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Original article

Effective bubble-based testing for SARS-CoV-2 using swab-pooling

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ABSTRACT

Objectives: Despite the success in developing COVID-19 vaccines, containment of the disease is obstructed worldwide by vaccine production bottlenecks, logistics hurdles, vaccine refusal, transmission through unvaccinated children, and the appearance of new viral variants. This underscores the need for effective strategies for identifying carriers/patients, which was the main aim of this study.
Methods: We present a bubble-based PCR testing approach using swab-pooling into lysis buffer. A bubble is a cluster of people who can be periodically tested for SARS-CoV-2 by swab-pooling. A positive test of a pool mandates quarantining each of its members, who are then individually tested while in isolation to identify the carrier(s) for further epidemiological contact tracing.
Results: We tested an overall sample of 25 831 individuals, divided into 1273 bubbles, with an average size of 20.3 ± 7.7 swabs/test tube, obtaining for all pools (≤37 swabs/pool) a specificity of 97.5% (lower bound 96.6%) and a sensitivity of 86.3% (lower bound 78.2%) and a post hoc analyzed sensitivity of 94.6% (lower bound 86.7%) and a specificity of 97.2% (lower bound 96.2%) in pools with ≤25 swabs, relative to individual testing.
Discussion: This approach offers a significant scale-up in sampling and testing throughput and savings in testing cost, without reducing sensitivity or affecting the standard PCR testing laboratory routine. It can be used in school classes, airplanes, hospitals, military units, and workplaces, and may be applicable to future pandemics. **Yuval Cohen, Clin Microbiol Infect 2022;28:859**

Introduction

The success in developing COVID-19 vaccines raised the hope for containment of the disease. However, the shortage in vaccine production, along with logistic issues and relaxation of preventive measures, led to a rise in the spread of the disease in many countries [1]. This underscores the notion that controlling the spread of the SARS-CoV-2 virus is a complex task involving a combination of preventive measures, diagnostic testing that involves complicated logistics, proper explanation to the public, and contact tracing. Moreover, it is confounded by issues of psychology of the masses, conspiracy theories, fake news, and lack of public trust [2,3] and the significant number of asymptomatic carriers [4–6].

Testing for the presence of the virus is key to the termination of viral transmission. Simple and fast detection methods are available, but the reference standard is still qRT-PCR of nasal or nasopharyngeal swabs, which typically takes several hours and is carried

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out in an authorized laboratory [7,8]. Several approaches were developed to increase the throughput of qRT-PCR testing, such as sample pooling, which enables a shorter turnaround time with a slight reduction in test sensitivity [9–14], as well as using lysis buffer for swab collection instead of viral preservation medium [15]. Although numerous studies have been published on sample pooling [9–13], only a few studies were published on swab-pooling [16–20], with only one large-scale study [21].

Here we describe the combination of the bubble (termed 'capsule' in Israel) concept [22,23] with a large-scale screening approach of swab-pooling into lysis buffer. Members of each bubble were assayed in parallel by swab-pooling and by individual testing, and the results of each swab-pool were compared to the individual testing results of its members.

Methods

Outline of the approach

We defined a bubble as a group of people who spend time together (e.g. students in a class, workers in a specific factory space), such that if one member of the bubble is identified as SARS-CoV-2 positive, all members of the bubble are sent into isolation. Here, the members of each bubble were assigned in advance and tested by swab-pooling; namely, a swab was taken from each person, and the swabs were all pooled into a single test tube. To increase sensitivity and safety, we used lysis buffer for swab-pooling. Up to 37 swabs can be placed in a 50-mL test tube containing 10 mL of lysis buffer, and up to 8 swabs can be placed in a standard 15-mL test tube with 3 mL lysis buffer (Fig. S1). For comparison, all members of each bubble were also sampled into individual 3-mL viral transport medium (VTM) tubes. All pools and individual samples were tested for SARS-CoV-2 using commercial qRT-PCR assays, as described under Methods. When a pool was declared PCR positive, all bubble members were sent into isolation, and the carrier(s), identified by the individual test underwent an epidemiology inquiry for contact tracing (Fig. 1).

