810                      | -10                                           | 0.99                                        |
|            | 10        | 100            | 1000                     | 100                              | 0                                         | 1000                     | -100                                          | 0.91                                        |
| 5%         | 2         | 100            | 200                      | 10                               | 180                                       | 20                       | 80                                            | 1.67                                        |
|            | 3         | 100            | 300                      | 15                               | 255                                       | 45                       | 155                                           | 2.07                                        |
|            | 4         | 100            | 400                      | 20                               | 320                                       | 80                       | 220                                           | 2.22                                        |
|            | 5         | 100            | 500                      | 25                               | 375                                       | 125                      | 275                                           | 2.22                                        |
|            | 6         | 100            | 600                      | 30                               | 420                                       | 180                      | 320                                           | 2.14                                        |
|            | 7         | 100            | 700                      | 35                               | 455                                       | 245                      | 355                                           | 2.03                                        |
|            | 8         | 100            | 800                      | 40                               | 480                                       | 320                      | 380                                           | 1.90                                        |
|            | 9         | 100            | 900                      | 45                               | 495                                       | 405                      | 395                                           | 1.78                                        |
|            | 10        | 100            | 1000                     | 50                               | 500                                       | 500                      | 400                                           | 1.67                                        |
| 1%         | 2         | 100            | 200                      | 2                                | 196                                       | 4                        | 92                                            | 1.92                                        |
|            | 3         | 100            | 300                      | 3                                | 291                                       | 9                        | 182                                           | 2.75                                        |
|            | 4         | 100            | 400                      | 4                                | 384                                       | 16                       | 268                                           | 3.45                                        |
|            | 5         | 100            | 500                      | 5                                | 475                                       | 25                       | 350                                           | 4.00                                        |
|            | 6         | 100            | 600                      | 6                                | 564                                       | 36                       | 428                                           | 4.41                                        |
|            | 7         | 100            | 700                      | 7                                | 651                                       | 49                       | 502                                           | 4.70                                        |
|            | 8         | 100            | 800                      | 8                                | 736                                       | 64                       | 572                                           | 4.88                                        |
|            | 9         | 100            | 900                      | 9                                | 819                                       | 81                       | 638                                           | 4.97                                        |
|            | 10        | 100            | 1000                     | 10                               | 900                                       | 100                      | 700                                           | 5.00                                        |
Table 5. Potential reduction in testing resources for different pool sizes.

| Pool Size                          | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|------------------------------------|---|---|---|---|---|---|---|---|---|----|
| Plastics                           |   |   |   |   |   |   |   |   |   |    |
| 1 mL tips Percent Reduction        | 0%| 1%| 13%|18%|22%|24%|26%|27%|28%|28% |
| compared to standard test          |   |   |   |   |   |   |   |   |   |    |
| 10-200μL tips Percent Reduction    | 0%| 50%|67%|75%|80%|83%|86%|88%|89%|90% |
| compared to standard test          |   |   |   |   |   |   |   |   |   |    |
| Total Tips                         | 0%| 15%|27%|34%|38%|40%|42%|43%|44%|45% |
| Deep well plates Percent Reduction | 0%| 29%|48%|57%|63%|67%|69%|71%|73%|74% |
| compared to standard test          |   |   |   |   |   |   |   |   |   |    |
| PCR Plates Percent Reduction       | 0%| 50%|67%|75%|80%|83%|86%|88%|89%|90% |
| compared to standard test          |   |   |   |   |   |   |   |   |   |    |
| Total Plates                       | 0%| 31%|50%|59%|65%|69%|71%|73%|75%|76% |
| Reagents                           |   |   |   |   |   |   |   |   |   |    |
| Extraction Percent Reduction       | 0%| 50%|67%|75%|80%|83%|86%|88%|89%|90% |
| compared to standard test          |   |   |   |   |   |   |   |   |   |    |
| qPCR reagents Percent Reduction    | 0%| 50%|67%|75%|80%|83%|86%|88%|89%|90% |
| compared to standard test          |   |   |   |   |   |   |   |   |   |    |

Table 6. Additional staffing and infrastructure requirements to double capacity of a lab currently processing 2500 samples a day.

