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Evaluation of sample pooling using the SAMBA II SARS-CoV-2 test

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ABSTRACT

Background: Screening of infectious asymptomatic or pre-symptomatic individuals for SARS-CoV-2 is at present a key to controling the COVID-19 pandemic. In order to expand testing capability and limit cost, pool testing of asymtomatic individuals has been proposed, provided assay performance is not significantly affected.
Methods: Combined nose and throat (N/T) swabs collected from COVID-19 infected or non-infected individuals were tested using SAMBA II individually and in pools of four (one positive and 3 negative). The evaluation was conducted by the manufacturer and an independent NHS site. Ct cycles of individual positives and pooled positives were determined by qRT-PCR.
Results: In 42 pools containing a single positive sample with Ct values ranging between 17 and 36, 41 pools (97.6 %) were found positive by the SARS-CoV-2 SAMBA II test. The false-negative pool by SAMBA was also negative by both reference methods used in this evaluation.The individual positive sample in this pool was positive by SAMBA (Orf only) and by one of the reference methods (S gene only, Ct 35) but negative by the second reference method indicating that the sample itself was very low viral load. All 78 pools containing 4 negative swabs were negative (100 % specificity).
Discussion: The preliminary data of the evaluation indicated a high level of performance in both sensitivity and specificity of the SAMBA II assay when used to test pools of 4 patient samples. The implementation of this pooled protocol can increase throughput and reduce cost/test when the prevalence of COVID is low.

1. Introduction

Until recently in the UK, emphasis has been placed on testing individuals with COVID-like symptoms (fever, cough, anosmia) in order to identify COVID-19 infection and subsequently isolate the individuals and their close contacts. This strategy has been applied in the community, in hospitals for triage of patients and in schools in order to decide on isolation of bubbles. However, the main drivers of the pandemic are asymptomatic and pre-symptomatic infections that remain undetected despite infectiousness similar to symptomatic cases (Arons et al., 2020; Rivett et al., 2020). A recent study estimated that at least 50 % of COVID-19 cases may have been contracted from asymptomatic individuals (Johansson et al., 2021). Therefore, screening of pre-symptomatic and asymptomatic carriers is crucial for SARS-CoV-2 infection prevention and in a hospital setting to diagnose SARS-CoV-2 infection in incoming patients regardless of symptoms. The SAMBA II SARS-CoV-2 Test is an accuracte point-of-care (POC) test for diagnosis of SARS-CoV-2 infection with a limit of detection of 250 cp/mL and high clinical sensitivity and specificity Assennato et al., 2020; Collier et al., 2020). Rapid POC tests, such as SAMBA II, with fast results are useful, so that those who are positive can be promptly isolated and attended. This cannot be achieved with centralised testing, with turnaround times of 24 h or more.

In order to limit cost and expand testing capability, Public Health England (PHE) recommended pooling to increase testing capacity and reduce reagent consumption when there is a low background prevalence in target groups where there is need and benefit from identifying positive individuals, eg asymptomatic patients and professionals, at a time of low positive prevalence (Hogan et al., 2020; Lohse et al., 2020; Mastrianni et al., 2020) (https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/09/C0777-sample-pooling-sop-v1.pdf]). DRW, the manufacturer of the SAMBA II test, investigated the

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performance of the SAMBA SARS-CoV-2 Test in pooled clinical samples using pools of four.

2. Materials and methods

2.1. Study design

The study was intended to examine the feasibility and reliability of the SAMBA II SARS-CoV-2 Test using pools of 4 samples in order to expand the availability of the assay without compromising its performance. The first phase was conducted in the manufacturer's facilities in collaboration with the Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Cambridge (CMPHL) using surplus frozen samples from the COVIDx study and additional fresh negative samples. SAMBA and PHE positive, well-characterised, individual samples were thawed and mixed with three negative swab samples by one operator to make positive pools and four negative samples were mixed to make negative pools. SAMBA testing was carried out by a second operator in a blinded fashion.

The second phase was carried out in the Royal Berkshire Foundation Trust POC testing laboratory. This pilot study consisted of selecting 10 patient samples which previously tested by the SAMBA-SARS-CoV-2 and deemed to be either strong positive (both Orf and N detected) or weak positive (Orf only), mixed with three known negative samples. Individual positive samples and pools were frozen and tested by two independent reference assays: Genesig RT-PCR at Molecular Laboratory at Royal Surrey NHS Foundation Trust (RSML) and the PHE reference test at CMPHL at Addenbrooke's hospital and results recorded as Ct cycles. PCR results were blinded to the technicians who carried out the SAMBA testing.

