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Sample pooling is efficient in PCR testing of SARS-CoV-2: a study in 7400 healthcare professionals

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ABSTRACT

SARS-CoV-2 pandemic shows the importance of having efficient virus diagnosis, especially in groups of particular relevance such as health care professionals, without involving a large economic expense. This is a prevalence study carried out in 7400 health care professionals in a 1350-bed hospital in Madrid, Spain. Pools of 10 samples were performed, using the Xpert® Xpress SARS-CoV-2 test for the diagnosis from clinical samples of nasopharyngeal exudate. A previous study was performed to evaluate the effect of the dilution in terms of sensitivity. The estimated sensitivity was over 95%. A total of 740 pools were performed, with a final result of 218 health care professionals being positive. Using the pooling system, the reagent cost reduction to the institution was 75.3%. It can be concluded that the described sample pooling system is a useful and efficient tool in the diagnosis of SARS-CoV-2 in certain groups, assuming a cost reduction without reducing the sensitivity.

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1. Introduction

The current SARS-CoV-2 pandemic has highlighted the need for a strong diagnostic capability of SARS-CoV-2 by health care hospitals. The most widely used technique in the diagnosis of SARS-CoV-2 is the detection of specific genes of the virus by polymerase chain reaction (PCR). The current available PCR methods require high technical capacity, providing results in several hours. For this reason, it is necessary to optimize a system that allows results to be obtained in a short period and in large numbers. Pooling systems for clinical samples have proven to be effective in terms of greater optimization of resources in obtaining results without a significant decrease in diagnostic accuracy in different infectious diseases (Bang et al., 2003, Pooling Self-Taken Pharyngeal, 2016). In the case of SARS-CoV-2 diagnosis, the pooling strategy has proven to be effective only as a tool to detect the virus at community level in circumstances of very low prevalence (Graham et al., 2020, Hogan et al., 2020).

Our study aimed to assess the reliability and savings of a pooling system for PCR of nasopharyngeal samples for SARS-CoV-2 in health

care workers with a moderate prevalence of the disease, using Xpert® Xpress SARS-CoV-2 (Xpert) test.

2. Materials and methods

Ours is a 1350-bed teaching institution serving an urban area of nearly 350,000 inhabitants in Madrid, Spain. More than 8000 health professionals work in our hospital. The SARS-CoV-2 pandemic has led to a significant increase in the workload, resulting in a reconversion of resources to adapt to the new situation. During the Covid-19 outbreak, about 20% of our health care workers acquired the disease.

During the months of April and May 2020, a SARS-CoV-2 prevalence study was carried out on all of these personnel. In this article, we analyze the data obtained with 7,400 health care professionals. The exclusion criteria to participate in the study were to present symptomatology compatible with COVID-19 at that time, or to have had a positive PCR for SARS-CoV-2 in the previous month. To the PCR determination, we used the Xpert® test, an effective technique with high sensitivity in the diagnosis of SARS-CoV-2, with a shorter response time than conventional PCRs and the advantage of being performed on direct sample (Loeffelholz et al., 2020).

For the screening of SARS-CoV-2, we set up a pooling system of nasopharyngeal exudate samples. As a previous step to the

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development of the sample pooling system for the SARS-CoV-2 screening program in our hospital, we had the need to perform a preliminary evaluation to make sure that this system did not imply a significant loss of diagnostic sensitivity. For this purpose, we chose a series of SARS-CoV-2 positive clinical samples at different cycle threshold (Ct) values, and whose PCR had been performed by Xpert® test. We then evaluated the detection of these samples in a 1:10 dilution formed by the positive sample together with 9 other negatives. The data derived from this previous study are collected in the Results section.

Initially, we performed a primary pool of 10 samples (Abid et al., 2020, Lohse et al., 2020) with a volume of 35 μ L per sample, with a final volume of 350 μ L. Then, 300 μ L of the total pool volume are added to Xpert® test, which is processed by a GeneXpert® Infinity System. In the event of a negative test, we consider the total of 10 samples in the primary pool to be negative. In the case of positivity, we proceed to perform 3 secondary pools consisting of two pools of 3 samples and a third of 4 samples. The volume contributed by each of the samples in the secondary pool is 110 μ L, adding 300 μ L of total final volume to an Xpert® test. The samples belonging to a negative secondary pool are also considered as negative. In the case of a positive secondary pool, we proceed to perform an individualized Xpert® test for each sample belonging to that positive pool.