| Area                                      | Required additional Staff/Infrastructure                                                                 |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Pre-analytic: samples arrive              | Additional sample booking stations and bench space for booking samples into LIMS                         |
| Pre-analytic: sample booking              | Additional class-I cabinet for sample neutralisation and swab removal and racking                        |
|                                           | Additional bench space to store racked samples                                                          |
| Pre-analytic: primary sample processing   | Additional automated liquid handling robot with barcode reader and at least 8 tip head for transfer      |
|                                           | from primary tubes to pool tubes OR Use of other readily available automation such as ELISA DS2 liquid |
|                                           | handling robot to transfer from primary tubes to low profile plates and capture tube barcode.          |
| Pooling: primary tube transfer            | Additional robotic 96 tip head for plate-to-plate transfer (10 minutes per pool, up to 10 samples per  |
|                                           | pool) OR Manual Pipetting (40 minutes per pool)                                                        |
| Pooling: plate pooling                    | Software to link the primary tube barcode to pool result.                                               |
| Pooling: sample tracking software         |                                                                                                        |
| Testing: extraction                       | Existing testing allocation                                                                            |
| Testing: amplification                    | Existing Roche 6800 testing allocation                                                                  |

drag-and-drop CSV file upload, generating two CSV exports to support the pooling workflow: (1) instructions for the liquid handler for re-arraying pooled samples that returned a positive or inconclusive result; and (2) a LIMS-compatible CSV-format file with all deconvoluted, individual negative results.
Technical feasibility of pooled testing

In order to assess the technical feasibility of the designed pooling workflow, nine hundred and twenty (920) patient samples were booked into North West London Pathology’s Sunquest LIMS test environment. The samples were then run through the pooling workflow (Figure 5) and final results were reported to the Sunquest LIMS test environment.

The results are shown in Figure 6 and Table 7, and on average pooling resulted in a loss of analytical sensitivity of 2.335 cycles in the ABY channel (S gene), 1.74 cycles in the FAM channel (ORF1ab) and 2.3 cycles in the VIC channel (N gene). Using our sensitivity analysis with our sVLPs, Sample 3 was at around the reliable N gene limit of detection (~467 copies) in our retest, and therefore would usually not be detectable in a 1-IN-10 pool. However, Sample 3 was still detected using our pooling strategy which could be due to the other gene targets of the ThermoFisher assay having lower a LLOD than that calculated by our N-gene sVLPs. In summary, we observed an expected increase in Ct value with pooled testing, however, due to our multi-target assay we were still able to detect samples with concentrations of below 1000 copies/mL.

Discussion

Here we describe a proof-of-concept study on the implementation of a plate-based pooling strategy for SARS-CoV-2 testing in an NHS diagnostic laboratory. We systematically examined the scientific, technical and operational constraints of implementing pooling in any frontline diagnostic laboratory. We not only considered the technical challenges in sample tracking of positive samples within pools and accompanying liquid handing, but modelled resources savings and analyser capacity increases and considered operational constraints such as additional infrastructure and staff requirements.

Group testing is an obvious proposition to increase testing capacity in reagent and analyser constrained settings. However, simpler solutions, such as swab pooling19, are not viable options for diagnostic laboratories where turnaround times preclude follow up retesting. Therefore, laboratory-based pooling, with its accompanying logistical and operational challenges remains the only viable option. Given that each laboratory has its own specific requirements, we focused on creating a generalisable approach that could be implemented elsewhere. We developed generalisable laboratory automation workflows and easily deployable software and our operational models can be altered based on assumptions relevant to a local laboratory context.

While we focused on plate-based pooling in this work, we also considered the use of tube-based end-to-end solutions (such as the Roche 6800). These platforms have already been approved for pooling of up to 6 samples per pool12 and many principles are shared between plate-based and tube-based pooling. We found that given the limited availability of Roche reagents worldwide, an optimal pooling approach could make use of the Roche allocation for pooling, with retest samples tested on a different platform to preserve test capacity on the instrument with limited supplies. Given a fixed allocation of tests for an analyser, this would substantially increase the number of samples that could be tested (Figure 3).