2.2. Molecular SARS-CoV-2 assays

2.2.1. SAMBA-II SARS-COV-2 Test

The SAMBA II platform and the SAMBA II SARS-CoV-2 Test kit are CE IVD marked for diagnosis of SARS-CoV-2 infection. The system and test have been previously described in detail (Assennato et al., 2020). The assay specifically amplifies two regions of the SARS-CoV-2 genome in the ORF1ab and nucleocapsid gene (N) with a visual readout on a test strip. The uppermost line detects the internal control, which ensure adequate test procedures, (IC), and the two lower lines represent specific targets of the SARS-CoV-2 genome, open reading frame 1ab (Orf1ab) and (N). The presence of either test line (ORF1ab or N) indicates a positive result in the presence or absence of IC. The presence of the internal control line alone indicates a negative result. The signal on the test strip is read and interpreted by an integrated camera in the SAMBA II machine with the result reported by the tablet. Each SAMBA II assay module can process one patient or one pool sample at a time with test times of 86 min for a strong positive to 101 min for a negative or weak positive result.

2.2.2. Reference testing at royal berkshire hospital

Samples collected in SAMBA SCoV buffer were extracted using a Kinfisher Plex and tested using the Genesig Coronavirus COVID-19 genesig® Real-Time PCR assay (Primerdesign Ltd, Chandlers Ford, UK) run on a MIC Real Time qPCR cycler (Biomolecular Systems, London, UK), targeting the ORF1ab genome region. Samples with Ct <35 were reported as positive, samples with Ct 35–37 were equivocal Ct >37 were reported negative. The limit of detection of the Genesig test is 0.58 copies/µl (580 copies /mL). Repeated samples were run using the original swab sample that had been stored in the refrigerator at 4−8 °C and brought to room temperature before running.

2.2.3. PHE reference test at CMPHL

The PHE reference test was performed at CMPHL. Samples used for the DRW pooling study were collected in April 2020 as part of the COVIDx study and tested using the Cambridge RdRp gene assay on the Rotor gene Q real-time PCR assay routinely used by CMPHL as previously described (Sridhar et al., 2020) but modified by switching the enzyme master mix used to Taqpath™ 1-Step RT-q PCR from Life Technologies (Cat No A15300). The samples used for the Royal Berkshire phase 2 pooling study were tested using an upgraded assay, which also amplifies the S gene target in addition to the RdRp gene as previously detailed (Skittrall et al., 2020). A reactive result for either or both genes below Ct 36 was considered a positive result on both assays run at CMPHL.

2.3. Specimen collection and handling of individual and pooled samples

Combined nose and throat (N/T) swab samples were re-suspended in 2 mL of SAMBA SCoV buffer, provided with the kit. The SAMBA SCoV buffer inactivates SARS-CoV-2 within 10 min (Assennato et al., 2020; Collier et al., 2020; Welch et al., 2020). It is therefore recommended that samples be incubated at room temperature for 10 min to inactivate the sample before loading it into the machine. The input volume for the SAMBA test is 300 µl of which 250 µl is used by the SAMBA II machine as input into the sample processing. For the purpose of this study four samples were pooled together by pipetting 75 µl of each of 4 samples into the SAMBA input tube to give a total volume of 300 µl. This pooled sample was then run in the SAMBA II and the result recorded. All pooled samples were also tested individually and the results recorded and compared to the pooled result.

2.3.1. Pooled samples run at DRW

Thirty-one (31) frozen surplus SARS-CoV-2 positive combined N/T swab samples from a previous evaluation (Collier et al., 2020) were used to prepare positive pools. These samples had previously been tested as positive for SARS-CoV-2 by both the SAMBA SARS-CoV-2 test and the PHE reference laboratory assay by CMPHL. These samples were all collected from symptomatic individuals and the Ct values ranged from 17 to 34 by the reference method. Fifty-two frozen surplus SARS-CoV-2 positive combined N/T swab samples from the same evaluation were used to prepare the positive and negative pools. These samples were negative individually by both SAMBA and the standard PHE test (Corman et al., 2020). In addition, 103 SAMBA negative N/T swab samples from healthy individuals without symptoms were used to prepare pools. In total 32 positive pools (containing 1 positive and 3 negative samples) and 44 negative pools (containing 4 negative samples) were generated. The content of each pool was blinded from the operator running and interpreting the SAMBA and results.

