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.

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.

JL and SYK contributed equally. The research described in this Correspondence was supported by the Korea Centers for Disease Control and Prevention. We declare no competing interests.

*Jaehyeon Lee, So Yeon Kim, Heungsup Sung, Sang Won Lee, Hyukmin Lee, Kyoung Ho Roh, Cheon Kwon Yoo, *Ki Ho Hong*
kihohongmd@gmail.com

Department of Laboratory Medicine, Jeonbuk National University Medical School and Hospital, Jeonju, South Korea (JL); Department of Laboratory Medicine, National Medical Center, Seoul, South Korea (SYK); Department of Laboratory Medicine, Asan Medical Center and University of Ulsan College of Medicine, Seoul, South Korea (HS); Korea Centers for Disease Control and Prevention, Cheongju, South Korea (SWL, CKY); Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, South Korea (HL); Department of Laboratory Medicine, National Health Insurance Service Ilsan Hospital, Goyang, South Korea (KHR); and Department of Laboratory Medicine, Seoul Medical Center, Seoul, South Korea (KHH)

1 Lohse S, Pfuhl T, Berkó-Göttel B, et al. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people. *Lancet Infect Dis* 2020; published online April 28. https://doi.org/10.1016/S1473-3099(20)30362-5.
2 Yelin I, Aharony N, Shaer Tamar E, et al. Evaluation of COVID-19 RT-qPCR test in multi-sample pools. *Clin Infect Dis* 2020; published online May 2. DOI:10.1093/cid/ciaa531.
3 Eis-Hübinger AM, Hönemann M, Wenzel JJ, et al. Ad hoc laboratory-based surveillance of SARS-CoV-2 by real-time RT-PCR using minipools of RNA prepared from routine respiratory samples. *J Clin Virol* 2020; **127:** 104381.
4 Life Technologies. Real-time PCR handbook. https://www.thermofisher.com/content/dam/LifeTech/global/Forms/PDF/real-time-pcr-handbook.pdf (accessed May 2, 2020)

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.

We declare no competing interests.

Baijayantimala Mishra, Bijayini Behera, Monalisa Mohanty, Akshatha Ravindra, Jai Ranjan
micro_baijayantimala@aiimsbhubaneswar.edu.in; bm_mishra@hotmail.com

Department of Microbiology, All India Institute of Medical Sciences, Bhubaneswar, Odisha 751019, India

1 Lohse S, Pfuhl T, Berkó-Göttel B, et al. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people. *Lancet Infect Dis* 2020; published online April 28. https://doi.org/10.1016/S1473-3099(20)30362-5.
2 Hogan CA, Sahoo MK, Pinsky BA. Sample pooling as a strategy to detect community transmission of SARS-CoV-2. *JAMA* 2020; **323:** 1967–69.
3 Department of Health Research, Indian Council of Medical Research. Advisory on feasibility of using pooled samples for molecular testing of COVID-19. https://www.mohfw.gov.in/pdf/letterregguidanceonpoolingsamplesfortesting001.pdf (accessed May 2, 2020).
4 Williams WV, Rosenbaum H, Weiner DB. Effect of RNA concentration on cDNA synthesis for DNA amplification. *PCR Methods Appl* 1992; **2:** 86–88.

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

Published **Online** July 14, 2020 https://doi.org/10.1016/S1473-3099(20)30467-9

Published **Online** July 14, 2020 https://doi.org/10.1016/S1473-3099(20)30463-1