Subjects and sampling

The study was conducted with daycare nannies from the municipality of Bnei-Brak, students and personnel from yeshivas for

Fig. 1. Outline of the bubble-based swab-pooling concept. (A) individuals in a workplace or a school are divided into bubbles, with each individual in a bubble (six in this example) tested with two swabs: one is placed in the swab-pool test tube and the second in an individual tube. Black person image, healthy individual; red image, SARS-CoV-2–infected individual, whose swab is coloured red. (B) Swab-pool A and swab-pool C are PCR negative, and therefore all individuals belonging to bubbles A and C are regarded as negative. Swab-pool B gave a positive PCR result, and therefore each individual belonging to swab-pool B is being isolated. (C) The individual test tubes for swab-pool B members are individually PCR tested to identify the SARS-CoV-2–positive individual. This is followed by contact tracing.

orthodox Jewish boys/young men, and elementary and secondary schools for orthodox Jewish girls, as well as staff working in nursing homes for the elderly, geriatric hospitals, assisted living facilities, and welfare institutions for populations at risk. The study was coordinated with the relevant authorities, and all participants gave verbal consent.

The study enrolled a total of 27 348 subjects. Of these, 1284 were excluded due to technical issues such as leakage from the test tubes or inconsistency in the barcodes between a pool and its individuals' tests, and 233 of the samples were excluded because we did not have their test results in the computerized data system. Overall, 25 831 were included in the final analysis (94.5%), including 1096 (4.2%) for whom age was not available. The sex and age distributions of the participants are presented in Table 1.

The study included 654 daycare nannies from different daycares. These were randomly divided into 47 groups (simulated bubbles). A total of 16 322 Yeshiva students and personnel were divided into 812 bubbles. A total of 8855 workers of institutions for the elderly were divided into 414 bubbles. Sampling was performed by Magen David Adom, the Israeli equivalent of the Red Cross.

Data analysis was done using Excel files that did not include any identifying personal information except for age, sex, and population group (daycare nannies, Yeshiva students and personnel, or workers at institutions for the elderly). This project was a quality enhancement project, and the analysis of the data was approved by the Research Ethics Committee of the Sheba Medical Center (reference number 9067-22-SMC).

Table 1
Demographic and swab-pools characteristics

Characteristics	Institutions for subjects at risk[a] (n = 8855)	Yeshivas[b] (n = 16 322)	Nannies[c] (n = 654)	Entire study (N = 25 831)
Sex, n (%)				
Female	6300	1053	654	8007
Male	2436	15 211	—	17 647
Unknown	119	58	—	177
Age (y), mean ± SD[d]				
All	51.7 ± 17.6	20.0 ± 6.0	NA[e]	30.9 ± 19.0[f]
Females	52.3 ± 17.2	20.7 ± 6.4	NA	47.7 ± 19.6
Males	50.7 ± 18.7	20.0 ± 6.0	NA	24.2 ± 13.7
Unknown	38.9 ± 8.4	20.4 ± 9.5	NA	33.2 ± 12.2
Pools, n	413	813	47	1273
Swabs/pool, mean ± SD	21.4 ± 7.5	20.1 ± 7.4	NA	20.5 ± 7.3[g]
Positive pools, n	4	47	2	53
Marginally positive, n	3	33	10	46
Negative, n	406	733	35	1174
Positive individuals/pool (pools), n[h]	0 (3), 1 (3), 3 (1)	0 (27), 1 (32), 2 (9), 3 (7), 5 (1), 6 (1), 7 (1), 8 (1), 11 (1)	1 (10), 2 (1),3 (1)	0 (30), 1 (45), 2 (10), 3 (9), 5 (1), 6 (1), 7 (1), 8 (1), 11 (1)