2.3.2. Pooled samples run at Royal Berkshire Hospital

In phase 1, samples already run by the operational SAMBA point-of-care service were selected and randomised by an operator into groups of 4. The aim was to test 50 pools, with at least 10 containing a positive sample. Of these 10 positives, 5 to contain a strong positive sample, defined as positive for both SAMBA targets (ORF1ab and N) and 5 to contain a sample with low level positive result, defined as a single target line only detected (ORF1ab). These patient samples were kept at 2−8 °C for 0–3 days before they were used to constitute pools with fresh negative samples.

The content of each pool was blinded from the operator running and interpreting the results. Pools including a positive sample that did not report positive were repeated. The positive pools and the positive individual samples were all also sent to the Molecular Laboratory at Royal Surrey Foundation Trust (RSML) for testing.

In Phase 2 10 positive pools (5 high and 5 low as described above) were run on the same day as the individual samples ranging from 1.5–7 h between the individual result and the pooled result. Aliquots of the ten positive individual samples and pools were frozen and sent to the RSML and CMPHL for reference testing.

2.4. Ethics

The samples used in this study were surplus volume from the sample taken as part of the patient's standard care, once all analysis had been completed. No additional tests were carried out on patients or clinical decisions made as a result of this work. This work is classified as a service evaluation.

3. Results

3.1. Pooled samples run at DRW

In total 32 positive pools (containing one positive and three negative samples) were tested along with 44 negative pools (containing four negative samples). The Ct value of the positive samples ranged from 17 to 34 according to the PHE Cambridge method described in 2.2.3 (Table 1). All 32 positive pools tested and all 44 negative pools were negative. SAMBA results were recorded by visually reading the test strip and by recording the camera results reported on the tablet. On the basis of this limited study, the sensitivity and specificity of the pool of four testing was 100 % respectively and camera and visual results were 100 % concordant.

3.2. Pooled samples run at Royal Berkshire Hospital

In phase 1, all 34 negative pools (containing 4 negative samples), tested negative confirming that the operational process of pooling did not introduce any contamination. Nine of the sixteen positive pools contained a strong positive sample (SAMBA Orf and N detected in individual test) all returned positive results in their pool (Table 2). Seven of the sixteen pools contained low level positive individuals (SAMBA only detected the Orf target in individual test). Three of these seven low level positive pools reported as SAMBA positive and four reported as SAMBA negative, and repeated as negative. The samples had been stored in the refrigerator for up to 3 days before being testing in a pool and when the individual samples were repeated they reported as negative by SAMBA. An aliquot of each expected positive pool (N = 16) and the associated individual positives samples (N = 16) were also sent to the RSML for testing by Genesig. Six of the16 individuals reported as not detected and 3 samples returned a Ct value above 37 and hence would be reported as negative. Ten of the 16 expected positive pools reported as not detected by Genesig, including the 4 pools not detected by SAMBA (samples 30c, 31c, 32c and 47d in Table 2). If any individual was not detected, the pool was also not detected by Genesig.

Both the Genesig and repeat SAMBA results suggest there has been significant degradation of the RNA content over time in these low level samples despite storage according to the DRW instructions. This is not realised during normal service operational activity as samples are run on average 43 min after the patient is swabbed. A 2nd swab was also taken on these patients within 24 h of the initial SAMBA sample, as part of their clinical care, and all had detectable levels of COVID-19, confirming the original positive SAMBA result.

In Phase 2 of the Royal Berkshire study the samples were pooled on the same day as the sample was collected and an aliquot immediately frozen after testing for comparator testing. In this phase a further 5 strong positives (Orf and N detected by SAMBA) and 5 low level (Orf only detected by SAMBA) positives were used to make pooled samples. The five pools containing a strong positive SAMBA sample reported as

Table 1
SAMBA II SARS-CoV-2 pooled testing results for positive pools.

