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

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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.
- 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.
- Mathematical models have demonstrated that pooling of samples could be done for detection of SARS-CoV-2 using RT-PCR.
- 12 studies with different pooling techniques were included. Diagnostic test results ranged from 60%-100% sensitivity, 100% specificity and 67% positive predictive value. However, one study reported a high false negative rate. These results were affected by differences in viral loads and pooling ratios utilized in the study.
- 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. However, the possibility of false negative must be taken into consideration if the viral load of the positive sample is low.

RESULTS

As of July 3, 2020, 12 cross-sectional studies on pooling of samples to increase throughput in the detection of SARS-CoV-2 using RT-PCR are available.[1-12] 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%.[1]

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.[2]

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 nasopharyngeal 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.[3]

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.[4]

Ben-Ami et al did both proof-of-concept study and testing for unknowns. Through the Dorfman pooling method, 184 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 validating the 8-sample Dorfman pooling, unknown samples from asymptomatic healthcare workers, personnel of essential industries, and residents and employees of nursing homes were tested using this pooling process. In the first three batches, 2,168 samples were tested, and five positive samples were identified and individually validated (prevalence of 0.23%). After this, implementation of pooling for screening of asymptomatic population followed. Among a total 26,576 samples tested, 31 positive tests were found (0.12% prevalence). The pooling process resulted to a “7.3-fold increase in throughput”.[5]

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%.[6]

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 (1X) added to nine negative samples or two positive samples (2X) 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.[7]

Hirotsu and co-authors used synthetic DNA and nucleic acids derived from SARS-Cov-2 positive and negative patients. They also tested nasopharyngeal swabs from 1,000 individuals – 195 healthworkers, 472 hospitalized patients for non-COVID-19 disorders and 333 patients suspected of COVID-19. Using the assay from the National Institute of Infectious Diseases, Japan, they tested the N1 and N2 sites of the N gene of SARS-CoV-2. They used three representative nucleic acid extracts from COVID-19 patients. These contained high, intermediate and low viral loads. Samples from SARS-CoV-2 positive patients were pooled with negative samples from healthy individuals in ratios of 1:4, 1:9 and 1:19. The N1 site was detected in samples containing high and intermediate viral loads diluted with negative samples, but not with low viral load. The N2 site assay in contrast was detected in all – high, intermediate and low viral loads. Samples from 555 individuals not suspected of COVID-19 (healthcare workers and patients hospitalized for non-COVID-19 conditions) were tested in 93 pools (containing 5 – 10 samples per pool) which resulted in 46% savings in reagent. Results were all negative and no symptoms were observed during follow-up for 10-12 days of all hospitalized patients.[8]

Cabrera et al collected nasopharyngeal swabs from workers and residents in Care Homes. Initial assessment consisted of testing 26 pools containing 20 samples/P20 (one positive sample mixed with 19 negative samples and 14 sub-pools of 5 samples/SP5 (one positive sample mixed with four negative samples). All the positive samples were detected in all pools and sub-pools (100% sensitivity). Furthermore, proof of concept was done through two simulations that were retrospectively tested through an algorithm using P20 and SP5. Individual testing was done if positive in SP5. The first simulation involved 100 samples with 2% prevalence of SARS-CoV-2. Five P20 were tested and two of these pools had positive results. From these two positive P20, eight SP5 were prepared and analyzed. Two out of the five SP5 turned out positive, thus 10 individual samples were tested. This resulted to 77% reduction in the number of tests. The second simulation involved 60 samples with 1.7% prevalence. Three P20 were tested, one of which had positive result. Four SP5 were prepared and tested. One SP5 tested positive, thus five individual tests were carried out. One of these tests turned out positive. Pooling in this second simulation resulted to 80% savings in the number of tests.[9]

Mulu et al used pooled individual clinical samples and nucleic acid (RNA) preparations. Several pool sizes for both clinical samples and RNA preparations which contained high and low viral loads were tested.

