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Pooling of six respiratory samples for the detection of SARS-CoV-2: A validation and cost study in a cohort in Lima, Peru

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ABSTRACT

Background: The continuous evolution of the SARS-CoV-2 pandemic has led to a high demand for diagnostic testing and major shortages in testing materials, especially in low- and middle-income countries. As an alternative to testing individual samples, pooling of respiratory samples has been suggested. Previous studies have assessed performance of pooling, mainly using nasopharyngeal samples for the detection of SARS-CoV-2, but few studies have examined the performance of pooling the more practical nasal swabs or saliva samples.

Objective: To evaluate the sensitivity, specificity, and potential cost reduction of pooling of nasal swab (NS) and saliva (SL) samples for detection of SARS-CoV-2 in a community-based cohort study in Lima, Peru.

Study design: A prospective cohort study was conducted in a community setting in San Juan de Lurigancho, Lima-Peru. NS and SL samples were collected from 132 participants twice-a-week for a 2-month period. Pools of 2 to 12 samples of the same type, from participants of the same household, were tested by RT-PCR. After pooled testing, all individual samples from positive pools and all individual samples from randomly chosen negative pools were evaluated. For assessment of diagnostic performance, pool testing results were compared with results from individual testing, which served as reference, and concordance in pooled and individual test detections was evaluated. Laboratory costs for both types of samples and testing were compared.

Results: A total of 2008 NS and 2002 SL samples were collected from 132 study participants. We tested 329 NS and 333 SL pools. The mean pool size for NS and SL pools was 6.22 (SD = 0.92) and 6.39 (SD = 1.71), respectively. Using individual testing as reference, NS pooling of 6 had a sensitivity and specificity of 94% and 100%, respectively, with kappa of 0.97 (CI 95%: 0.93–1.00). The corresponding values for SL pooling of 6 were 83%, 100%, and 0.90 (CI 95%: 0.83–0.97). Compared with individual testing, pooling resulted in a cost reduction of 74.8% for NS and 72.4% for SL samples.

Conclusions: Pooling easy-to-collect respiratory samples, especially NS, demonstrated very high diagnostic performance for detection of SARS-CoV-2 with substantial cost savings. This approach could be considered in large population screening programs, especially in LMIC.

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1. Introduction

The COVID-19 pandemic has precipitated an unprecedented public health and financial crisis throughout the world, with the greatest negative impact in low- and middle-income countries (LMIC) [1]. With the recent increase of infections associated with the Omicron variants, the availability of COVID-19 testing became very limited in some settings, partly due to limited availability of reagents and supplies for the detection of SARS-CoV-2, resulting in major challenges for identification of infections and adoption of isolation and quarantine, especially in LMIC [2].

Individual testing of respiratory samples using reverse-transcription polymerase chain reaction (RT-PCR) has been the gold standard for diagnosing COVID-19 since it was authorized for its use in February 2020 [3]. However, during periods where the number of cases increases, individual level testing has been disrupted by limited laboratory capacity and shortages in testing supplies. Pooling samples, in which several samples are combined into a single sample that is tested for SARS-CoV-2, offers a practical alternative to reduce reagents, supplies, and costs associated with individual testing. If the pool test result is positive, individual testing of the samples included in the pool is then performed. While several reports of pool testing have focused on assessments of nasopharyngeal specimens (NP), performance of pooled testing for other specimens that are more practical and acceptable to patients and research study participants, such as nasal swabs (NS) or saliva (SL) samples, has been less studied. A few prior studies suggested that pooling SL and NS samples can be used for the detection of SARS-CoV-2, with significant cost reduction in processing [4–6]. However, most of those studies have been done in selected samples from individuals presenting to healthcare facilities or in the hospital or using stored residual laboratory samples from symptomatic cases; few from well-characterized cohorts have been conducted in the community, including both symptomatic and asymptomatic individuals. Furthermore, very few studies have examined the potential logistical and economic advantages of pooling strategies.

We examined samples collected during a prospective cohort study that systematically collected respiratory samples twice-a-week from participants with or without respiratory symptoms over a two-month period [7]. In the current study, we evaluated the sensitivity, specificity, and potential cost reduction of pooling of NS and SL samples for the detection of SARS-CoV-2 compared to individual sample testing.