	Individual result					Pooled result			
Pool number	IC line[1]	ORF1ab line[1]	N line[1]	SAMBA tablet result[1]	PHE RdRp Ct[3]	IC line[1]	ORF1ab line[1]	N line[1]	SAMBA tablet result[1]
1	+	+	–	Positive	30	+	+	+	Positive
2	+	+	+	Positive	32	+	+	+	Positive
5	+	+	–	Positive	32	+	+	+	Positive
7	+	+	–	Positive	34	+	+	–	Positive
10	+	+	+	Positive	25	+	+	+	Positive
13	+	+	+	Positive	26	+	+	–	Positive
14	+	+	–	Positive	22	+	+	+	Positive
15	+	+	–	Positive	27	+	+	–	Positive
18	+	+	+	Positive	26	+	+	–	Positive
22	+	+	+	Positive	22	+	+	–	Positive
23	+	+	+	Positive	29	+	+	–	Positive
27	+	+	+	Positive	19	+	+	–	Positive
28	+	+	+	Positive	25	+	+	–	Positive
33	+	+	+	Positive	22	+	+	–	Positive
34	+	+	+	Positive	17	+	+	–	Positive
35	–	+	–	Positive	20	+	+	–	Positive
47	–	+	–	Positive	20	+	+	+	Positive
48	NR[2]	NR[2]	NR[2]	Positive	20	+	+	–	Positive
51	+	+	–	Positive	29	+	+	–	Positive
52	+	+	–	Positive	24	+	+	–	Positive
54	+	+	–	Positive	31	+	+	–	Positive
57	+	+	+	Positive	20	+	+	–	Positive
58	+	+	+	Positive	21	+	+	+	Positive
59	+	+	+	Positive	28	+	+	–	Positive
61	+	+	+	Positive	22	+	+	–	Positive
62	+	+	+	Positive	25	+	+	–	Positive
64	+	+	+	Positive	18	+	+	+	Positive
65	+	+	+	Positive	25	+	+	–	Positive
67	+	+	+	Positive	27	+	+	+	Positive
69	+	+	+	Positive	27	+	+	–	Positive
70	+	+	+	Positive	28	+	+	–	Positive
71	+	+	–	Positive	31	+	+	+	Positive

[1] SAMBA results can be interpreted visually and also reported electronically via the tablet. The ORF1ab target is more sensitive than the N target. Detection of either target results in a positive diagnosis.
[2] NR – eye read not recorded.
[3] For PHE Cambirgde method Ct <36 is considered positive for SARS-CoV-2. All samples were positive.

Table 2
SAMBA II SARS-CoV-2 pooled testing results for Berkshire phase 1.

	Individual sample					Pool sample					
Pool Number	IC line[1]	ORF1ab line[1]	N line[1]	SAMBA result	Genesig ORF1ab Ct[3]	IC line	Orf1ab line	N line	SAMBA result	Genesig ORF1ab Ct[3]	Time between individual and pool
6a		+	+	Positive	37.24	+	+	+	Positive	35.28	0 days
8a	+	+	+	Positive	ND	+	+		Positive	ND	0 days
13c	+	+	+	Positive	32.94	+	+	+	Positive	34.24	2 days
16c		+	+	Positive	34.75	+	+	+	Positive	34.48	1 day
22b		+	+	Positive	31.42	+	+	+	Positive	32.21	0 days
27c	+	+	+	Positive	42.75	+	+	+	Positive	ND	0 days
37a	+	+	+	Positive	37.51	+	+		Positive	ND[2]	1 day
38c	+	+	+	Positive	36.37	+	+	+	Positive	37.55	1 day
41b	+	+	+	Positive	33.71	+	+	+	Positive	36.32	1 day
30c	+	+		Positive	ND[2]	+			Negative	ND[2]	3 days
31c	+	+		Positive	ND[2]	+			Negative	ND[2]	3 days
32c	+	+		Positive	ND[2]	+			Negative	ND[2]	0 days
40a	+	+		Positive	35.82	+	+		Positive	ND[2]	1 day
46b	+	+		Positive	37.19	+	+		Positive	ND[2]	1 day
47d	+	+		Positive	ND[2]	+			Negative	ND[2]	1 day
48c	+	+		Positive	ND	+	+		Positive	ND[2]	1 day

[1] SAMBA results can be interpreted visually and also reported electronically. The ORF1ab target is more sensitive than the N target. Detection of either target results in a positive diagnosis. Tests with both Orf and N detected were categorised a "strong positives" for this study and samples with ORF1ab but no N were categorised as "weak positives" for this study.
[2] ND = not detected.
[3] With Gensig Ct 35–37 is considered equivocal. Ct >37 is negative.

Table 3
SAMBA II SARS-CoV-2 pooled testing results for Berkshire phase 2.

