Should pooled sample testing using RT-PCR be used in screening patients suspected to have COVID-19?

Authors: Tumanan-Mendoza, Bernadette (bernadette.tumanan@gmail.com); Genuino, Rowena (rfgenuino@post.upm.edu.ph), Bermudez-delos Santos, April Ann (abdelossantosmd@gmail.com)

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KEY FINDINGS

Based on the current data, pooling of samples for the detection of SARS-CoV-2 using RT-PCR may warrant consideration especially among communities with low prevalence of Covid-19.

- Individual testing using RT-PCR is the recommended method for the laboratory confirmation of COVID-19 infection (presence of SARS-CoV-2). However, due to shortage of reagents and testing capacity, pooled sample testing is being studied as a screening test to track early community transmission and identify which group of patients will need to proceed to individual testing for confirmation of positive results.[1-2]
- In 2011, pooling of samples for the detection of influenza virus using RT-PCR was shown to be feasible and could be useful in populations with low influenza prevalence.[3]
- Mathematical models have demonstrated that pooling of samples could be done for detection of SARS-CoV-2 using RT-PCR.[4-9]
- Seven studies were conducted to determine the feasibility and efficiency of sample pooling for detection of SARS-CoV-2.[10-16] Due to different pooling techniques and sampling, they reported different diagnostic test results: 100% sensitivity [10], 90% sensitivity [11], 67% positive predictive value [12], 100% sensitivity if the prevalence is 1.3% [13], 100% sensitivity and specificity [14], 60-70% sensitivity [15], and 86.7 - 100% sensitivity (depending on the viral load) with no difference in the PCR cycle threshold between the pooled and individual specimens.[16]
- The number of samples per pool varied from laboratory to laboratory and pooling is deemed useful if the prevalence of COVID-19 in the sample is low.[10-16]

RESULTS

As of May 16, 2020, seven cross-sectional studies on pooling of samples to increase throughput in the detection of SARS-CoV-2 using RT-PCR are available.[10-16] The study by Abdalhamid et al first did a proof-of-concept or preliminary study where known samples were used for testing. A web-based application was used to determine the most efficient pool size. A pool size of 5 containing 1 known positive and 4 known negative samples was used in 25 pools (from 125 individual samples). Despite the dilution, all the 25 positive samples were detected (100% sensitivity, 95% CI 86.3, 100). The pooling technique was re-tested among 60 unknown samples. It detected two positive pools which were confirmed to be true positives on individual testing (100% sensitivity). In pooling, individual testing is not done for negative test results, hence in this study, resources for 38 RT-PCR tests for SARS-CoV-2 were saved. The study also showed the different optimum pool size and expected testing efficiency with different prevalence rate. It concluded that pooling will result to increase testing efficiency by at least 69% if the incidence of SARS-CoV-2 is less than 10%.[10]

Yelin et al also did a proof-of-concept study. They prepared different samples of different dilutions, whereby a known positive sample was combined with 1, 3, 7, 15, 31 and 63 known negative samples. They showed that a pool containing 32 samples (1 positive and 31 negatives) had a sensitivity of 90% (95% CI 55.5, 99.8) and 10% false negative rate.[11]

A brief report on pooling by Hogan et al did not present a proof-of-concept study before testing for unknown samples. Two hundred ninety-two pools (9-10 samples/pool) of 2,740 nasopharyngel and 148 bronchoalveolar lavage samples which were previously collected were screened for the presence of SARS-CoV-2. The authors reported positive results in two pools which were confirmed true positives through individual testing. On the other hand, one pool which tested positive turned out to be negative (false positive). Only the positive predictive value (67% 95% CI 22,93.4) can be calculated, since the true results of the 289 negative pools cannot be ascertained.[12]

Another proof-of-concept study was done by Shental et al. They developed “Pooling-Based Efficient SARS-CoV-2 Testing (P-BEST), a method whereby one sample is part of several pools. Left-over samples taken from naso and oropharyngeal swabs from 384 patients with known PCR-based tests results were used to create 48 pools containing 48 samples (one patient’s sample was used to make six replicates). Through P-BEST logarithmic approach, several sets of 384 samples which contained 2-5 positive samples were tested. Results showed that the sensitivity and specificity were both 100% in those experiments that contained 2–4 positive samples among 384 samples. Simulations were still able to show 100% sensitivity (95% CI 47.8, 100) if there were 5 positives (5/384, 1.34% prevalence), with “average number of false positives” less than 2.75 and “average number of false negatives” less than 0.33. Pooling increased efficiency by 8-fold.[13]

