Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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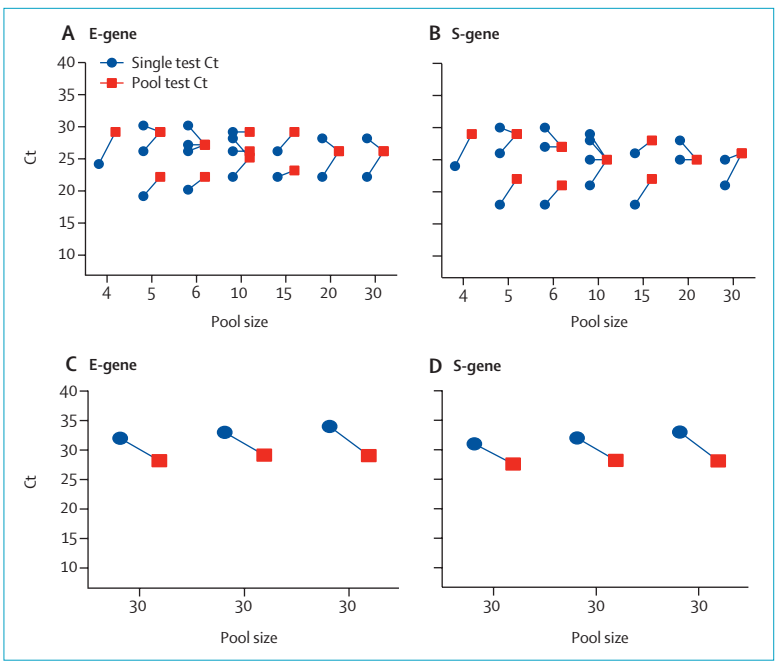

Figure: **Ct values of single versus pooled samples**
Absolute Ct values of positive pools (13 of 164 tested pools) in relation to pool size and corresponding Ct values of individual positive samples for the E-gene assay (A) and the S-gene assay (B). Absolute Ct values were below 30 for all pool sizes. Three positive individual samples with Ct values greater than 30 were spiked into negative pools of 30 samples and tested with E-gene (C) and S-gene (D) assays. We hypothesise that the lower Ct values of pools than of single samples were because of the carrier effect of the higher RNA content in pools. Connecting lines show positive single samples and their corresponding pools. Ct=cycle threshold. E-gene=envelope protein gene. S-gene=spike protein gene.

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.

See Online for appendix

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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 results[2,3] 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 specimens 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

and pooling size were small and so could probably be explained by random variation in the PCR or pooling process with small volumes. By contrast, the difference between the expected and observed Ct values in Loshe and colleagues' study was large and so could not be attributed to random variation. A decrease in Ct value after pooling with negative specimens might cause a false-positive result and would be regarded as contamination in a clinical setting.

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Stefan Lohse and colleagues[1] described a sample pooling strategy for testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) via RT-PCR to meet the unprecedented demand for laboratory testing. Lohse and colleagues evaluated a range of pool sizes (four to 30 samples per pool) in asymptomatic people. The additional time to deconvolute the larger pools yielding a positive result into sub-pools precludes the use of this strategy in patients with severe acute respiratory illness and high-risk contacts. Moreover, most of the studies on pooled sample testing have not discussed the crucial technical points. Here, we raise certain technical issues pertaining to SARS-CoV-2 pool testing.

First, sample collection for SARS-CoV-2 testing in field settings is done without supervision and dependent on the skill of the people doing the test. The crucial pre-analytical variable in SARS-CoV-2 testing is the amount of host RNA (detected by RNAse P). Before we adopted pool testing in our laboratory, approximately 3% of individual samples tested showed no RNAse P amplification, indicating inadequate sample collection, which was resolved on repeat sample collection. Such a sample would have been missed in pooled testing and might have been reported as negative despite an inadequate amount of clinical material.

Second, Lohse and colleagues attributed the lower Ct values of pools than of single samples to the carrier effect of the higher RNA content in pools. If the same hypothesis is applicable to the adequacy of a sample, then inadequate samples in a large pool will be falsely reported as negative.

Third, different RNA extraction kits recommend different volumes of sample, ranging from 140 µL to 200 µL. It is not clear how Lohse and colleagues addressed the issues of total amount of pooled sample and the minimum amount of each sample to be added in pool sizes ranging from four to 30 samples. In a large pool of up to 30 samples, if we take 5–10 µL of each sample, there is every chance of missing borderline-positive single samples.[2]

In our laboratory, as suggested by the Indian Council of Medical Research,[3] we are testing four samples in a pool. Some pools have been inadequate (RNAse P not detected), which was resolved when individual testing was attempted. Absence of RNAse P in a pool might be due to an inhibitory effect of concentrated RNA samples on reverse transcription.[4] Large-scale validation of SARS-CoV-2 sample pooling strategies addressing these technical issues is needed to reach a consensus strategy.

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We read with interest the Correspondence by Stefan Lohse and colleagues,[1] who evaluated the practicability of pool testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Pooling of samples yields considerable savings of test kits when the prevalence of infection is low because pools with all-negative samples can be discarded with a single test. Lohse and colleagues' findings suggest that pooling up to 30 samples is technically feasible with currently used and

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