



Correspondence

Interpretation of pooled sample testing of COVID-19 for efficient use of resources

Sir,

We read with interest the article by Prahraj *et al*¹. The authors have concluded that the pooling of five COVID-19 suspected samples for SARS-CoV-2 detection by real-time reverse transcription polymerase chain reaction (RT-PCR) may be an acceptable strategy without much loss of sensitivity (88%) even for low viral loads compared to higher number of false negatives with 10-sample pools. Based on the current evidence, we would like to offer our additional inputs on the interpretation of the five-sample pool testing strategy for efficient detection of cases to limit missing out of positive cases.

In India, the first case of COVID-19 was reported on January 30, 2020 from Kerala². Following which, a seropositivity rate of 0.73 per cent was observed (6,468,388 adult infections) during May 11 to June 4, 2020³. As of now (April 13, 2021), a total of 13,689,453 cases with 1.25 per cent mortality is reported⁴. The percentage of positivity is still low (7.0%). Hence, the pooling strategy of five samples will be more helpful in country like India having a large population with minimal resources.

Prahraj *et al*¹, have shown that an average Ct value obtained with the five-sample pools exceeds an individual sample testing by 2.18 ± 1.86 cycles, while Ct value obtained with 10-sample pooling exceeds an individual sample testing by 3.81 ± 2.26 cycles. At ICMR-Regional Medical Research Centre, Gorakhpur, India, a total of 206,232 samples were tested for SARS-CoV-2, with around 2.5 per cent positivity. Ct value of 100 pools of five-sample pools and their respective individual samples were analyzed. It was observed that an average Ct value obtained with the five-sample pooled testing exceeded an individual sample testing by 2.62 ± 1.40 and 2.76 ± 1.63 with *E* and

ORF1ab genes, respectively (unpublished data), which was concordant with the findings of Prahraj *et al*¹.

Hence, while determining the positive pools for segregation, the cut-off value should be adjusted as RT-PCR kit recommended Ct cut-off value plus four cycles in case of five-sample pools for interpretation of the real-time RT-PCR results. This recommendation may help reduce the possibility of missing COVID-19-positive cases. An elucidation from the authors about the results and interpretations taking into account the above observations will benefit the scientific community.

Conflicts of Interest: None.

Niraj Kumar, Kamran Zaman, Brij Ranjan Misra & Rajni Kant*

COVID-19 Laboratory, ICMR- Regional Medical Research Centre, Gorakhpur 273 013, Uttar Pradesh, India

*For correspondence:
rajnikant.srivastava@gmail.com

References

1. Prahraj I, Jain A, Singh M, Balakrishnan A, Dhodapkar R, Borkakoty B, *et al*. Pooled testing for COVID-19 diagnosis by real-time RT-PCR: A multi-site comparative evaluation of 5- & 10-sample pooling. *Indian J Med Res* 2020; 152 : 88-94.
2. Varghese GM, John R. COVID-19 in India: Moving from containment to mitigation. *Indian J Med Res* 2020; 151 : 136-9.
3. Murhekar MV, Bhatnagar T, Selvaraju S, Rade K, Saravanakumar V, Thangaraj JWV, *et al*. Prevalence of SARS-CoV-2 infection in India: Findings from the national serosurvey, May-June 2020. *Indian J Med Res* 2020; 152 : 48-60.
4. Ministry of Health and Family Welfare, Government of India. Available from: <https://www.mohfw.gov.in>, accessed on April 13, 2021.