Title: Increasing SARS-CoV-2 RT-qPCR testing capacity by sample pooling

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Abstract

Objectives: Limited testing capacity has characterized the ongoing COVID-19 pandemic in Spain, hampering a timely control of outbreaks and the possibilities to reduce the escalation of community transmissions. Here we investigated the potential of using pooling of samples followed by one-step retrotranscription and quantitative PCR (RT-qPCR) to increase SARS-CoV-2 testing capacity.

Methods: We first evaluated different sample pooling (1:5, 1:10 and 1:15) prior to RNA extractions followed by standard RT-qPCR for SARS-CoV-2/COVID-19 diagnosis. The pool size achieving reproducible results in independent tests was then used for assessing nasopharyngeal samples in a tertiary hospital during August 2020.

Results: We found that pool size of five samples achieved the highest sensitivity compared to pool sizes of 10 and 15, showing a mean (± SD) Ct shift of 3.5 ± 2.2 between the pooled test and positive samples in the pool. We then used a pool size of five to test a total of 895 pools (4,475 prospective samples) using two different RT-qPCR kits available at that time. The Real Accurate Quadruplex corona-plus PCR Kit (PathoFinder) reported the lowest mean Ct (± SD) shift (2.2 ± 2.4) among the pool and the individual samples. The strategy allows detecting individual samples in the positive pools with Cts in the range of 16.7-39.4.

Conclusions: We found that pools of five samples combined with RT-qPCR solutions helped to increase SARS-CoV-2 testing capacity with minimal loss of sensitivity compared to that resulting from testing the samples independently.

Keywords: COVID-19; SARS-CoV-2; testing capacity; scalability; sample pooling

Introduction

The SARS-CoV-2 pandemic causing COVID-19 continue imposing a heavy burden on healthcare systems worldwide because of a shortage of consumables and the demand for scaling up efficient screening approaches. To limit the escalation of cases and amplification of infections, increasing the capacities and developing alternatives to the one-step reverse transcription and real-time quantitative PCR (RT-qPCR) for regular testing of SARS-CoV-2 is key (Mina *et al.* 2020). We have assessed a direct heating method of nasopharyngeal (swab) samples to bypass the RNA extraction step for increasing the testing capacity (Alcoba-Florez *et al.* 2020a,b).

In areas with low COVID-19 prevalence, such as the Canary Islands until June 2020 (Pollán *et al.* 2020), testing of samples in pools is another approach that can efficiently increase SARS-CoV-2 testing capacity. Simulation studies support the value of testing on sample pools and the speed of reporting, while the impact of the sensitivity of tests is comparable smaller (Larremore *et al.* 2020). Supporting this, a pooled test of SARS-CoV-2 of four samples has received Emergency Use Authorization from the USA Food and Drug Administration (FDA COVID-19 Update 2020).

Here we aimed to evaluate the RT-qPCR testing on pooled swab samples with the goal to demonstrate its feasibility as an option to increase SARS-CoV-2 testing capacity in the region.

Materials and Methods

The study was conducted in two stages at the University Hospital Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain) during August 2020. Nasopharyngeal swab samples were collected in 2 mL of viral transmission medium (VTM) (Biomérieux).

RNA extractions were conducted from 200 µL of pooled VTMs using the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche) or the STARMag Viral DNA/RNA 200C kit (Seegene) as described elsewhere (Alcoba-Florez *et al.* 2020a,b). Two RT-qPCR solutions were used in the study period: The Real Accurate Quadruplex corona-plus PCR Kit (PathoFinder), and the TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific). We focused on the results for the N target gene for both kits (Alcoba-Florez *et al.* 2020b). The RT-qPCR was performed in 10 µL final volume reactions (5 µL of sample) using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) following the thermal cycling specifications of each solution. Samples were considered negative when the SARS-CoV-2 target had Ct >40. Positive and negative controls were included in all experiments as described elsewhere (Alcoba-Florez *et al.* 2020a,b).

In a pilot stage, we used the Real Accurate Quadruplex corona-plus PCR Kit for testing 15 pools made by combining 5, 10, or 15 retrospective samples, each containing equal volumes of a SARS-CoV-2/COVID-19 positive sample and the respective amounts of negative samples to complete the pool size. In a validation stage, we used the two RT-qPCR kits available on swab samples from prospective subjects using the pooling size achieving optimal results. Then, individual samples from each positive pool were subjected to RNA extraction followed by RT-qPCR using the above-mentioned methods to validate the results.

