Concentrating Pooled COVID-19 Patient Lysates to Improve Reverse Transcription Quantitative PCR Sensitivity and Efficiency

To the Editor:

The SARS-CoV-2 virus has spread globally, with >95 million reported cases and 2 million deaths. Although, reverse transcription quantitative PCR (RT-qPCR) has emerged as the gold standard method for diagnosis and, by extension, for early identification and quarantine, upscaling these tests to meet current demand is difficult. Furthermore, the per-test cost is prohibitive for many laboratories and government health districts around the world. One means of reducing costs while simultaneously increasing throughput is to perform pooled testing (1). However, the main shortcoming of pooled testing lies in the loss of sensitivity associated with diluted samples, leading to false-negative results (2, 3). If we were to overcome the dilution limitation, pooled testing would serve as an attractive alternative for COVID-19 diagnostic testing. In this letter, we describe a fast and efficient method to overcome this deficit by concentrating pooled lysates.

It is known that coronaviruses have large RNA genomes, with SARS-CoV-2 having a length of 29.9 kb (approximately 9 million Da) (4). Given this property, we hypothesized that concentrating pooled lysates with centrifugal concentrators would restore the lost sensitivity. We evaluated a range of large molecular-weight cutoff (MWCO) centrifugal concentrators (10 000 Da and 100 000 Da), followed by head-to-head comparisons between individual PCRs and PCRs performed following concentration of pooled lysates.

We present a simple 1-step concentrating protocol using centrifugation for improved detection of pooled oro-nasopharyngeal lysates preidentified at the John Radcliffe Hospital using the Abbott m2000 assay for detection of SARS-CoV-2. This method readily slots within the existing workflow of most diagnostic laboratories conducting SARS-CoV-2 screening. Following heat inactivation, the samples were prepared for RNA extraction in a guanidine thiocyanate–based buffer (Buffer RLT/OBL, Qiagen RNeasy® Plus 96 Kit). Finally, RT-qPCR was performed using the CDC assay (Integrated DNA Technologies) with the TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific), following the manufacturer's protocol.

A 1:10 dilution of Twist Synthetic SARS-CoV-2 RNA Control 1 (Twist Bioscience), mimicking standard pooling protocols for 10 samples, demonstrated that 100 000 MWCO filters were the most accurate at replicating the quantification cycle (Cq) values identified with undiluted samples (in addition to being the fastest in comparison to 10 000 MWCO). Therefore, we decided to pursue our future work with patient lysates using a 100 000 MWCO, for which 300 µL of one SARS-CoV-2–positive lysate was mixed with 9 × 300 µL of SARS-CoV-2–negative lysates, yielding a final volume of 3 mL (1:10 ratio; 340 individual patient samples, 34 pooled lysates). Samples were processed using a swing bucket centrifuge at 3000*g* for 15 min at room temperature, resulting in a final volume of about 400 µL.

As seen in Fig. 1, we identified that across a spectrum of patient samples that were SARS-CoV-2 positive and negative (Cq ranging from 14 to 38), concentration of pooled lysates (n = 34) did not result in a loss of RT-qPCR sensitivity (r^2 = 0.90). The overall Cq values for concentrated lysates were not found to be significantly different from values obtained through standard processing techniques for each single positive sample (paired, 2-tailed *t*-test; $P = 0.80$). This was true for patients with high viral loads and those with lower viral loads. Our data show that pooling and concentration of lysates from patients with COVID-19 could indeed be implemented for high-throughput screening.

Interestingly, 48% of our positively tested lysates displayed a decrease in the Cq values when concentrated (e.g., shifting the Cq value from 37.8 to 33.5). Moreover, this effect was particularly apparent with lysates containing lower viral loads. We found that 70% of patient samples with Cq values >30 displayed significant Cq improvements (2.1-Cq average decrease; paired, 2-tailed *t*-test; $P = 0.02$) following pooling and concentration, compared with just 26% that showed improvements with Cq values <30. These data suggest that pooling and concentrating samples can both reduce the workload and, importantly, enhance RT-qPCR sensitivity.

The pooled testing approach has been utilized for the detection of many infectious DNA and RNA viruses such as HIV (5). Our reported findings demonstrate the great potential of pooled testing to improve screening. However, it should be acknowledged that the usefulness of pooled testing diminishes as the prevalence of the disease increases because of the increased

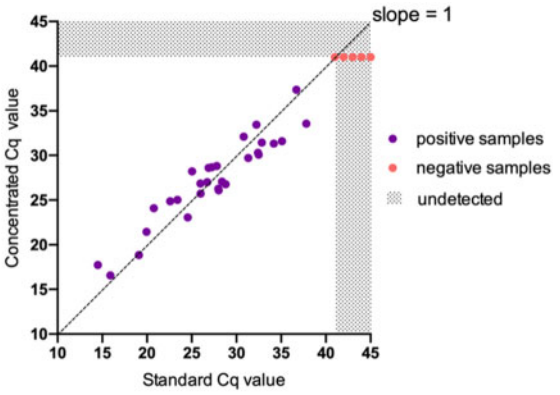

Fig. 1. A spectrum of COVID-19 pooled patient samples with and without subsequent concentration using 100 000 MWCO filters (340 samples, 34 pooled lysates).

need for individual tests for deconvolution. Consequently, a pooling strategy should take community prevalence of the virus into account.

Overall, we show that our concentrating approach could aid in improving the sensitivity of pooled testing and reduce the use of laboratory resources by up to 90% while adding only 15 min to the existing protocol. Given its simplicity, with no need for additional sophisticated instrumentation, our concentration approach could be used successfully to identify SARS-CoV-2 and all viral infections (old and novel).

Nonstandard Abbreviations: RT-qPCR, reverse transcription quantitative PCR; MWCO, molecular-weight cutoff; Cq, quantification cycle.

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