Eventually, they recommended a pooling ratio of 4 biological samples in 1 pool and RNA pooling of 8 samples per pool.[10]

Arvind et al used “repeatedly tested” positive and negative sample elutes in different pooling ratios. These pools contain one positive sample mixed with negative samples which ranged from 1 – 47 to determine the optimal pool size. A pool size of 6 was found to have 97.8% sensitivity (95% CI 94.9, 99.3), 100% specificity, and negative predictive value of 97.2.[11]

Testing for RT-qPCR of pooled RNA samples was done by Gan et al. Two positive samples containing high viral load (positive results for three COVID-19 probe genes) and one containing low viral load (positive results in only two COVID-19 probe genes) were mixed with negative samples in different dilution ratios and were tested in triplicates. Both RNA pools with high viral loads showed 100% sensitivity (same results in triplicates) for the following dilution ratio of positive to negative samples: 1:1, 1:4, 1:9, and 1:19. On the other hand, for the dilution ratio 1:49, the sensitivity of these two RNA pools were 90 - 100%. However, the performance of the pool with low viral load was unsatisfactory (0 – 90% sensitivity) even at a dilution ratio of 1:1. In view of the high false negative rate of the positive sample with low viral load, Gan et al stated that the use of pooling for large-scale surveillance “requires careful consideration” and is dependent on the viral loads of the positive samples.[12]

In summary, 12 studies on pooling are reported in this review. Three studies were done in Israel and two each for United States and Spain, while one study was conducted in each of the following countries: Thailand, Japan, Ethiopia, India and China. All studies except the Hogan et al study did proof-of-concept studies. High sensitivity (90% - 100%) was reported in most studies except that of Torres et al who reported sensitivity of 60% - 70%. Gan et al reported high sensitivity (90% - 100%) in samples containing high viral loads but high false negative rates for a positive sample with low viral load. The largest study in this review was done by Ben-Ami et al, who after conducting a proof-of-concept study reported screening of 26,756 asymptomatic individuals through pooled samples.[1-12]

The other details of the 12 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). <u>RNA extraction</u> 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	<u>Phase 1</u> (Known samples) Nasopharyngeal specimens from the community (Nebraska state); Positive test within a range of -1.1 Ct to 5.09 Ct	<u>Phase 1:</u> Pooled sample (1:4) (n=25)	<u>Phase 1:</u> 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.
							<u>Phase 2</u> (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. https://doi.org/10.1101/2020.03.26.20039438	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 samples (as control) 9 pools of mixed positive samples	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 (E_Sarbeco_R: ATATTGCAGCAGTACGCACACA, E_Sarbeco_F: ACAGGTACGTTAATAGTTAATAGCGT, E_Sarbeco_P: ACACTAGCCATCCTTACTGCGCTTCG)	Pooled sampling, of up to 32 samples, has a sensitivity of 90% and specificity of 100%. 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	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,	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	Positive or negative results	Pooled testing containing 9-10 samples showed 2 true positive and 1 false negative result.

			for confirmation			2020, they started testing these samples for SARS-CoV 2.			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.		
4	Shental N, Levy S, Wuvshet V, et al. Efficient 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.	Clinical Diagnostic laboratory of the 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)		Cross-sectional study	University of Soroka Medical Center, Israel	included left-over samples that were previously clinically tested for COVID-19	384 known samples	Used P-BEST Algorithmic approach where 384 samples were used to create 48 pools containing 48 samples (one sample was used to create 6 replicates).	Results of the pools were compared to 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.	Positive or negative test; Number of tests saved	Pooled testing had 100% sensitivity if the prevalence was 1.3% or less. Efficiency increased by 8-fold.
5	Ben-Ami R, Klochendler A, Seidel M, Sido T, Gurel-Gurevich O, Yassour M, Meshorer E, Benedek G,	For matrix pool design: MagNA Pure 96 kit (Roche Lifesciences) using Roche platform	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	<u>Phase 1:</u> Used samples from symptomatic patients from hospital and	<u>Phase 1:</u> Dorfman: 184 samples divided in 23 pools of 8 samples each	<u>Phase 1:</u> Individual testing done in pools with positive result	<u>Phase 1:</u> Positive Indeterminate negative <u>For phase 2:</u>	Demonstrated two simple pooling methods that can increase testing capacity to 5 to 7.5 fold in

	Fogel I, Oiknine-Djian E, Gertler A, Rotstein Z, Lavi B, Dor Y, Wolf DG, Salton M, Drier Y, The Hebrew University-Hadassah COVID-19 diagnosis team, Large-scale implementation of pooled RNA extraction and RT-PCR for SARS-CoV-2 detection, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.06.009.	For 1:8 pool design: QIAsymphony DSP Virus/Pathogen kit on Qiasymphony platform Real-Time Fluorescent RT-PCR kit (BGI)				screened asymptomatic healthcare personnel and employees of essential industries	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 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	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	Matrix: Individual testing done in pools with +ve result <u>Phase 2:</u> Individual testing done on pools with positive samples	Indeterminates were retested with a different kit to confirm if positive or negative Criteria: Positive: ≤35 Indeterminate: >35 to <38 Negative: ~38 and above	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. Method implemented in routine clinical diagnosis setting of asymotomatic populations, testing a total of 26,576 samples. Throughput was increased 7.3 fold, identifying a total of 31 positives (0.12%)

							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) RNA extraction was performed using the DSP virus Pathogen Minikit on the QiaSymphony	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 10 samples (4/4 and 3/3, respectively.

