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Correspondence

Evaluation of SARS CoV-2 RT-PCR in a multiple sample pool

Sir,

We read with interest the article on pooled testing for COVID-19 diagnosis by Prahraj *et al*[1]. In this study, the authors performed comparative analysis of pooled testing for 5 and 10 sample pools across the 10 virus research and diagnostic laboratories established in various parts of India. At each laboratory, 10-sample pools were created and real-time RT-PCR for SARS-CoV-2 was performed for both individual and pooled samples. Concordance between individual sample and testing in the 5 or 10 sample pools was analyzed; the results showed that pooling five samples was an acceptable strategy without significant loss of test sensitivity[1].

This is a well-conducted study and we appreciate the research group for conducting a timely study, particularly amidst the ongoing pandemic. The pandemic reached India in March 2020 and that time the testing capacity of nation was less than 1000 samples/day. On April 13, 2020, the Ministry of Health and Family Welfare, Government of India, in collaboration with Indian Council of Medical Research (ICMR), New Delhi, issued an advisory recommending pooled COVID-19 testing of five-sample pools is areas where the disease prevalence was <5 per cent[2]. Sample pooling conserves RT-PCR kits, consumables and significantly decreases work force requirement. At Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India, we performed 400,000 COVID-19 RT-PCR till date using 5- and 10-sample pool strategy, and recently published data from our centre[3] which suggested that five-sample pooling was practically possible with the current prevalence. This saves 60 per cent kits and increases the testing capacity 2.5 times using the same infrastructure and workforce[3].

We wish to raise a few concerns regarding the methodology used by the investigators. First, in this study, RNA extraction was performed at participating centres using different kits and technology[3]. Two of the centres used supramagnetic bead-based technology (MGI Easy Nucleic Acid Extraction Kit, MGI Technology, China) and the remaining eight centres used spin column-based technology (QIAamp Mini Viral RNA Kit, Qiagen, Germany/HiPura Viral RNA Extraction Kit, Hi-Media, India/Purelink viral DNA Kit, Thermo Fischer scientific, USA). The Qiagen/Hi-Media spin column kits are optimized for 140 µl of clinical specimen and manufacture recommends that for samples larger than 140 µl, the amount of lysis buffer and other reagents need to be standardized; however, the Thermo Fischer kit is optimized for 200 µl samples[4]. In this study, the majority of laboratories took 200 µl of pooled viral transport medium for RNA extraction using QiaAmp Mini Viral RNA Kit[1]. An important question that remained unanswered was that whether a uniform RNA extraction protocol was followed at all centres or did each centre standardize its own protocol. The study results documented a large variation in concordance between individual sample and pooled sample at different centres; the 10-sample pool concordance was only 50 per cent at Regional Medical Research Centre, Bhubaneshwar, and Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, whereas it was 90 per cent at one centre. A clarification regarding this will be helpful to readers as most of the Indian laboratories are trying to perform pooled sample testing for COVID-19.

In this study[1], four different RT-PCR kits were used (*E* gene screening NIV assay, TIB Molbiol 2019 nCoV Kit, Standard diagnostics nCoV Real Time Detection Kit and COVID-19 Mylab, India kits). All four kits use similar primer probes targeting Sarbeco *E* gene[5]; however, the reverse transcriptase and amplification cycles are different for all kits as master mix used in NIV screening kit is AgPath-ID™ One-Step RT-PCR, whereas TIB Molbiol kit uses SuperScrip III Platinum

One-Step master mix. Similarly, the cut-off threshold (C_t value) for NIV screening kit is 35 and for TIM Molbiol and Mylab kit is 40. The authors have used positive C_t<36 for defining positive samples in this study; instead of defining one fixed C_t value. It would have been better approach if authors used kit protocol for result analysis. This might also explain the low concordance between individual sample and five-sample (69% concordance) and 10-sample pool (27% concordance)[1].

Further, the authors reported that C_t value of five-sample pool exceeded individual sample C_t value by 2.18±1.86 cycles, while C_t values of 10-sample pooling exceeded individual sample C_t value by 3.81±2.26 cycles. We have also documented similar findings from our centre[3]. It has been observed that if pooled sample are showing sigmoid-shaped graph in RT-PCR after cut-off threshold, then are should test the deconvoluted individual samples.

***Conflicts of Interest*:** None.

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References

1. Praharaj 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. Ministry of Health and Family Welfare, Government of India. *Advisory on feasibility of using pooled samples for molecular testing of COVID-19*. Available from: *https://www.mohfw.gov.in/*, accessed on April 14, 2021.
3. Garg J, Singh V, Pandey P, Verma A, Sen M, Das A, *et al*. Evaluation of sample pooling for diagnosis of COVID-19 by real time PCR - A resource saving combat strategy. *J Med Virol* 2020. doi: 10.1002/jmv.26475.
4. QIAamp Viral RNA. *Mini Kit Handbook protocol for purification of viral RNA from plasma, serum, cell-free body fluids, and culture supernatants*. Available from: *www.qiagen.com,* accessed on October 5, 2020.
5. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, *et al*. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 2020; *25* : 2000045.