Ben-Ami et al did both proof-of-concept study and testing for unknowns. Through the Dorfman pooling method, 183 known samples were pooled into 23 pools containing 8 samples per pool. The results showed that all true positives and true negatives were all detected. Testing for “indeterminates” was also done by placing one “indeterminate” in five pools. One of these five pools had a negative result which suggested a small decrease in sensitivity. After having validated the Dorfman pooling, 2,168 unknown samples from asymptomatic healthcare workers and personnel of essential industries were tested. Using pools containing eight samples/pool, five positive samples were identified and individually validated (prevalence of 0.23%). Through this pooling technique, only 311 testing kits were used which represented 14% of the total kits had they used individual testing.[14]

A brief report by Torres et al on their proof-of concept study showed low sensitivity compared to the other studies. They used 20 mini-pools containing 5 or 10 samples from left-over nasopharyngeal specimens (30 known negative and 10 known positive samples). The sensitivity ranged from 60-70%.[15]

A study by Wacharapluesadee et al used archived specimens from nasopharyngeal and throat swabs. Fifty negative samples were combined into a single negative specimen. 0.1 ml (1X) or 0.2 ml (2X) from positive specimens of varying viral concentrations were combined with either 0.9 ml or 0.8 ml negative sample, respectively to create 1.0 ml of pooled samples. This represents either one positive sample added to nine negative samples or two positive samples with eight negative samples. Forty-nine positive samples were used to make 49 pools of 5 different combinations of 1X and 2X. Thirty-one pools had 1X while 18 had 2X. In the 1X ratio, weakly positive, low, and high viral concentrations were seen in 15, 12 and 4 pools, respectively. Samples with weakly positive results were re-tested individually (results of some of these re-tests showed negative results). In the 18 pools containing 2X (2 positive samples), 5 pools had two low viral concentrations, another 5 had two high viral concentrations and 8 pools had 1 high and 1 low viral concentration. For the 1X pools, all those with high and low viral concentrations, showed positive results (100% sensitivity). In addition, the PCR cycle threshold of the pooled and individual specimens did not show significant difference. The same results (100% sensitivity and no significant difference in PCR cycle threshold) were seen in all the 2X pools. In the 15 1X pools with weakly positive viral concentrations, 2 were found to be false negative. Savings in the cost of tests (25–89% reduction in cost) through pooling of specimens for 4 prevalence rates, 0.1–10% were reported. Pooling is shown to be more efficient in population with low prevalence of Covid-19.[16]

The other details of the seven studies are given in the table on the characteristics of included studies (Appendix).

Pooling samples is a complex procedure that may vary from laboratory to laboratory. The number of samples per pool must be ascertained in order to detect a positive result despite the dilution process. Moreover, the usefulness of pooling is affected by prevalence of the disease. Lastly, approval from the local regulatory bodies is required.

CONCLUSION

Based on the current data, pooling of samples for the detection of SARS-CoV-2 using RT-PCR may warrant consideration especially among communities with low prevalence of Covid-19.

Declaration of Conflict of Interest

No conflict of interest

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Table 1. Characteristics of included studies

No.	Title/Author	Test kit	Definition of a positive test	Study design	Country/Setting/ Prevalence of COVID-19	Inclusion criteria	Population	Intervention Group(s)	Comparison Group(s)	Outcomes	Key findings
1	Abdalhamid B, Bilder CR, McCutchen EL, Hinrichs SH, Koepsell SA, Iwen PC. Assessment of specimen pooling to conserve SARS CoV-2 testing resources. Am J Clin Patho; (April 18). doi:10.1093/ajcp/aqaa064.	CDC (2019-nCoV) Real-Time RT-PCR Diagnostic Panel kit (CDC, Atlanta, GA). RNA extraction QIAGEN EZ1 Virus Mini Kit v2.0 (QIAGEN, Germantown, MD) or the QIAGEN manual extraction kit	When both nucleocapsid targets (N1 and N2) reached a defined threshold prior to an amplification cycle of 40.	Cross-sectional study	USA (Nebraska) Public health laboratory	COVID-19 positive specimens with a range of cycle threshold (Ct) values from 18.23 to 37.96 for N1 and from 17.33 to 38.65 for N2	Phase 1 (Known samples) Nasopharyngeal specimens from the community (Nebraska state); Positive test within a range of -1.1 Ct to 5.09 Ct	Phase 1: Pooled sample (1:4) (n=25)	Phase 1: Individual samples (n=105): Positive =84 Negative=21	Positive or negative test; Number of tests saved	Group testing may result in the saving of reagents and personnel time with an overall increase in testing capability of at least 69% when the positive laboratory test rate is 10% or less.
							Phase 2 (Unknown community samples) 60 specimens from individuals at risk for COVID-19 as determined by the public health department	Pooled samples (1:4 ratio) (n=12)	Individual samples (n=10)		
2	Yelin I, Aharony N, Tamar ES, et al. Evaluation of COVID-19 RT-qPCR test in multi-sample pools.	AgPath-IDTM One-Step RT-PCR Reagents (Thermo Fisher Scientific)		cross-sectional	Israel		Patients with suspected COVID-19 infection	Pooled samples (n=10) mixed as 1 pool of mixed negative	Individual samples (Positive samples n=5 Negative samples n=67)	Detection of SARS-CoV-2 RNA using RT-qPCR Bio-Rad CFX 96 qPCR machine with WHO primers and probe	Pooled sampling, of up to 32 samples, has a sensitivity of 90% and specificity of 100%.