2.1. Statistics

The resulting different data were incorporated into a Microsoft® Excel database for Windows and analyzed using the IBM SPSS Statistics® software.

2.2. Ethical and legal aspects

The Research Ethics Committee of the HGUGM approved the study protocol entitled "PROYECTO COVI-STATUS MARAÑÓN", version 7, on 29 May, 2020.

3. Results

During the study period, 7400 health care professionals from our hospital were screened for SARS-CoV-2 using the pooling system described in the Methods section. In order to estimate the diagnostic sensitivity of our sample pooling system, we carry out a prescreening study. First, we selected 8 samples that had been individually analyzed by the Genexpert PCR system. These samples corresponded to patients in our hospital who had attended the emergency department the same week as this pooling system validation study was carried out. The samples were properly stored at a temperature of -80° C. The Ct of the samples ranged from 28.4 to 41.4 for the E and N2 genes. We chose samples with elevated Cts to more realistically evaluate the robustness of the pooling system in maintaining its

detection capability. Secondly, we diluted 35 μ L of each of these positive samples together with 35 μ L of 9 negative samples, in pools of 10 samples in total. Then, 300 μ L of the total pool were added to a Genexpert cartridge and compared the detection and Cts of the samples tested individually versus testing them in a diluted manner in the pools of 10 (Table 1).

In order to validate the 1:10 dilution process, we applied the Ct delays to a cohort formed by all the clinical samples analyzed using Xpert® test before starting the prevalence study. The selected cohort was 199 nasopharyngeal samples from 199 patients of which 80 (40.2%) were positive for one of the two genes detected (63 E(+)/N2 (+) samples, 4 E(+)/N2(-) samples and 13 E(-)/N2(+) samples) and 119 were negative.

Since the 1:10 dilution of a sample increases the Ct values of the E and N2 genes of a positive sample by a median of 3.8 and 3.5, respectively, the percentage of the 80 positive samples in the cohort that would have continued to be detected as positive by GeneXpert was 90.0% (72 samples). Therefore, the estimated sensitivity of pooling with respect to individual testing would be 90.0%. To increase this sensitivity, three positive samples with Ct out of detection range by pooling were selected and processed by GeneXpert (Table 2).

As expected, GeneXpert did not detect any of the three diluted samples, however, when the endpoints (level of fluorescence detection of each gene at the end of 45 PCR amplification cycles) of the two genes were analyzed it was found that two of the samples (66.6%) had an N2 gene endpoint value of 3, which was abnormally high when compared to the values of the selected negative sample cohort. Specifically, only 8.4% of the negative samples had values equal to or greater than 3. On the other hand, the endpoint values of the E gene obtained in the three samples analyzed were within the central zone of the value distribution since the 90th percentile was composed of ≥ 7 values.

Therefore, in order to increase the sensitivity of the technique without significantly increasing the percentage of false positives, negative results with endpoint values for the E and N2 genes ≥ 3 and/or ≥ 7 , respectively, were selected to be analyzed together with the positive results. The estimated sensitivity of the procedure is 96.7%.

After checking the accuracy of our preliminary study with 1:10 dilution, we performed 740 primary pools out of 7400 samples from 7400 health care workers.

We performed 740 primary pools, of which 580 were negative, corresponding to 5800 samples, 132 positive, corresponding to 1320 samples and 28 undetermined by Endpoint, corresponding to 280 samples. The 580 negative pools correspond to 5800 workers who were directly issued a negative PCR result. We rejected from the prevalence study those professionals who had previously tested positive for SARS-CoV-2.

Of the 132 positive pools, 396 secondary pools were performed, formed by pools of 3, 3 and 4 samples from the 10 samples of the

Table 1
Study of sensitivity of the 1:10 dilution at different cycle threshold values from clinical samples.