Differences between the pooled Ct and the positive sample Ct in the pool (Ct shift) for the two different RT-qPCR kits were assessed by Mann-Whitney U-test using the R v4.0.3 software. When more than a positive sample was present in the pool, the mean Ct of the positive samples was used in the calculation. Sensitivity and the 95% Confidence Interval (CI) was assessed with MedCalc (MedCalc Software Ltd.).

Results

In a pilot study, detection of SARS-CoV-2 was achieved with pool size of five (one positive) in 13 out of 15 independent pools (sensitivity = 86.7% [95% CI= 59.5-98.3]), where detected positives had a maximum Ct value of 36.2 (**Figure 1**). A mean (± SD) Ct shift of 3.5 (± 2.2) was obtained between the pool of five samples and the individual positive samples. False negatives increased for pool sizes of 10 and 15 samples, both showing larger mean Ct shifts (5.3 ± 2.4 and 7.2 ± 4.4, respectively).

A validation study included samples from 4,475 prospective subjects between August 18th 2020 and August 31st 2020. Samples were tested in 546 pools (447 negatives) for the Real Accurate Quadruplex corona-plus PCR Kit and 349 (286 negatives) for the TaqPath COVID-19 CE-IVD RT-PCR Kit. A total of 162 pools were tested positive among the two kits, 118 containing just one positive sample and 44 containing more than one positive sample (**Figure 2**). Compared to the independent samples present in the positive pools, the mean Ct shift upon pooling was 2.2 ± 2.4 for the Real Accurate Quadruplex corona-plus PCR Kit and 3.1 ± 2.9 for the TaqPath COVID-19 CE-IVD RT-PCR Kit (p=0.006, Mann-Whitney U-test). Both RT-qPCR kits allowed to sensitively detect positive samples with a maximum Ct value of 39.4.

Discussion

In this study, we describe the feasibility of RT-qPCR in pools of five swab samples with a minimal loss of sensitivity compared to the assessment of samples independently. Consistent with these findings, others have recently demonstrated that pooling of up to 10 samples result in a slight shift in Ct (around 3), and therefore a drop of sensitivity (Das *et al.* 2020).

A major limitation of the study is that sensitivity was only evaluated in the pilot stage. However, despite the small Ct shift between the positive pools and the individual positive samples in the prospective study, a high impact of false negatives is not

expected (Cherif *et al.* 2020). Our data suggest that our sample pooling strategy is able to detect COVID-19 positive cases with Ct values ≤39.4. Pooling with compressive sampling designs, which involves repetitive tests (Shental *et al.* 2020), and the use of alternative testing approximations (Peto *et al.* 2020) may help to further lessen the impact of the dilution factor on pooling and continue increasing health capacity building.

Authors' contributions

JAF and CF designed the study. JAF, HGC, DGM, and ODG participated in data acquisition. JAF, LC, and CF performed the analyses and data interpretation. LC, AVF, RGM and CF wrote the draft of the manuscript. All authors contributed in the critical revision and final approval of the manuscript.

Acknowledgments

We deeply acknowledge the University Hospital Nuestra Señora de Candelaria board of directors and the executive team for their strong support and assistance in accessing diverse resources used in the study.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research was funded by Cabildo Insular de Tenerife [grants CGIEU0000219140 and "Apuestas científicas del ITER para colaborar en la lucha contra la COVID-19"]; the agreement with Instituto Tecnológico y de Energías Renovables (ITER) to strengthen scientific and technological education, training research, development and innovation in Genomics, Personalized Medicine and Biotechnology [grant number

OA17/008]; Ministerio de Ciencia e Innovación [grant numbers RTI2018-093747-B-100 and RTC-2017-6471-1], co-funded by the European Regional Development Fund (ERDF); Lab P2+ facility [grant number UNLL10-3E-783], co-funded by the ERDF and "Fundación CajaCanarias"; and the Spanish HIV/AIDS Research Network [grant number RIS-RETIC, RD16/0025/0011], co-funded by Instituto de Salud Carlos III and by the ERDF. The funders had no role in the study design, collection, analysis and interpretation of data, in the writing of the manuscript or in the decision to submit the manuscript for publication.

Ethical Approval

The University Hospital Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain) review board approved the study (ethics approval number: CHUNSC_2020_24).