	Indiviual sample							Pooled samples						
sample ID	IC line[1]	ORF1ab line[1]	N line[1]	SAMBA result	Genesig ORF1ab Ct[3]	PHE RdRp Ct[4]	PHE S gene Ct[4]	IC line[1]	Orf1ab line[1]	N line[1]	SAMBA result[1]	Genesig ORF1ab Ct[3]	PHE RdRp Ct[4]	PHE S gene Ct[4]
2LP-01	+	+	+	positive	24.13	22.36	20.23	+	+	+	positive	27.58	24.37	22.66
2LP-02	+	+	+	positive	29.2	29.39	25.63	+	+	+	positive	32.26	34.76	28.36
2LP-03	+	+	+	positive	27.11	28.78	21.85	+	+	+	positive	25.17	29.03	22.3
2LP-04	+	+	+	positive	29.64	34.37	27.37	+	+	+	positive	33.31	35.39	29.07
2LP-05	+	+	+	positive	29.59	29.51	27.23	+	+	+	positive	34.67	33.23	29.67
1LP-01	+	+		positive	ND[2]	Neg	34.45	+	+		positive	ND[2]	Neg	Neg
1LP-02	+	+		positive	ND[2]	Neg	35.18	+			negative	ND[2]	Neg	Neg
1LP-03	+	+		positive	37.64	41.89	35.65	+	+		positive	ND[2]	Neg	Neg
1LP-04	+	+		positive	36.77	39.74	33.02	+	+		positive	37.39	Neg	34.24
1LP-05	+	+		positive	37.92	Neg	35.27	+	+	+	positive	37.79	Neg	34.29

[1] SAMBA results can be interpreted visually and also reported electronically. The ORF1ab target is more sensitive than the N target. Detection of either target results in a positive diagnosis. Tests with both Orf and N detected were categorised a "strong positives" for this study and samples with ORF1ab but no N were categorised as "weak positives" for this study.
[2] ND – not detected.
[3] With Gensig Ct 35–37 is considered equivocal. Ct >37 is negative.
[4] For PHE Ct >36 is considered negative for that target. The S gene target is more sensitive than the RdRp gene target. Detection of either target with Ct <36 results in a positive diagnosis.

positive (Table 3). Four of the five pools containing a low level positive were positive and one (1LP-02) was negative (Table 3).

The five high level positives were also positive by the Genesig and PHE tests both individually and in pools (Table 3). Of the five low level positives four were negative by Genesig (ND or Ct>37) individually and all were negative in pools (Table 3). All five low level positives were negative by the PHE RdRp gene (Neg or Ct>36) both individually and in pools (Table 3). However, the S gene target of the PHE test was positive for all five individual low level samples (Ct 33.02–35.65) and for two of the five pools (1LP-04 and 1LP-05) (Table 3). The one positive pool that returned a negative result by SAMBA (1LP-02) was also negative in the pool by Genesig and PHE tests. The positive sample in this pool was not detected by Genesig or the PHE RdRp assay but was positive for the PHE S gene (Ct 35.18) indicating that it was a very low positive.

Results obtained in the Royal Berkshire branch of the evaluation confirmed the 100 % specificity of pool testing obtained at DRW. Despite a degree of uncertainty regarding the low positive samples, 90 % of samples were correctly identified when pooled and individual samples were run on the same day.

4. Discussion

Pooling techniques enable screening of greater numbers of individuals while preserving testing resources. Numerous publications recently pointed out sample pooling as a method to reduce cost and maximising efficiency (10–12) and investigated an optimum balance between test performance and number of samples in a pool. Studies reporting on SARS-CoV-2 testing in pools were conducted in pools of 5, 8 or 10 samples (Praharaj et al., 2020; Chhikara et al., 2021; Torres et al., 2020). In pools of five (the closest to the pools of four described here), the expected number of tests performed was 57 % less than in individual testing at a 5% prevalence rate and a 100 % sensitivity with LOD of 1,

000–3,000 copies/mL (Abdalhamid et al., 2020). Another study using the Cepheid Xpert® Xpress SARS-CoV-2 assay could detect positive samples of Ct 20–28 when run in a pool or 4 or 6 with a median change of Ct value of 2.0 and 2.9 respectively but samples with higher Ct values were not tested (Graham et al., 2021). Another report examined the options of pooling before or after nucleic acid extraction and did not find a significant difference between the two (Chhikara et al., 2021). In our case, for simplicity and time saving, pooling swab samples prior to extraction was adopted and samples of Ct value < Ct35 (PHE S gene) were detected in pools of 4 samples. It should be noted that Ct values vary between different tests and between different targets, and should not be compared since without standardisation Ct values are not equivalent.