There are limitations to our study, pooling of samples leads to the loss of a sample adequacy control which can lead to false negative results. Our laboratory uses an assay that uses a spike-in processing control which would control for severe inhibition of the reaction. Nevertheless, this is one disadvantage of pooling that should be considered before implementation. Pooling would usually only be implemented when there are resource constraints that make it the most viable option to keep a diagnostic service running or when there are clear advantages over testing samples individually.

With the roll out of the vaccine, decreasing disease prevalence and a growing interest in surveillance for variants of concern,
we see laboratory-based pooling as being an attractive option. Pooling will not only optimize the use of testing resources including the use of reagents and labware but also increase capacity for the testing of asymptomatic staff and patients. Here we have shown that laboratory-based pooling is scientifically, technically and operationally feasible in an NHS diagnostic laboratory. We believe that this work provides a generalisable approach for other NHS diagnostic laboratories to implement adaptive pooling strategies in their own specific settings.

**Figure 5.** The designed and implemented pooling workflow for plate-based SARS-CoV-2 testing.

**Figure 6.** Positive samples: Ct values when tested individually versus Ct of respective positive pool for all 3 assay targets (S gene, ORF1ab and N gene). Sample Ct values found to the right and below the dotted line were found in pooled wells with other positive samples with higher viral loads.
**Data availability**

Underlying data
Zenodo: Rapid design and implementation of an adaptive pooling workflow for SARS-CoV-2 testing in an NHS diagnostic laboratory: A proof-of-concept study, [https://doi.org/10.5281/zenodo.554241](https://doi.org/10.5281/zenodo.554241).

This project contains the following underlying data:
- design_standardcurve.xlsx
- Detailed process map for plate-based pooling developed by NWLP and Imperial College.pdf
- rawdata_pooling_pooledplate.csv
- rawdata_pooling_retest_plate1.csv
- rawdata_pooling_retest_plate2.csv
- rawdata_standardcurve.csv

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0).

More detailed data, including that uploaded to the LIMS test environment, cannot be provided as although it contains anonymised container IDs, these can be linked back to patients if someone has access to the laboratory LIMS environment.

**Ethics statement**
Surplus clinical material was used to validate the assay as per normal practice and does not require ethical review.

**Author contributions**
- Conceptualization - MAC, PR, AA, PP, PSF
- Data curation - MAC, PR, PP
- Formal analysis - MAC, PR, PP
- Funding acquisition - MAC, SMC, PP, PSF
- Investigation - MAC
- Methodology - MAC, PR, AA, PP
- Project Administration - MAC, SMC, LAP, PP, PSF
- Resource - PR, PP
- Software - MAC, ZH
- Supervision - LAP, PP, PSF
- Validation - MAC, PR, PP

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**Table 7. Summary of positive results from 92 pools of 10 samples and the subsequent deconvolution and retesting of positive pools.**

| Positive Samples | Pooled Sample Well | Pooled Sample Ct Values | Retest Sample Well | Retest Sample Ct Values |
|------------------|--------------------|-------------------------|--------------------|-------------------------|
|                  | ABY | FAM | VIC | JUN | ABY | FAM | VIC | JUN |
| Sample 1         | A10 | 35.54 | 33 | 35.59 | 27.01 | D09 | 32.51 | 31.77 | 32.33 | 26.32 |
| Sample 2         | A11 | 29.56 | 31.33 | 25.75 | D10 | 27.44 | 28.32 | 25.28 |
| Sample 3         | B02 | 35.35 | 26.79 | 25.98 | F10 | 34.13 | 32.94 | 34.64 | 25.85 |
| Sample 4         | B03 | 28.17 | 26.75 | 28.65 | 25.98 | D12 | 24.82 | 24.37 | 27.36 | 26.12 |
| Sample 5         | B03 | 28.17 | 26.75 | 28.65 | 25.98 | F11 | 34.32 | 26.97 |
| Sample 6         | B06 | 30.18 | 29.11 | 30.14 | 26.22 | G01 | 28.43 | 27.79 | 29.61 | 26.88 |
| Sample 7         | B09 | 35.2 | 31.87 | 36.71 | 26.66 | E02 | 31.6 | 30.96 | 33.41 | 26.14 |
| Sample 8         | C05 | 32.39 | 35.42 | 26.41 | E03 | 30.03 | 32.48 | 26.15 |
| Sample 9         | D12 | 33.49 | 32.11 | 33.46 | 26.22 | E04 | 31.29 | 30.47 | 31.4 | 26.47 |
| Sample 10        | D12 | 33.49 | 32.11 | 33.46 | 26.22 | D05 | 33.41 | 33.6 | 34.56 | 26.67 |
| Sample 11        | E07 | 28.88 | 28.65 | 30 | 27.1 | E05 | 27.86 | 27.01 | 27.74 | 26.22 |
| Sample 12        | G06 | 22.45 | 20.92 | 24.16 | 25.2 | E06 | 20.73 | 19.83 | 22.59 | 27.52 |
| Sample 13        | H01 | 24.19 | 23.77 | 25.95 | 28.26 | E07 | 22.18 | 21.73 | 23.17 | 28.33 |
Visualisation - MAC, ZH