	https://doi.org/10.1101/2020.03.26.20039438							samples (as control) 9 pools of mixed positive samples		(E_Sarbeco_R: ATATTGCAGCAGTACGCACACA, E_Sarbeco_F: ACAGGTACGTTAATAGTTAATAGCGT, E_Sarbeco_P: ACACTAGCCATCCTTACTGCGCTTCG)	PPV of 100% and NPV of 50%.
3	Hogan CA, Sahoo MK, Pinsky BA. Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2. JAMA. 2020;E1-E2.	did not mention name of kit used but pointed to a reference where kits made in Germany were used	screening was performed using reverse transcriptase–polymerase chain reaction targeting the envelope (E) gene.3 Positive pools were deconvoluted and individual samples tested for both E and theRNA-dependentRNA polymerase (RdRp) gene for confirmation	cross-sectional study	San Francisco Bay Area, California, U.S.A.	retrospective study that used previously collected nasopharyngeal and bronchoalveolar lavage samples from Jan 1, 2020 to February 26, 2020 for routine respiratory virus testing in Stanford Health Care Clinical Virology Laboratory. By Feb 26, 2020, they started testing these samples for SARS-CoV 2.	did not state inclusion criteria but just included all samples submitted for routine respiratory virus as mentioned in previous column	Nine or 10 individual samples were pooled, screened 292 pools - containing 2740 nasopharyngeal samples and 148 bronchoalveolar lavage samples	Detected 2 true positive pools and 1 false positive pool The results of these pools were confirmed by individual testing. The 2 positive samples showed detection Of E and RdRp. Sanger sequencing revealed100 % identity with the SARS-CoV-2 E gene. Only 1 pool showed a positive E signal that was not reproducible with testing of the individual samples of that pool.	Positive or negative results	Pooled testing containing 9-10 samples showed 2 true positive and 1 false negative result.
4	Shental N, Levy S, Wuvshet V, et al. Efficient	Clinical Diagnostic laboratory of the		Cross-sectional study	University of Soroka Medical Center, Israel	included left-over samples that were previously	384 known samples	Used P-BEST Algorithmic approach where	Results of the pools were compared to	Positive or negative test;	Pooled testing had 100%

	high throughput SARS-CoV-2 testing to detect asymptomatic carriers. *MedRxiv Prepr.* 2020; (April 20). doi:https://doi.org/10.110/2020.04.14.20064618.	University of Soroka Medical Center using a clinically approved COVID-19 PCR-based diagnostic protocol that included an RNA extraction stage. This laboratory uses the clinically approved SARS-CoV-2 detection kits of SeeGene (California, USA)				clinically tested for COVID-19		384 samples were used to create 48 pools containing 48 samples (one sample was used to create 6 replicates).	the known results of the samples (4 of the 384 subjects had positive results). Four experiments were conducted using 2, 3, 4 or 5 positive samples in the pools.	Number of tests saved	sensitivity if the prevalence was 1.3% or less. Efficiency increased by 8-fold.
5	Ben-Ami R, Klochendler A., Seidel M, et al. Pooled RNA extraction and PCR assay for efficient SARS-CoV-2 detection. MedRxiv pre-print 2020; (April 22) https://doi.org/10.1101/2020.04.17.20069062.	For matrix pool design: MagNA Pure 96 kit (Roche Lifesciences) using Roche platform For 1:8 pool design: QIAsymphony DSP Virus/Pathogen kit on Qiasymphony platform Real-Time Fluorescent RT-PCR kit (BGI)	Positive: if the viral genome is detected at threshold cycle (Ct) values ≤35	Cross-sectional study	Hadassah Medical Center, Israel	Not mentioned, but included nasopharyngeal swab specimens of routinely tested screened asymptomatic healthcare personnel and employees of essential industries	<u>Phase 1:</u> Used samples from symptomatic patients from hospital and from community Testing of pooling methods (to confirming which one the center will use for overall pooling of samples) Method 1: Simple Dorfman pooling 184 consecutive samples divided into 23 pools of 8 samples each Method 2: Matrix pooling "where n2 samples are ordered in an n x n matrix. Each	<u>Phase 1:</u> Dorfman: 184 samples divided in 23 pools of 8 samples each Matrix: 75 lysates, 3 matrices (5x5), 30 pools Per pool is 25 lysates with 1 lysate as known positive <u>Phase 2:</u> 2168 samples divided into 3 batches Batch 1 & 2 720 samples, 90 pools, 8 samples per pool Batch 3 728 samples, 91 pools, 8 samples per pool	<u>Phase 1:</u> Individual testing done in pools with positive result Matrix: Individual testing done in pools with +ve result <u>Phase 2:</u> Individual testing done on pools with positive samples	<u>Phase 1:</u> Positive Indeterminate negative <u>For phase 2:</u> Indeterminates were retested with a different kit to confirm if positive or negative Criteria: Positive: <u><</u>35 Indeterminate: >35 to <38 Negative: ~38 and above	Demonstrated two simple pooling methods that can increase testing capacity to 5 to 7.5 fold in populations with low infection rate Pooled testing using Dorfman method (for this study: 1 pool, 8 samples) have a 100% sensitivity and 99.6% specificity, with a PPV of 80.0 and NPV of 100.