Utility of pooling for SARS-CoV-2 will depend on the turnaround time and sensitivity of the test and prevalence of infection in the setting. SARS-CoV-2 testing strategies would be different for symptomatic and asymptomatic patients. Symptomatic individuals should be tested individually, but asymptomatic individuals can be tested in pools of four. When a pool is positive, each individual sample should be tested in order to identify the person whose sample is responsible for the positive pool result. Therefore, the utility of pool testing is closely associated with turnaround time for results, if a test result is obtained on SAMBA in less than 2 h and can be immediately repeated with individual samples, only 4 h have elapsed and maximum efficacy of prevention can be obtained making it a feasible option.

With regards to sensitivity, the SAMBA II SARS-CoV-2 Test has a limit of detection of 250 copies/mL and therefore pooling four samples will likely raise this to 1000 copies/mL. Viral loads below such levels appear to be a small minority and to be far below the infectivity level estimated around 100,000 copies/mL (Wölfel et al., 2020). This evaluation was carried out in two distinct settings: one internal to the SAMBA manufacturer, the other external in a NHS point of care testing setting. The combined specificity between the 2 sites was 100 % (95 % CI: 96.2–100 %) in 78 negative pools constituted of 312 individual negative samples. When samples were tested the same day as collection or frozen on the day of collection the sensitivity was 100 % (95 % CI: 92.61–100 %) in 39 samples with Ct <35 and 97.6 % (95 %CI: 87.4–99.9) in all 42 positive pools containing one positive sample (Ct 17–35.65). Studies have shown that patients with Ct values >34-35 were unlikely to be infectious (La Scola et al., 2020; Singanayagam et al., 2020) and therefore the sample that was missed in the pool (Ct 35.18), would likely not have resulted in onwards transmission of the virus. In total 5 samples with Ct 34–35.65 were tested in pools and four out of five were detected.

Data interpretation however needs to take into consideration for the Royal Berkshire data that the SAMBA testing was performed on fresh individual swabs and pools. The individual and pooled samples were immediately frozen after SAMBA testing and were tested by Genesig at RSML after 8–18 days and by PHE at CMPHL after 11–21 days. In the DRW study the SAMBA individual samples were tested fresh and samples frozen the same day. The pools were tested by SAMBA after around 4 months. Therefore, samples used for pooling by SAMBA must be tested on the same day of collection (within 7 h) or frozen and tested at a later date to ensure that the low level positive samples are detected.

The utility of a diagnostic strategy using pooled samples also holds close relation to the prevalence of infection in the proposed setting. In high prevalence scenarios, a greater number of pools need to be retested, rendering the strategy more costly and time-consuming. Several mathematical models indicated that pool testing was cost-effective below an acute infection prevalence of 10–30 % (Mutesa et al., 2020; Mallapaty, 2020; Aragón-Caqueo et al., 2020). Although the exact cost-benefit of the pooling approach needs to be individually assessed based on circumstances, it appears reasonable to pool samples of 4 individuals if the SARS-CoV-2 if prevalence in the target population is below 10 % with predicted test reduction in test numbers and hence cost of 35, 55 and 71 % at prevalenceof 10, 5, and 1 % respectively.

5. Conclusions

Both arms of the evaluation study concluded the high performance of the SAMBA II SARS-CoV-2 Test using pools of 4 samples. Furthermore pooling four samples was found to be operationally acceptable in an NHS point of care setting as the turnaround time of SAMBA means that results are delivered within 4 h if the pool needs to be repeated. One positive sample (Ct 35.18) was missed in the SAMBA pool testing but the low viral load of this patient likely indicates very early infection or lingering viral presence and they are likely no longer infectious. Therefore, combining a pooling strategy with clinical understanding of a patients history i.e. evidence of past infection, presence of current symptoms or risk of exposure has a role when balanced against the need of high throughput screening of patients with a sensitive molecular tests.

Author contribution

Katy Heaney: Conceptulisation, Methodology, Supervision, Resources, Validation, Writing-Reviewing and Editing

Allyson Ritchie: Conceptulisation, Methodology, Supervision, Investigation, Validation, Writing-Original draft preparation

Rowan Henry: Investigation

Adam Harvey: Investigation, Writing-Reviewing and Editing

Martin Curran: Investigation, Writing-Reviewing and Editing

Jean-Pierre Allain: Conceptualisation, Validation, Writing-Original draft preparation

Helen Lee: Conceptulisation, Supervision

All authors had access to the data and agreed with the content of the manuscript.

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Declaration of Competing Interest

Dr AV Ritchie, AJ Harvey and Dr HH Lee are employees of Diagnostics for the Real World (DRW), Little Chesterford, UK. Prof JP Allain is emeritus professor of Transfusion Medicine, University of Cambridge and consultant to DRW.

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