NA, not available; SD, standard deviation.
[a] Including geriatric hospitals, assisted living facilities, and welfare institutions for populations at risk.
[b] Including students and personnel from regular yeshivas for orthodox boys and young man, and the Beis Yaakov elementary and secondary schools for orthodox Jewish girls.
[c] Daycare nannies from the city of Bnei Brak.
[d] Mean age excluding a total of 1096 individuals whose age was unknown. These were distributed as follows: nursing homes for the elderly—246 females, 72 males, 8 unknown; Yeshivas—27 females, 81 males, 8 unknown; Nannies—654 females. Entire study—927 females, 153 males, and 16 unknown.
[e] These data were not collected for the nannies.
[f] Mean age of all individuals excluding the nannies, for whom age was not available.
[g] Mean numbers of swabs/pool for all pools, except those of the nannies group, which were not available.
[h] Positive pools with matching positive individuals. For example, 0 (3), 1 (3), and 3 (1) stand for three positive pools with no corresponding individual test, three pools with one corresponding positive individual test, and one pool with three corresponding individual positive tests, respectively. Marginally positives were counted as positive.

qRT-PCR analysis

All samples (pooled and individual test tubes) were delivered to the MyHeritage lab, Israel, and assayed on the same day using the commercial BGI Real-Time Fluorescent RT-PCR kit for detecting SARS-CoV-2. PCR-positive pools were transferred to the Central Virology Laboratory (CVL) of the Ministry of Health at the Sheba Medical Center and retested for confirmation using the Seegene Allplex 2019-nCoV commercial assay, yielding identical results. The details of the qRT-PCR analysis are presented in the Supplementary Methods.

Statistics

Sensitivity and specificity of the pooled tests, relative to the individual tests, are presented with 95% lower confidence bounds. False positive and false negative rates are presented with 95% upper confidence bounds. All confidence bounds are exact and computed by the Clopper-Pearson method.

Two methods were used to assess a possible relationship between pool size and the effectiveness of the pooled tests. First, when positive results were obtained both from individuals and from the pool, Pearson correlation was computed relating pool size to the difference between the pooled Ct and the summary of the pool's individual Ct values, compensated for the four-fold dilution of the pooled samples compared to the individual sample (see details in the Supplementary Methods). Second, logistic regression was used to assess whether the tendency for false positive results and false negative results at the pool level were related to pool size.

Results

General features of the swab-pools

For all Ct values of the tests that were positive or marginally positive in the swab-pool, the individual test or both are listed in the supplementary Excel Table. The swab-pools had Ct values of 14.8 to 42.2, and the individual tests Ct 18.7 to 40.0. Typically, Ct $\geq$ 35.5 were marked marginally positive by the testing laboratory and considered positive for the statistical analysis (see Supplementary Data for the details of qRT-PCR analysis and the Discussion section on the potential significance of marginally positive results).

The pools ranged in size from 1 to 37 pooled swabs, with a mean of 20.3 ± 7.7 (mean ± SD) and median of 22 ± 5 (median ± MAD). The most common pool size was 25 swabs/pool (211 pools), reflecting the size of common bubbles (school classes). For pools found positive by both pooled and individual testing, we observed no correlation between pool size and its performance ($r = 0.004$ between pool size and Ct value (pool summary)).

Sensitivity and specificity of swab-pooling

All individual test results were considered the reference to which the swab-pool qRT-PCR test results were compared. The test results of all 1273 pools compared to the individual test results of all 25 831 participants are summarized in Table 2. Of the 1273 pools, 1221 were fully concordant (95.9%), including 1163 negative results, 45 positive results, and 13 marginally positive results (Table 2). When considering all samples, based on the individual test results,

1193 swab pools should have yielded negative results, when in fact 1163 swab-pools were negative, implying a specificity of 97.5% (lower bound 96.6%). Eighty swab-pools should have been positive, when in fact 69 swab-pools were positive, indicating a sensitivity of 86.3% (lower bound 78.2%) (Table 3). We found no association between pool size and false positive pool results ($p = 0.75$, logistic regression). However, false negative results, although rare, were more likely as pool size increased (OR 1.27, 95% CI 1.12–1.44, $p < 0.001$, logistic regression), illustrated in Fig. 2(A), which shows a plot of the estimated sensitivity as a function of pool size. Because false positive results increased with pool size, and because the most common pool contained 25 swabs/pool, we conducted a post hoc analysis for pools with ≤25 swabs (Table S1). In that analysis, 56 pools with ≤25 swabs should have been positive, when in fact 53 were positive, implying a sensitivity of 94.6% (lower bound 86.7%; Table S1). Of 940 pools that should have yielded negative results based on the individual tests, 914 pools were negative, implying a specificity of 97.2% (lower bound 96.2%; Table S1).