							row and each column are pooled" Pooled 75 samples into 5x5 matrices (30 pools) Phase 2: Used the Dorfman method for 2168 samples from routinely tested asymptomatic healthcare workers and essential industry workers				
6	Torres I, Albert E, Navarro D. Pooling of Nasopharyngeal Swab Specimens for SARS-CoV-2 Detection by RT-PCR. J. Med Virol 2020 May 5. doi:10.1002/jmv.25971.	RT-PCR (REALQUALITY RQ-2019-nCoV from AB ANALITICA; Padua, Italy, performed on the Applied Biosystems 7500 instrument) RT-PCR assay targets the E (envelope) and RdRp (RNA dependent RNA polymerase) genes of SARS Cov-2 in a single reaction with LODs of 125 and 150 copies/ml, respectively (according to the manufacturer)	Cycle threshold values (CT) ranging from 23.4 to 38.8 for the E gene, and 21.8 to 35.8 for the RdRP gene	Cross-sectional study	Valencia, Spain	10 RT-PCR positive NP specimens yielding cycle threshold values (CT) ranging from 23.4 to 38.8 for the E gene, and 21.8 to 35.8 for the RdRP gene	Left-over specimens (both negative and positive)	Negative: A total of 30 leftover specimens testing negative for SARS CoV-2 Positive: 10 RT-PCR positive NP Specimens 20 mini-pools with pooling ratio of 1:5 or 1:10	Results of the pools were compared to known results of the samples (Proof of concept study on preexisting lab samples with known results on RT-PCR testing)	Positive detection Negative detection	Positive specimens yielding CT <32 for the E gene (6 out of 10) or <35.2 for the RdRP gene (7 out of 10) were detected in mini-pools of both sizes. In contrast, most NP samples displaying CTs > 35.8 for the E gene or 35.7 for the RdRP gene remained undetected in mini-pools of 5 specimens (3/4 and 2/3, respectively) or in mini-pools of

		RNA extraction was performed using the DSP virus Pathogen Minikit on the QiaSymphony Robot instrument (Qiagen, Valencia, CA, USA)									10 samples (4/4 and 3/3, respectively.
7	Wacharapluesadee S, et al. Evaluating efficiency of pooling specimens for PCR-based detection of COVID-19. J Med Virol. 13 May 2020; https://doi.org/10.1002/jmv.26005	Real-time PCR (qPCR) for detection of SARS-CoV-2 was performed using a commercial kit which targets the ORF1ab gene as per the manufacturer's protocol (BGI, Shenzhen, China).	The protocol's stated limit of detection of ORF1ab real-time PCR was 100 copies/mL and the cutoff PCR cycle threshold (Ct) was 38.	Cross-sectional	Thailand between February 1, and March 31, 2020. A	NT specimens used in this study had been collected from patients under investigation (PUI) for COVID-19 infection at King Chulalongkorn Memorial Hospital, specimens with Ct values between 26 – 35 were considered to have low concentrations of viral RNA, while those with Ct values lower than 26 were considered to have of high-concentrations viral RNA. Ct values higher than 35 were considered weakly positive. As	50 SARS-CoV-2 negative NT specimens in VTM from routine diagnoses (1.0 mL each), as determined by real-time PCR (BGI, Shenzhen, China), were pooled, and this pooled negative NT-VTM served as the negative portion of all samples tested	49 pooled samples (5 pooling ratios)	Individual samples 49 PCR positive NT specimens (Ct ranging from 12.91 to 37.10 50 negative	Positive or Negative results and savings in costs depending on the prevalence of the disease.	Sensitivity was 86.7 – 100% (depending on viral load) was seen in pooled testing (containing 10 samples/po. No significant difference was seen in the PCR cycle threshold of the pooled specimens as compared to individual specimens. Cost in savings was reported for 4 prevalence rates (0.1-10%) and efficiency was shown to be high if pooling is used in population with low prevalence of Covid-19.

						per the laboratory's protocol, samples that test weakly positive are re-tested for confirmation.					