Sample number	Undiluted sample		Diluted sample 1/10 (pool)		Cycle difference	
	E gene cycles	N2 gene cycles	E gene cycles	N2 gene cycles	E gene cycles	N2 gene cycles
20092626	28.4	31.1	32.1	34.8	+3.7	+3.7
20095160	31.4	34.6	33.7	37.5	+2.3	+2.9
20095161	34.4	37.0	42.1	40.0	+7.7	+3.0
20095166	28.3	31.0	31.0	34.1	+2.7	+3.1
20095167	34.3	37.6	39.0	No detection	+4.7	Not calculable
20095696	28.4	30.9	31.3	34.2	+2.9	+3.3
20092738	28.6	31.4	32.5	36.2	+3.9	+4.8
20092739	41.4	40.6	No detection	No detection	Not calculable	Not calculable
Median	30.0	33.0	33.1	36.9	3.8	3.5

The cycle difference values that were not calculable were included as maximum values for the calculation of the median.

Table 2
Detection of cycle threshold and Endpoint values in clinical samples.

Sample number	Undiluted sample		Diluted sample 1/10			
	E gene cycles	N2 gene cycles	E gene		N2 gene	
			Cycles	Endpoint	Cycles	Endpoint
20091421	0	43.4	No detection	2	No detection	-1
20104499	0	42.3	No detection	5	No detection	3
20104576	41.3	42.7	No detection	5	No detection	3

primary pools. 215 secondary pools were negative and 181 were positive. Finally, the 181 positive secondary pools were analyzed as individual samples, and the result was 193 individual positive samples. There were 28 undetermined pools by Endpoint, with the subsequent 84 secondary pools, of which 24 were positive and 60 negative. The samples belonging to the positive secondary pools were analyzed individually, detecting 25 positive samples.

Regarding the time of the reporting of samples results, 78.4% of the results (those samples from the 580 negative primary pools) were reported in approximately 1 hour after the samples reception in the laboratory, 12.6% (those from the 275 negative secondary pools) in 2 hours and 9% (those from the 205 positive secondary pools including all the 218 positive samples) in 3 hours.

Overall of the 7400 health care workers, 218 (2.9%) turned out to be positive by PCR. If we had made individual PCR determinations for each of the health care workers, the number of cartridges used would be 7400. The cost of the Xpert® cartridge for our laboratory is 40€, so the 7400 samples would have cost 296,000€. With our pooling system, we required 1828 cartridges (740 primary pools, 480 secondary pools and 608 individual determinations), with a total cost of 73,120 €, which translates into a reagent cost per determination of 9.89€. Our pooling system saved the institution 222,880€, a 75.3% reduction in reagent cost.

Regarding the PCR Ct for the genes analyzed, the median Ct for the E gene was 36 and for the N gene 39.5.

4. Discussion

The SARS-CoV-2 pandemic has meant an exponential increase in the workload in our Clinical Microbiology laboratory. Since March, we have been processing around 1000 samples per day for SARS-CoV-2 PCR. Since the beginning of the pandemic, we have performed more than 90,000 PCRs, being one of the hospitals in Spain with the highest number of determinations, increasing by 1200% the number of samples processed by the Virology Department of our laboratory. This has also led to an increase in the staff dedicated to COVID-19, with a team of more than 15 people dedicated exclusively to processing clinical samples of suspected SARS-CoV-2 24 hours a day.

The arrival of the Xpert® test in our laboratory has meant a turning point in the diagnosis of the virus. This automated system shortens the duration of the process to less than 1 hour from the reception of the sample to the report of the result. The technique is performed on direct sample and processed in GeneXpert® Infinity System. This process includes the extraction and purification of viral RNA, the PCR technique and the subsequent detection of the specific amplified genes. The target genes incorporated in the Xpert® test PCR are the specific SARS-CoV-2 gene N and the gene E, common to the subgenus Sarbecovirus (Corman et al., 2020). The same GeneXpert® Infinity system device, using automated interpretation software, performs the result of Positive or Negative.