		Robot instrument (Qiagen, Valencia, CA, USA)									
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 per the laboratory's protocol, samples that test weakly	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.

						positive are re-tested for confirmation.					
8	Hirotsu Y, Maejima M, Shibusawa M, et al. Pooling RT-PCR test of SARS-CoV-2 for large cohort of “healthy” and infection-suspected patients: A prospective and consecutive study on 1,000 individuals ORCID: 0000-0002-8002-834X (Yosuke Hirotsu) No DOI information 06 May 2020	MagMax Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher Scientific, Waltham, MA one-step real-time quantitative RT-PCR according to the NIID protocol with minor modification (version 2.7) The primer/ probe set testes two sites (N1 and N2) of the *N* gene of SARS-CoV-2 RT-PCR assays were conducted on a StepOnePlus Real-Time PCR Systems (Thermo Fisher Scientific)	Threshold line 0.2	Cross-sectional study	Japan Yamanashi Central Hospital RT-qPCR showed the prevalence of COVID-19 was 3.6% (12/333) of infection-suspected patients and none in both healthcare workers and hospitalized patients in our distinct	Not mentioned, but included nasopharyngeal swab specimens of healthcare personnel and patients with confirmed positive SARS-CoV2 infection	1000 samples from 1000 individuals, mixed patients and hospital staff Pooling: 538 samples (445 individuals, 93 pools) examined how far SARS-CoV-2 could be detected when multiple samples were pooled	SARS-CoV-2 positive and negative samples were mixed in ratios of 1:4, 1:9, and 1:19 → pooled samples of 5-, 10- and 20-fold dilution were created Phase 1: serial dilution using plasmid control and SARS-CoV-2 negative samples Plasmid at 100 1000 10,000 100,000 copies Diluted at 1:9 N1 site detected at 10,000 to 1,000 copies of plasmid N2 site detected at 100 copies of plasmid Spike-in assay using SARS-CoV-2 positive and negative			

								health individuals Prepared at 1:4, 1:9, and 1:19 (5-, 10- and 20-fold dilutions)			
9	Cabrera JJ, Rey S, Perez S, et al. Pooling For SARS-CoV-2 Control in Care Institutions. *MedRxiv Prepr.* 2020 (June 2). doi: https://doi.org/10.1101/2020.05.30.20108597.	Results of samples individually tested by cobas® SARS-CoV-2 test were compared with results of samples tested in pools by the STARlet instrument (Microlab) with STARMag 96 x 4 Universal Cartridge Kit for automated extraction (200 µL of sample and added RNA IC) and the Allplex™2019-nCoV Assay PCR set-up.	Sensitivity was 31.25 copies/µl (6.75 copies/reaction) for cobas® SARS-CoV-2 test on the cobas® 6800 system, 125 copies/µl (6.67 copies/reaction) for Allplex™2019-nCoV assay after nucleic acid extraction with MagCore® HF16 Plus system and 250 copies/ml (4 copies/reaction) for Allplex™ 2019-nCoV assay after nucleic acid extraction with STARlet system (Hamilton (USA)).	Cross-sectional	Galicia, Spain 3.36% prevalence (852 positive out of 25,386 people from 306 Galician Care Homes: 16477 residents, 8,599 workers and 310 not specified)	Residents and workers from institutionalized homes	institutionalized people (CARE homes) Mean age of workers and residents was 44.25 years (min 18, max 69) and 80.07 years (min 3, max 109), respectively	Positive samples were selected between those originally tested by cobas® SARS-CoV-2.	Test performance of **26** P20 s and **14** SP5 was studied *Pools of 20 samples (P20) and sub pools of 5 samples (SP5)		
10	Mulu et al Evaluation of Sample Pooling for Screening of SARS CoV-2	Novel Coronavirus 2019-nCov PCR Kit-	Positive SARS CoV-2 result is determined	Proof-of-concept	Ethiopia an experimental prevalence	No info	No info	6 positive specimens	54 pools of 6 specimens, each containing one positive		

