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Poolising of samples for testing for SARS-CoV-2 in asymptomatic people

The ongoing coronavirus disease 2019 (COVID-19) pandemic is a substantial challenge for health-care systems and their infrastructure. RT-PCR-based diagnostic confirmation of infected individuals is crucial to contain viral spread because infection can be asymptomatic despite high viral loads. Sufficient molecular diagnostic capacity is important for public health interventions such as case detection and isolation, including for health-care professionals.

Protocols for RNA RT-PCR testing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became available early in the pandemic, yet the infrastructure of testing laboratories is stretched and in some areas it is overwhelmed. We propose a testing strategy that is easy to implement and can expand the capacity of the available laboratory infrastructure and test kits when large numbers of asymptomatic people need to be screened. We introduced the pooling of samples before RT-PCR amplification, and only in the case of positive pool test results is work-up of individual samples initiated, thus potentially substantially reducing the number of tests needed.

Viral load during symptomatic infection with SARS-CoV-2 was investigated by Zou and colleagues. To analyse the effect of pooling samples on the sensitivity of RT-PCR, we compared cycle threshold (Ct) values of pools that tested positive with Ct values of individual samples that tested positive.

We isolated RNA from eSwabs (Copa Italia, Brescia, Italy) using the Nuclisens easy MAG Instrument (bioMérieux Deutschland, Nürtingen, Germany) following the manufacturers’ instructions. PCR amplification used the RealStar SARS-CoV-2 RT-PCR Kit 1.0 RUO (Altona Diagnostics, Hamburg, Germany) on a Light Cycler 480 II Real-Time PCR Instrument (Roche Diagnostics Deutschland, Mannheim, Germany) according to the manufacturers’ instructions.

Our results show that over a range of pool sizes, from four to 30 samples per pool, Ct values of positive pools were between 22 and 29 for the envelope protein gene (E-gene) assay and between 21 and 29 for the spike protein gene (S-gene) assay. Ct values were lower in retested positive individual samples (figure A, B). The Ct values for both E-gene and S-gene assays in pools and individual positive samples were below 30 and easily categorised as positive. Ct value differences between pooled tests and individual positive samples (Ct_pool< Ct_positive sample) were in the range of up to five. Even if Ct values of single samples were up to 34, positive pools could still be confidently identified (figure C, D). Sub-pools can further optimise resource use when infection prevalence is low. Generating a pool of 30 samples from three sub-pools of ten samples can reduce retestings.

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If the large pool is positive, the three sub-pools are reanalysed, and then the individual samples of the positive sub-pool. In our analyses during March 13–21, 2020, testing of 1191 samples required only 267 tests to detect 23 positive individuals (prevalence 1.93%). The rate of positive tests was 4.24% in our institution during this period.

These data suggest that pooling of up to 30 samples per pool can increase test capacity with existing equipment and test kits and detects positive samples with sufficient diagnostic accuracy. We must mention that borderline positive single samples might escape detection in large pools. We see these samples typically in convalescent patients 14–21 days after symptomatic infection. The pool size can accommodate different infection scenarios and be optimised according to infrastructure constraints.

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**Challenges and issues of SARS-CoV-2 pool testing**

We read with interest Stefan Lohse and colleagues’ Correspondence about sample pooling for testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in asymptomatic people.1 Some of the findings Lohse and colleagues report do not seem to be consistent with other research results2,3 nor our experiences.

In panels C and D of the figure in Lohse and colleagues’ letter,1 which show the three pooled samples, there is one positive sample in 30 negative samples in each pool, and the pooled samples show lower Ct values than do single samples, which suggests the RNA concentration increased after pooling. Considering that concentrations of RNA had been reduced to 1/31 in the pooled specimens, the Ct values were expected to increase by five compared with single samples. However, in figures C and D, the actual Ct values of the pooled samples were approximately six values lower than expected, corresponding to a 60-fold increase in RNA concentration.4 By contrast, we found that when testing pooling of 50 nasopharyngeal and oropharyngeal samples, Ct values (RdRp gene) increased with pool size (appendix).

Lohse and colleagues attribute the decreased Ct values to the carrier effect from a higher RNA content in the pool; however, we did not observe a similar phenomenon in 600 tests. Lohse and colleagues did not describe clearly whether the experiment was done with media pooling or swab pooling in a single tube. To our knowledge, the NucliSens easyMAG instrument does not use carrier RNA or DNA for extraction, and there was no evidence to support the carrier phenomenon in the Correspondence.

During our experiments, we observed a few instances wherein the Ct value decreased despite an increased pool size. However, the changes in Ct value...