Comparison between Ct values of swab-pools and individual test results

To further estimate the ability of a swab-pool test to identify a single positive individual and to compare sensitivity, we compared the Ct values for the 69 bubbles in which both the swab-pool and the individual Ct results were positive (including marginally positive pools). The pool Ct was compared to the expected Ct value from the pool (Fig. 2(B)). The pool results showed lower Ct values than corresponding individual tests, with a difference of 2.0 ± 3.9 (mean ± SD) and a 95% confidence interval of 1.1–3.0. Surprisingly, the observed difference was in favour of the pool tests and significantly lower than the expected value of 0 (p value < 0.001). This suggests a biochemical advantage to the pool test in lysis buffer over the individual tests in VTM. The mean difference was 2.4 ± 4.1 in the 24 pools with multiple positive individuals, slightly larger than the mean difference of 1.8 ± 3.8 in the 45 pools with a single positive individual. The difference is within the bounds of statistical uncertainty (95% CI for the difference, −1.4 to 2.6). In all 24 cases where more than one positive individual was part of the pool, the pool tested positive.

Discussion

Having performed both individual testing (one swab) and swab-pool testing (another swab), we observed, after adjusting for the dilution factor, a sensitivity difference between pools and individual tests of about two Ct values in favour of the pool tests. This might be explained by the different media that we used for individual tests (VTM) and pool tests (lysis buffer with RNA preservative), as previous work has showed an advantage in sensitivity of lysis buffer over VTM [15]. This higher sensitivity of the pool may result from the accumulation effect of viral RNA from several positive samples in the pool, each having a viral load that was below detection level when tested individually, but rendering the pool positive.

The specificity of swab-pooling based on all pools was high (97.5%) and similar to the specificity calculated post hoc for pools with ≤25 swabs (97.2%), with low false negative rates of 0.94% and 0.33%, respectively. In contrast, the sensitivity was significantly affected, with pools with ≤25 swabs exhibiting a post hoc calculated high sensitivity of 94.6% (Table S1). The lower sensitivity (86.3%) found when all pools were included in the analysis (≤37 swabs/pool) might have resulted from an overload in the test tube, which caused a decrease in the effectiveness of viral extraction from the swabs. The false positive rate was 30.3% for all pools with a post hoc calculated rate of 32.9% for pools with ≤25 swabs. The true false positive rate is probably much lower, because the 30 positive pools that did not have a positive individual test all had very high Ct values > 35 (26 of these pools had Ct values ≥ 36), suggesting that most if not all of these cases were false negatives of the individual tests due to the low viral titre and variation in sampling and not false positive pools. In any case, because a positive pool test mandates individual testing, the false positive result is less of a problem. Taken together, pools of 25 swabs or less are generally recommended.

Marginally positive results are analytically positive because they detect viral RNA. Typically, in the initial stage of COVID-19 the viral load rapidly increases after the first positive PCR test, whereas it slowly diminishes in the very late stages of the disease [24]. Therefore, when Ct values of a test are high, retesting a day or two later can indicate whether the tested individual is in the initial phase of infection (Ct will decrease upon retesting) or towards the end of infection (Ct will stay unchanged, increase, or RNA will be undetected). Such an approach can help in deciding whether to send an individual to isolation and for how long.

Compared to sample pooling, our results demonstrate that swab-pooling does not result in loss of sensitivity, whereas pooling several liquid samples together typically results in a loss in sensitivity of 2 to 3 Ct compared to individual testing [11,12,25]. Importantly, the implementation of sample pooling in PCR testing laboratories requires significant modification of standard operating procedures, notably the stage of preparing the pools, using a dedicated robotic platform, whereas swab-pooling is much simpler

Table 2
Comparison of swab-pool to individual test results[a]

	Swab-pool test results		
	Positive	Marginally positive[b]	Negative
Individual test results[c]			
Positive	45	6	4
Marginally positive[b]	5	13	7
Negative	3	27	1163

[a] Summary of the qRT-PCR results of 1273 swab pools compared to the results of individual testing of subjects in these pools. A cohort of 25 389 subjects was tested.
[b] Marginally positive test results typically indicate Ct ≥ 35.5.
[c] One or more individual positive result of samples that were pooled are counted as one occurrence.