2. Study design

2.1. Study population

This analysis is a substudy derived from a larger prospective cohort study conducted in the San Juan de Lurigancho district of Lima, Peru, between December 2020 and March 2021 as described elsewhere [7]. Households were eligible for participation if they included at least three consenting members; one child (<18 years), one young adult (18–50 years) and an older adult (>50 years), who were available during weekday working hours to be visited at home or in a nearby working area and had no plans to moving out of the area within the planned study follow-up period. Identification of eligible households was performed through a house-to-house screening census within the study area [7]. A total of 132 participants from 44 households (44 participants of each age group) were enrolled and followed through twice-a-week household visits for 2-months. The follow-up period coincided with high viral activity and circulation of the Lambda SARS-CoV-2 variant in Peru [7]. Research procedures were in accordance with the Helsinki Declaration for protection of human subjects from research risks and written consent of participants was obtained and formally recorded prior to data collection. The study was approved by the Comité Institucional de Ética en Investigación of Instituto de Investigación Nutricional (CIEI-IIN), the ethical approval reference number is N° 404–2020/CIEI-IIN and Institutional Review Board Vanderbilt, the ethical approval reference is IRB Number 191271.

2.2. Sample collection and pools preparation

Paired NS (collected in viral transport media (Remel®)) and SL (collected in sterile flask) samples were collected from the same individuals during the same household visit, twice-a-week, at home, by trained field workers. Samples were transported in cold packs to the study laboratory where aliquots were prepared and stored at −80 °C until testing [7].

NS and SL pools were prepared combining samples taken from individuals of the same household in a period of one or two weeks. Most households were constituted by 3 members, each pool was determined by the quantity of samples taken during each twice-weekly household visit, having some cases where a sample could not be taken. Pool sizes from 2 to 12 samples were prepared with equal volumes of each sample in order to obtain at least 400 μL of pool volume. For the preparation of pool sizes from 2 to 3 individuals, we used 200 μL of each sample; from pool sizes from 4 to 9, 100 μL of each sample; and for pool sizes from 10 to 12 samples, we used 50 μL of each sample, obtaining between 500 μL and 600 μL of pool volumes. After mixing, 200 μL from each pool were tested by RT-PCR (see below). After pool testing, all individual NS or SL samples from pools that were positive or indeterminate for SARS-CoV-2 were subsequently tested. All samples from households with a SARS-CoV-2 positive member were also processed individually. In order to evaluate possible false-negative pools, we randomly selected a sample of negative pools and tested all individual samples. When a negative pool had individual samples that tested positive, those pool samples were considered as a false negative sample. If a pool tested positive and all of their individual samples were negative, we considered those pool samples as false positives.

Table 1
Characteristics of samples collected in a peri-urban community. Lima 2021.

Characteristics	N (%)
Sample type	
Nasal swabs	2008 (100)
Saliva	2002 (100)
Nasal swab pooling size	
2	1 (0.3)
4	6 (1.8)
5	12 (3.7)
6	288 (87.5)
7	1 (0.3)
8	2 (0.6)
9	19 (5.8)
Saliva pooling size	
2	1 (0.3)
3	3 (0.9)
4	51 (15.3)
5	20 (6.0)
6	210 (63.1)
7	2 (0.6)
8	24 (7.2)
9	13 (3.9)
10	1 (0.3)
11	2 (0.6)
12	6 (1.8)
Mean of pooled samples‡	
Nasal Swab	6.10 ± 0.83
Saliva	6.01 ± 1.52

‡Mean and standard deviation.

2.3. Detection of SARS-CoV-2 by real-time RT-PCR

RNA extraction from pools and individual samples was performed using the *MagMax™ Viral/Pathogen Nucleic Acid Isolation* kit in the *KingFisher DUO Prime* equipment following the manufacturer's instructions, 200 μL of sample was used to obtain a final volume of 50 μL of eluted RNA [8,9].

For the qualitative detection of SARS-CoV-2 in pools and individual samples, the *TaqPath™ COVID*-19 CE-*IVD RT-PCR Kit* [10] was used following the manufacturer's instructions in the QuantStudio 5 equipment. The software from the QuantStudio 5 equipment was used for the interpretation of results. Samples with indeterminate results underwent a second real time RT-PCR test using the *IDT 2019-NCOV RUO* kit [11] following the recommended CDC protocol [12]. Samples with persistent indeterminate results were not included in subsequent analyses.