Regarding the diagnosis of SARS-CoV-2 by sample pooling system, there is little published information on this subject. The main limitations of the literature published so far are the small number of cases processed by this system or that it is based on mathematical models (Aragón-Caqueo et al., 2020; Brynildsrud, 2020), and that there is no

experience in the use of Xpert® test for PCR from sample pooling. In all cases, the PCR performed from the pooling, is a conventional PCR with previous RNA extraction (Rogers et al., 2020; Torres et al., 2020) assuming a longer time to obtain results, an important limitation in the development of SARS-CoV-2 population-level screening programs. It is important to take into account the economic aspect when establishing SARS-CoV-2 diagnostic programs in large population groups. Early detection of potential transmitters of the virus is important in order to achieve appropriate social isolation of them and thus prevent the spread of the virus and the emergence of new outbreaks of the disease. However, such population-based screening programs can be very costly to implement. The use of sample pooling systems such as the one proposed in this study and carried out in our care center, is presented as an effective tool in those population groups where a low prevalence of the virus is suspected. In our case, the commercial house provides us with the individual Xpert® cartridge at an approximate price of 40€. If we had made individual determinations using this technique to each of the 7400 workers in our hospital, the total cost would have been 296,000€, without taking into account the cost of hiring staff dedicated to processing the samples. Using the pooling system, the total number of cartridges needed was 1828, assuming a total cost of 73,120€, which translates into a cost reduction of 75.3%. As for the personnel hired for the processing of the samples in the laboratory, it was two laboratory technicians and two microbiologists, divided into two morning and afternoon shifts. According to our calculations, the estimated workload by pooling system has been practically the same as the one estimated for the individual processing of the samples, not assuming an increase in the staff hired for the study.

As for the time of reporting of results through the pooling system, varies between 1 and 3 hours, depending on whether the sample is part of a negative primary pool or whether an individual determination is needed from a positive secondary pool. In this sense, an option to reduce response time would have been to perform individual test on all positive primary pools to avoid making secondary pools. However, the total number of tests that would have been done would be 2180, almost 20% more tests and an extra cost of 14,080€.

Our GeneXpert infinity system had a capacity of 24 cartridges and the average time for a test is 1 hour, the system was only capable of performing 24 tests per hour while with the pooling system that allows for a 75.3% reduction in the cartridges needed, it is estimated that it could perform an average of $24/(1-0.753)=97.2$ tests per hour. Therefore, in a scenario where more than 24 samples are accumulated to be processed per hour and taking into account that the workload of pooling versus the traditional individualized system is similar, we could conclude that the response time of pooling would be less than the individualized system.

Moreover, assuming that there has been no need to increase the number of personnel hired, and that the reduction in the cost of reagent material for the performance of SARS-CoV-2 PCRs has been significant, we conclude that this is a cost-effective system. That translates into a reduction in the budget for the implementation of the screening program, without losing diagnostic sensitivity in a significant manner.

Regarding the limitations of our study, it is important to take into account that as with any pooling system, in which a dilution of the clinical samples is carried out, there is the possibility of reducing the

diagnostic sensitivity (Abdalhamid et al., 2020, Yelin et al., 2020). However, our experience with the previous study, in which we added a positive sample with a late amplification cycle (higher in some cases than the 42 CT) within a pooling of 10 samples made up of that positive sample and another 9 negative ones, confirmed the detection of that positive sample within the pooling in almost 100% of the cases. For this reason, we assume that the balance between potential loss of sensitivity and the high performance of the technique in terms of cost savings and efficiency is positive.

5. Conclusions

In view of our results, we conclude that the sample pooling system is an effective method for population-based screening for SARS-CoV-2 in groups where the prevalence of infection is low. It represents a considerable cost reduction without significantly increasing the workload in the laboratory and without reducing diagnostic sensitivity. It is a method that is easy to implement in conventional clinical microbiology laboratories. The Xpert® test system has high diagnostic sensitivity, and its processing characteristics from direct sample, integrated RNA extraction and subsequent PCR in less than an hour, make it a technique of choice in this type of program, due to its speed and reliability.

Finally, we believe it is necessary to carry out new studies using this methodology with the aim of implementing it in the future as a tool for the population diagnosis of SARS-CoV-2 in certain selected groups.

Author contributions

A.E.: Formal analysis, writing-original draft and editing. P.C., R.A., M.M., P.M., E.B.: Conceptualization, data curation, formal analysis, methodology. L.A.: Conceptualization, data curation, formal analysis, methodology, writing-review and editing.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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