Table 3
Sensitivity and specificity of swab-pool test results[a]

	Swab-pool test results	
	All pool sizes (≤37 swabs/pool)	
	Positive	Negative
Individual test results[b]		
Positive	69	11
Negative	30	1163
	Sensitivity[c] = 86.3%	
	Lower boundary[d] = 78.2%	
	False negative rate = 0.94%	
	Specificity[c] = 97.5%	
	Lower boundary[d] = 96.6%	
	False positive rate = 30.3%	

[a] Results for the entire study. When post hoc analysis is performed for pools ≤25 swabs/pool, sensitivity increases to 94.6% (lower bound 86.7%), while specificity remains essentially the same (see Table S1).
[b] One or more individual positive or marginally positive result of samples that were pooled are counted as one occurrence.
[c] Relative to the individual test results, which were taken as the reference.
[d] 95% Confidence lower bound.

and requires minimal adjustments in procedures. It does require adaptation during swab collection and appropriate barcoding for pool and individual samples, as well as corresponding data collection and transfer. This simplicity is a big advantage, which greatly facilitates implementation of this method.

The efficiency of swab-pooling, like any pooling methodology, depends on the prevalence of COVID-19 [26–28]. Table S2 lists the recommended numbers of swabs/pool under various prevalence values. When prevalence is 0.02% or lower, pooling of up to 25 swabs/test tube of 10 mL can be used, enabling up to over 20-fold increased sampling efficiency. The efficiency strongly drops when prevalence is 2%, but it still provides 3.65-fold better efficiency than individual testing. For a prevalence of 0.10% to 0.25%, 16 to 25 swabs can be pooled, and for 0.50% to 2.0% pools of 8 to 16 swabs are recommended (Table S2).

Swab-pooling can be carried out in several configurations. In simple swab-pooling, a single swab is taken from each bubble member, and the swabs are pooled in a single test tube that is delivered for testing. Individual testing of the members of a positive bubble is done while they are in isolation and should preferably include all household members.

Fast swab-pooling involves sampling each member of the bubble twice: One swab is used for the pooling test tube and the other for an individual test tube. If a pool tests negative, all individual test tubes are discarded. If it tests positive, the corresponding individual test tubes are assayed to identify the infected person. It allows rapid detection of the positive individual, facilitating contact tracing and isolation of the appropriate individuals. This method is currently applied in Israel in most of the routine swab-pooling protocols. Other configurations such as double swab-pooling (a swab pool of all bubble members and a smaller sub-bubble of that pool) are possible as well.

A limitation of the study is that no cost–benefit analysis was performed and no modelling was performed to show the outcome in Israel if the strategy had not been adopted. In addition, no experimental comparison to sample pooling was performed. Of course, the