2.4. Cost analysis

Cost analysis included labor and supplies used in the process of pools grouped in 6 samples and, due to budget limitations, a part of their respective individual samples for results confirmation (852 NS and 606 SL samples). The cost of pooling analysis until the individual final samples result, was compared with the cost of testing each one of the individual samples without pooling. Direct costs included all laboratory expenses such as laboratory personnel, supplies, equipment, basic services, and indirect costs included personal protection equipment and general services. The unit cost was calculated including the cost of materials, supplies (including the number of reactions per kit and their cost per sample considered), basic services, equipment and the paid laboratory personnel time required for testing. Costs were expressed in US$ with the exchange rate of March 25th, 2021 (S/.3.73 Peruvian soles per dollar). More specific details of the cost items included in the calculations are provided in the Supplement table 2.

2.5. Statistical analysis

Sensitivity and specificity of the pools were calculated using the individual sample results as the reference. To complement these validity assessments, we also determined the concordance in viral detections between pooled and individual testing approaches using the Kappa statistic [13]. Sensitivity, specificity and Kappa were calculated with their respective 95% confidence intervals using STATA16 (StataCorp, College Station TX).

Table 2
SARS-CoV-2 detection by real time RT-PCR in pools of saliva and nasal swabs samples.

	Positive (%)	Negative (%)	Indeterminate (%)	Total number of pools (%)
Saliva pool	16 (4.8)	313 (94.0)	4 (1.2)	333 (100)
Nasal swab pool	12 (3.7)	314 (95.4)	3 (0.9)	329 (100)

Table 3
Sensitivity, Specificity and Kappa correlation of pools of 6 versus individual nasal or saliva samples.

Individual Nasal swab samples	N	Pooled Nasal swab samples		Sensitivity	Specificity	Kappa	CI 95%
		Detected	No detected				
Positive	34	32	2	0.94	1.00	0.97	0.93–1.00
Negative	818	0	818				
Individual Saliva samples	N	Pooled Saliva samples		Sensitivity	Specificity	Kappa	CI 95%
		Detected	No detected				
Positive	41	34	7	0.83	1.00	0.90	0.83–0.97
Negative	564	0	564				

3. Results

3.1. Study samples

A total of 2008 NS and 2002 SL samples were collected from 132 study participants. With these samples we prepared a total of 329 NS and 333 SL pools. The average pool size for NS and SL pools was 6.10 (SD = 0.83) and 6.01 (SD = 1.52), respectively. The distribution of the number of pools by size and type of samples and summary frequencies are presented in Table 1.

A total of 12 NS pools (3.7%) and 16 SL (4.8%) pools were positive for SARS-CoV-2 (Table 2). Among them, a mean of 2.83 (SD = 1.75) individual samples (median = 2; range 2–4) were positive from NS positive pools and 2.94 (SD = 2.05) individual samples (median = 2; range 1–5) were positive from SL positive pools.

We randomly selected 160 NS and 171 SL pools that were negative for SARS-CoV-2, and tested their individual samples, totaling 1068 NS and 1192 SL samples tested.

3.2. Performance of pooling

Given the low number of pools of different sizes (see supplement Table 1), we selected pools of 6 samples to evaluate its sensitivity and specificity compared with individual testing results. The sensitivity of the NS pools was 94% and of the SL pools was 83%. The corresponding specificity was 100% for both NS and SL pools. In addition, pooling and individual testing for NS and SL samples demonstrated excellent concordance in viral detection with both yielding Kappa values > 0.9 (Table 3).

3.3. Cost analysis

We estimated that with a cost of $54.75 US dollars per individual test (see supplement Table 2), there was a 74.8% savings for NS pools as compared with the cost of testing all individual samples, and 72.4% savings for SL pools (Table 4).

4. Discussion

We demonstrated that using pooled testing of NS and SL samples was highly sensitive and specific for the detection SARS-CoV-2 and was associated with substantially reduced testing costs compared with individual testing of samples. Furthermore, the two testing approaches demonstrated excellent concordance in viral detections. These findings from a large community cohort with systematic testing, regardless of the presence of respiratory symptoms, complement observations from other studies conducted in healthcare and testing centers which largely focused on evaluation of symptomatic individuals [14,15].

A major advantage of SL samples is that they are less invasive and have greater acceptability as compared to NS and especially NP sampling [15]. In this study, we observed greater sensitivity values in pooling NS samples than pooling SL samples. This may be due to a lower viral load of SL samples (higher Cts) as compared to NS or NP samples reported in prior studies [16–18]. Nevertheless, the specificity of both approaches was very high. The preferred approach selection would need to consider the anticipated performance of the sample type and the underlying prevalence of infections to provide estimates of the expected number of false negatives in large testing operations.