Writing - original draft: MAC, ZH, LAP, PSF

Writing - review & editing: MAC, PR, ZH, LAP, PP, PSF

MAC, PR and PP have accessed and verified the underlying data

Acknowledgements

We would like to thank Fiona Powell and Rina Azad for their help in running experiments. We would like to thank Ruth Thomsen, Ailsa Willens and Jane Fryer from NHS England for their support of this project. We would also like to thank Robin Vickers, Jo Thomson, Rob Bell, Timothy Brown and Resham Govan from KPMG for their project management support.

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Open Peer Review

Current Peer Review Status: ✓ ✓

Version 1

Reviewer Report 11 November 2021

https://doi.org/10.21956/wellcomeopenres.19037.r46676

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Nei-Yuan Hsiao

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In this proof of concept study designed in the UK NHS environment, Crone and colleagues described the impact of pooled NAT approaches on sensitivity, efficiency and capacity of high-throughput SARS-CoV-2 molecular testing while demonstrating its feasibility and merit. The authors assessed the many important factors associated with the pooling approach of SARS-CoV-2 testing including scientific, technical and operational. The methods that outlined the feasibility, both conceptual and physical, are appropriately designed and well implemented, with detailed and rigorous analyses. I found the description of work conducted can be inaccessible at times due to unfamiliarity with jargon and knowledge with specific automation platforms but overall the authors were able to accurately communicate the complexity around pooled NAT in the setting of SARS-CoV-2 testing with variable population prevalence and laboratory constraints.

The work is entirely based on the assumption that automation is available and unfortunately does not tackle the feasibility of pooling in a low-resource setting where pooling needs to be done by hand. In the analyses, the impact of contamination and assay failure rate was also not considered. These factors may be highly context-dependent and thus may be beyond the scope of research. It would be useful to include these considerations in the study limitation section of the discussion, as often the setting that would most likely consider pooled NAT are often those with limited resources.

Overall the data and findings of the study are highly informative and definitely worthy of publication.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical virology, diagnostic research, Molecular diagnostic, viral epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 25 October 2021

https://doi.org/10.21956/wellcomeopenres.19037.r46415

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The article is very comprehensive. Pooling of samples for SARS-CoV-2 testing, depending on the existing prevalence of infection and to study the impact of pooling on the analytical sensitivity of the RT-PCR assay, is the critical part of the study.

Considerations like the impact on the workflow - whether as part of an independent (with and without reagent crunch) or an integrated pooling workflow - are often overlooked and were very clearly validated as shown by the data provided.

Balancing staffing and infrastructure requirements on the one hand, with planning an optimum workflow with an efficient validated pooling system so as to optimise benefits on the other, together with a software that helped decode the pooling strategy (clear and concise data and figures provided) have all contributed to this beautifully planned and executed study.

As the authors pointed out in the discussion, false-negative reports are a risk when the pooling of
samples is done. Hence, implementation of this strategy can be done ONLY when the prevalence of the infection is below an acceptably low level for that population specifically. Especially in resource-poor nations, with larger populations having acquired the infection and more vaccine roll-out, this does offer a very useful method to get greater numbers of the population screened more rapidly.

**Is the work clearly and accurately presented and does it cite the current literature?**  
Yes

**Is the study design appropriate and is the work technically sound?**  
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**  
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**  
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**  
Yes

**Are the conclusions drawn adequately supported by the results?**  
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Infectious Disease, immunogenetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.