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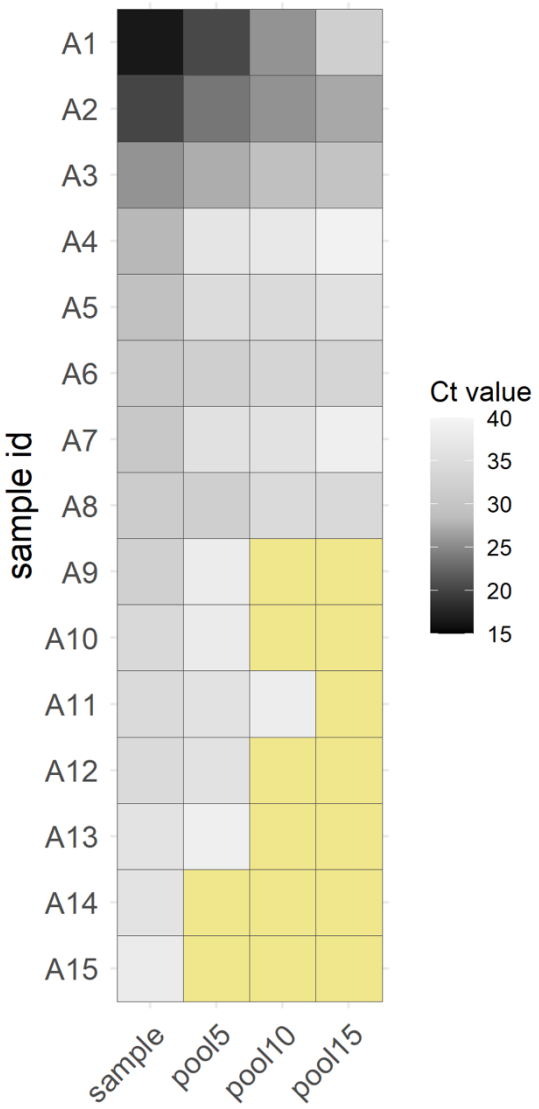

Figure 1. Heatmap representation of RT-qPCR test positiveness on a Ct scale (the darker, the lower the Ct) on the pilot study with 1:5 (pool 5), 1:10 (pool 10), and 1:15 (pool 15) pooling of retrospective swab samples. Negative results (Ct>40) are highlighted in pale yellow. The Real Accurate Quadruplex corona-plus PCR Kit (PathoFinder) was used in the experiment.

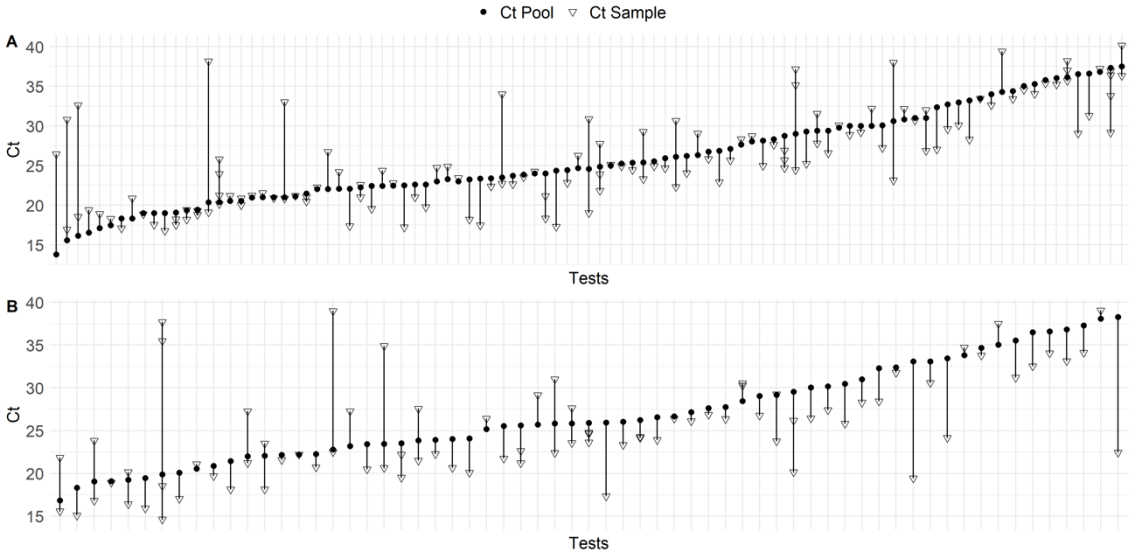

Figure 2. Scatter plot showing Ct values and Ct shifts for all prospective COVID-19 positive cases detected by RT-qPCR on 1:5 pooling tests (full circle) and on individual samples (empty triangle) using (**A**) the Real Accurate Quadruplex corona-plus PCR Kit and (**B**) the TaqPath COVID-19 CE-IVD RT-PCR Kit.