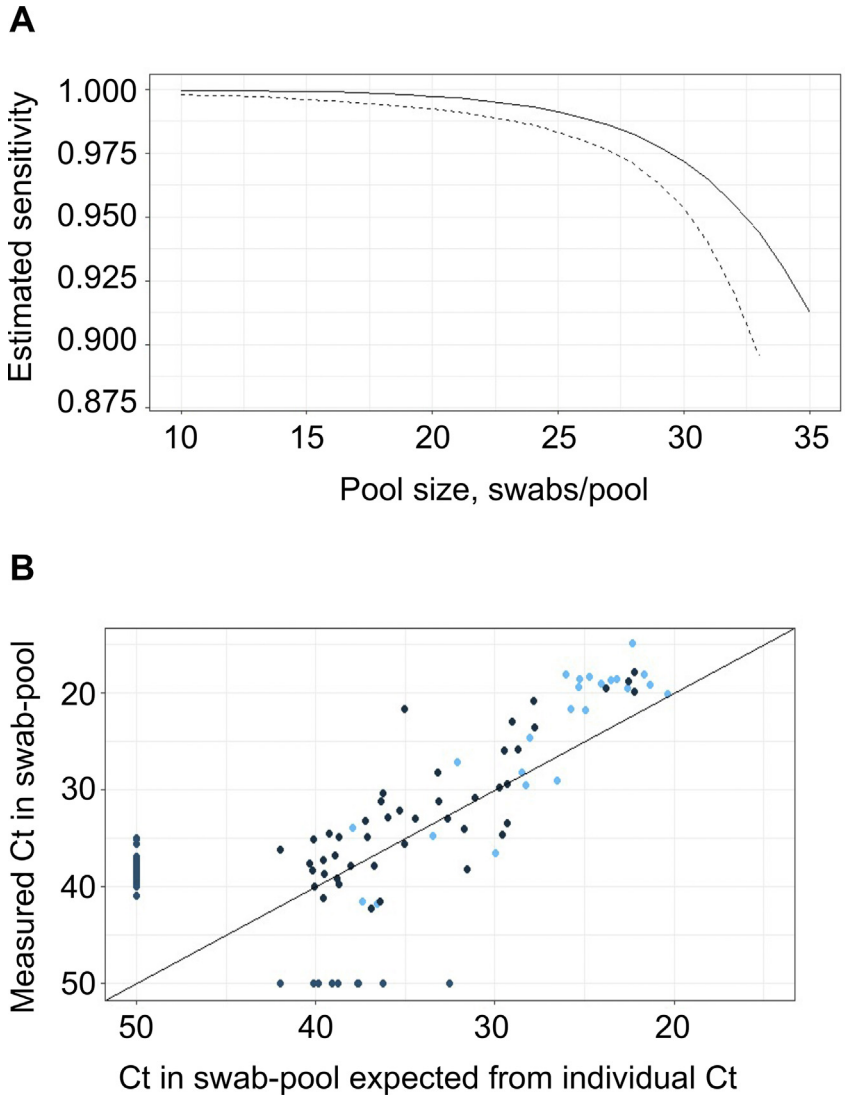

Fig. 2. Association of pool size to estimated sensitivity and comparison of Ct values of pool versus expected values from individual Ct. (A) Sensitivity as a function of pool size, as estimated by the logistic regression (solid line), with pointwise 95% lower confidence bounds for sensitivity (dashed line). (B) Comparison of the pooled Ct value to the expected Ct for the pool, computed from the Ct results for the individuals in the pool and adjusting for the extra dilution in the pooled sample. The 69 pools for which the pool gave a positive numerical result and positive individuals were identified are plotted along the line Y = X (black dots). The 11 pools that were negative but included positive individuals are represented by blue dots parallel to the X axis (i.e. a false negative pool). The 30 pools that were positive but included no positive individuals appear as blue dots parallel to the Y axis (i.e. a false positive pool).

success of swab-pooling relies on the quality of sample collection, the logistics of transportation conditions, and testing.

To conclude, the results presented here suggest that bubble-based swab-pooling provides a dramatic scale-up in sampling and testing throughput, about an order of magnitude of savings in testing cost, while minimally affecting test accuracy and the standard PCR testing laboratory routine workflow. It enables frequent testing and can be effectively used in school classes, airplanes, hospitals, and military units as well as workplaces that are naturally arranged or can be organized in bubbles. The bubble-based swab-pooling approach was adopted by the Israel Ministry of Health and by Ministry of Education and implemented in the Education Shield program for schools, the Mothers and Fathers Shield program for populations at risk in welfare institutions, and for passengers of international flights. By mid-August 2021, a total of approximately 1.5 million samples had been collected across the country, helping to control transmission rates.

Transparency declaration

There is no conflict of interest to declare. Funding was provided by the Ministry of Health and in part by the Ministry of Defense.

Author contribution

YC, NB, and OM equally contributed to the study. YC, SF, YK, IV, DMS, DZ, OM, and ZL conceptualized and designed the study. NB, RW, AY, DOL, OE, OM collected the samples, performed the tests, and organized the data. DSM and DZ did the statistical analysis. YC, OM, EM, and ZL supervised the study. ZL, OM, DMS, and DZ wrote the manuscript. All co-authors commented on the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.02.016.

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