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.

commercially available SARS-CoV-2 RT-PCR kits.[1]

But is bigger always better? Is it really efficient to pool 30 samples? First discussed around 80 years ago by Dorfman[2] in the context of large-scale syphilis testing, the matter is complex and optimal pool sizes depend on the prevalence of infection in the population. Furthermore, there are more sophisticated pooling schemes than the one originally discussed by Dorfman, which use multiple stages of pooling or test samples in rows and columns of a matrix.[3]

See Online for appendix

We propose an adaptive approach that uses different pooling schemes depending on the estimated prevalence in a population.[4] Our exhaustive comparison of testing schemes shows that three different schemes with initial pool sizes of 16, nine, and three samples are optimal for a prevalence of up to 3·5%, 3·5–12%, and 12–30%, respectively (appendix). The first two schemes are three-staged, meaning that if a pool tests positive it is further divided into sub-pools of four or three samples, before then testing samples individually. These schemes have a consistently higher testing efficiency than the method proposed by Lohse and colleagues, who used a three-staged scheme with initial pools of 30 samples and sub-pools of ten samples (appendix). For a prevalence of 2%, as in the population tested by Lohse and colleagues,[1] our proposed testing scheme (pool size of 16 and four sub-pools of four samples) uses around 20% fewer tests. At higher prevalence, differences become even more pronounced and smaller pool sizes are optimal. For prevalence over 18%, pools of 30 samples are even less efficient than individual testing, whereas small pool sizes of three samples still yield a considerable improvement in efficiency (appendix).

See Online for appendix

Hence bigger is not always better. Rather, it is preferable to choose one of the three proposed testing schemes based on the estimated underlying prevalence.

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Authors' reply

We appreciate the comments on our letter,[1] in which we described a strategy to identify asymptomatic people infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in large populations of uninfected people when testing capacity is low and laboratory infrastructure is overwhelmed. We proposed pool testing to screen for individuals who might spread SARS-CoV-2 without showing any symptoms. He and colleagues[2] reported temporal patterns of viral shedding and inferred from their data that viral load peaks 0·7 days before symptom onset and estimated that 44% of SARS-CoV-2 infections occur during the pre-symptomatic stage of the index case. Studies indicate a steady decline of viral loads and infectiousness after symptom onset.[2,3]

We recommended restricting pool testing to asymptomatic people, excluding patients with severe acute respiratory illness and high-risk contacts, and for when testing capacity is limited.[1] For symptomatic individuals we use individual testing so not to miss patients with low or borderline SARS-CoV-2 viral loads and to obtain diagnostic results as quickly as possible.

We proposed that pool sizes should "accommodate different infection scenarios", which should "be optimised according to infrastructure constraints", and suggested that "sub-pools can further optimise resource use when infection prevalence is low."[1] We investigated 164 pools in total and mainly used pool sizes of five (81 pools) or ten (45 pools), as further illustrated in the appendix. Beyond that, we explored the pooling of 30 samples with sub-pools of ten samples (five pools; appendix). During the period of our study (March 13–21, 2020), the positivity rate was below 2% in samples examined by pool testing. We agree with Jens Eberhardt and colleagues that pool sizes should be smaller than 30 when prevalence is higher than 2%.

We agree with Baijayantimala Mishra and colleagues that the swab sampling procedure is of paramount importance. The testing for host nucleic acid might help to confirm accurate sampling and improve the accuracy of a negative test result. Yet, as SARS-CoV-2 is released in mucous or saliva,[3] the absence of cellular control signals does not necessarily prove that mucous or saliva is missing and that swab taking needs to be redone.

Jaehyeon Lee and colleagues highlighted another point in our letter. In our study, we used the Copan Liquid Amies Elution Swab Collection and Preservation System for sampling and pooled media before RNA extraction. We performed nucleic acid extraction from 400 µL of our single or pooled samples. From theoretical considerations one would expect dilution effects and a resulting increase of Ct values in pools. We observed a puzzling increase of detection sensitivity in pools containing single samples with high Ct values (appendix). This increase did not occur randomly but was reproducible, systematic, and

significantly associated with higher Ct value samples for both E-gene and S-gene RT-PCRs (appendix). To explain our observation, we hypothesised that samples with higher Ct values might have gained detection efficiency through an RNA carrier effect in pools from the other negative samples with potentially higher cellular RNA content. We are currently addressing this interesting question in a further study, as well as whether different swab collection systems affect this phenomenon. The concern raised by Lee and colleagues that this phenomenon might cause false-positive results is not supported by our data obtained with now more than 3900 pools assessed in our institution since March, 2020.

In a broader context, several distinct steps contribute to accurate test results. Major contributing factors are adequate sample collection, quality of swabs, transport media, efficient nucleic acid extraction from a sufficient amount of material, and a highly sensitive detection method. All these steps need to be optimised and validated within the laboratory to obtain optimal pool testing efficiency and accuracy.

Owing to highly diverse laboratory settings, it might be difficult to harmonise worldwide pool testing protocols for SARS-CoV-2. However, we would be grateful if national authorities could guide SARS-CoV-2 pool testing procedures as has been done for blood donor pool testing in Germany[4] and recently been announced by the US Food and Drug Administration for SARS-CoV-2.[5]

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Macrolide consumption and resistance in *Mycoplasma genitalium*

Dorothy Machalek and colleagues published a systematic review and meta-analysis[1] detailing the alarming increase in the prevalence of mutations associated with macrolide resistance in *Mycoplasma genitalium*. Machalek and colleagues hypothesised that this increase and large heterogeneity in prevalence between countries could be due to differences in national protocols for sexually transmitted infection treatment or differences in the consumption of macrolides at the population level. To test this hypothesis, we used Spearman's correlation to assess the association between the country-level prevalence estimates of resistance that Machalek and colleagues generated and national macrolide consumption. The prevalence of resistance was defined as the prevalence of resistance-conferring mutations in the 23S ribosomal RNA gene at positions 2058 or 2059, in all isolates that were successfully characterised. Country-level macrolide consumption data were obtained from IQVIA (IQVIA, Danbury, CT, USA). IQVIA uses national sample surveys to generate estimates of antimicrobial consumption, reported as defined daily doses per 1000 population per year.[2] We calculated the median year of data collection for the studies that contributed to resistance prevalence estimates shown by Machalek and colleagues. Our measure of antimicrobial consumption was taken from 1 year before the year used to provide resistance prevalence.[3] For the sensitivity analysis, we repeated the analyses using average macrolide consumption for 3 years before the median year used to provide resistance estimates.

Data on macrolide consumption were available for 18 of 21 countries with macrolide resistance estimates. Considerable differences in macrolide consumption and resistance were evident, and both positively correlated with antimicrobial consumption in the preceding 1 year (ρ=0·51; p=0·032; appendix p 1) and the preceding 3 years (ρ=0·49; p=0·038). Low prevalence of resistance was seen in Belgium (6·5%) and France (11·3%) relative to high levels of macrolide consumption. However, these prevalence estimates were based on data collected between 2003 and 2016. More recent publications,[4,5] not included in the systematic review, found that the prevalence of macrolide resistance was 74% in Belgium (in the general population) and 58% in France (in the pre-exposure prophylaxis cohort).

Although these results could be due an ecological inference fallacy, taken together with similar findings from other bacteria such as *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*, they suggest that the promotion of macrolide stewardship in the general population and core groups should be considered as a strategy to counter the further emergence of macrolide resistance in *M genitalium*.[3]

We declare no competing interests.

See Online for appendix