		fluorescent PCR method of Da An Gene Co., Ltd, China NA extraction and Purification Reagent, DAAN Gene Co., Ltd,	when both targets reach a defined Ct value of less than 40, along with defined Ct value of less than 32 and 40 for positive control and internal control, respectively.		rate of SARS CoV-2 in Ethiopia to be 0.05% (as observed positive rate within the tested individuals is reaching to 0.66% in the last 5 weeks),				sample were group tested. pooling in two arms (direct clinical samples arm and nucleic acid arm) and each reaction was done in triplicate. experimental pools were created using SARS CoV-2 positive clinical samples spiked with up to 9 negative samples prior to NA extraction step to have a final extraction volume of 200µL (maximum dilution factor of 10). Viral NA was also subsequently extracted from each pool and tested using the SARS CoV-2 RT-PCR assay		

11	Arvind K, Abhishek P, et al. Optimal size of sample pooling for RNA pool testing: an avant-garde for scaling up SARS CoV 2 testing medRxiv preprint doi: https://doi.org/10.1101/2020.06.11.20128793 version posted June 14 2020	Viral nucleic acid extraction using Qiasymphony DSP virus / pathogen mini kit (Qiagen GmBH, Germany) RT-qPCR: AgPath-IDTM One-step RT-PCR Reagents (Thermo Fisher) using an Applied Biosystem (ABI) 7500 Real Time PCR system (ThermoFisher Scientific) and LightMix SarbecoV E-gene (TIB MOLBIOL) If positive for E gene, confirmation for detection of specific RdRp gene of SARS-CoV-2: LightMix Modular SARS-CoV-2 RdRP (TIB MOLBIOL)		Cross-sectional study	Institute of Liver and Biliary Sciences, New Delhi, India	Nasopharyngeal and oropharyngeal swabs	Control: 8th dilution series of 11 pools using PCR grade water	48 negative sample elutes to make 8 series of 11 pools of increasing number of elutes. Each 11 pool was mixed with 1 positive elute with increasing dilution (1:2 up to 1:48) Overall, 88 pools: 77 with one positive sample elute For calculation of specificity: pooling a total of 11 pools of 2, 4, 6, 8, 10, 12, 16, 20, 24, 32 and 48 SARS CoV 2 E & RdRp gene negative samples elutes were tested All 11 pools were negative	11 without positive (Control)		
12	Gan Y, Du L, Faleti OD, Huang J, Xiao G, Lyu X. Sample Pooling as a Strategy of SARS-COV-2 Nucleic Acid	RNA extraction kit (Shanghai ZJ Bio-Tech), QIAamp Viral RNA Mini Kit (QIAGEN Biotech) in a	No info	Cross-sectional	Guangdong, P.R. China. 3 positive out of 8097 throat swap samples	Patients who came for routine medical examination or fever	Case 1 - had a fever with body temperature at 39.3 degrees Celsius (□), which was	Three positive samples; two with high viral load (Case 1 and Case 2) and one with low viral	RNA samples "pooling." Each positive RNA samples were mixed with COVID-19 negative RNA or		

	Screening Increases the False-negative Rate. *MedRxiv Prepr.* 2020 (June 28). doi: https://doi.org/10.1101/2020.05.18.20106138.	SLAN-96P qPCR machine (Sansure Biotech) using WHO primers and probe			March 4 to April 26, 2020		accompanied with headache and muscle soreness Case 2 had abdominal pain for two days with l diarrhoea (three to four times each day). The patient also had low-grade fever of 37.3 Case 3 had a fever for a week followed by coughing (with sputum) and sore throat. The body temperature was between 37.1□ and 38	load (Case 3), and 8094 negative samples were used for the study.	ddH2O to form RNA pools. Three positive samples; two with high viral load (Case 1 and Case 2) and one with low viral load (Case 3), and 8094 negative samples were used for the study. The positive RNA sample Case1 or Case 2 was mixed with equal volumes of 1, 4, 9, 19, 49, 99, 199, 499, 999 negative RNA samples in order to dilute into 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, 1:1000. Using double distilled water (ddH2O), other dilution ratio of 1:2000, 1:4000, 1:8000 were made. The third positive RNA sample, Case 3, was mixed with equal		

									volumes of 1, 4, 9, 19, 49, 99, 199 negative RNA samples to make 1/2, 1/5, 1/10, 1/20, 1/50, 1/100, 1/200 dilution. Each RNA "pool" was detected using RT-qPCR test as one RNA template.		