The number of individual samples per pool in our study was 6; we could not evaluate other pool sizes due to low numbers (see supplement Table 1). In a study done with 25 experimental pools created with one positive sample, compared with 12 pools with all negative samples, a pool size between 5 and 6 samples was considered acceptable when screening populations with an expected prevalence $\leq$10% of the population [4].

Table 4
Estimated cost reduction of pools of 6 versus individual samples.

Sample type	Total samples	Number of pools	Positive pools (%)	Unit cost per sample (USD)[a]	Total cost - individual testing approach (USD)[b]	Total cost - pool testing approach (USD)[c]	Direct cost reduction of total samples (USD)[d]	Savings[e] (%)
Nasal swab	852	142	8.5%	$54.75	$ 46,647.00	$ 11,716.50	$ 34,930.50	74.8%
Saliva	606	101	10.9%	$54.75	$ 33,178.50	$ 9,143.30	$ 24,035.30	72.4%

a Calculated from direct and indirect costs related to laboratory analysis.
b Total costs for individual analysis (Total samples * unit costs).
c Total costs for analysis by pools (Total test * unit costs) + (positive pools*6*unit costs).
d Cost reduction (b-c).
e Percentage (d/b).

An important finding from our study has been the substantial savings of pooled testing representing between 74.8% and 72.4% reductions in the estimated cost relative to individual testing of NS or SL samples. In addition to cost savings, testing pools vs individual samples allows a much faster screening of large populations, allowing timely release of results while saving resources. While several factors may influence the overall savings of the pooling approach, the combination of volume of samples and the prevalence of infection are relevant. In particular, the prevalence of infections in the population to be screened is important: if the prevalence is high, the positivity rate would also be high, and therefore, it would be necessary to test more individual samples, increasing the cost; and vice versa (see supplement Table 3).

Our study used data from one of the few prospective longitudinal studies carried out at a community level, with systematic twice-a-week testing of both symptomatic and asymptomatic individuals during a period with relatively high viral activity. Importantly, while this study with intense and systematic follow-up allowed a precise identification of incident infections in the cohort, most infected persons did not seek medical attention or testing, and therefore, most detected infections were not detected by the regular passive surveillance system operating in the country [7].

Our study has a number of limitations. We only created pools for NS and SL samples but did not evaluate NP samples. Moreover, our pool size was determined by the samples available on a week period from the same household, and not by a prespecified number. Some estimates were based on small number of detections and had limited precision. Also, our study does not allow us to make a proper comparison between pools below to 6 and over to 6 samples. It is also important to mention that there could be an overestimation of the pooling sensitivity since it was done among members of the same household. If a member of the household is positive, the other household members could also be infected, increasing the sensitivity of pooling. Given these limitations, additional evaluations of pooling samples should be done in other settings. Finally, our study was conducted in a community in Lima, Peru, and testing in a specialized research laboratory during a period of increasing viral activity (Lambda variant). Thus, our findings may not be directly applicable to other populations, settings or other viral variants.

We conclude that our data provide important, 'real world' findings from samples collected in a community-based household study of both asymptomatic and symptomatic individuals. The pooled testing of nasal or saliva samples demonstrated high diagnostic performance for detection of SARS-CoV-2 infections, yielding timely results and substantial cost savings and could be considered as an important tool when screening large populations for SARS-CoV-2 infections, particularly in LMIC.

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Author contribution statement

Mayra Ochoa; Bia Peña; Omar Flores: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ana I. Gil; Lucie Ecker; Rubelio Cornejo; Leigh M. Howard; Carlos G. Grijalva; Claudio F. Lanata: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Claudio F Lanata, MD, MPH is a member of WHO COVID-19 vaccine effectiveness working group, and report grant funding from CureVac AG, PATH and HilleVax from work outside the submitted work, and consultancy fees from Valneva. Carlos G Grijalva MD, MPH, reports consultancy fees from Pfizer, Merck, and Sanofi-Pasteur; grants from Campbell Alliance/Syneos Health, CDC, NIH, the Food and Drug Administration, AHQR, and Sanofi, outside the submitted work. Leigh M Howard MD, MPH, reports grants funding from the Infectious Disease Society of America Education and Research Fund supported by Pfizer and from NIH, outside the submitted work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18904.

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