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LETTER TO THE EDITOR

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# Detecting SARS-CoV-2 RNA in fecal specimens: The practical challenges

To the Editor,

We read with great interest the short communication by Fumian et al. with the title of "SARS-CoV-2 RNA detection in stool samples from acute gastroenteritis cases, Brazil" recently published in the Journal of Medical Virology.[1] The authors reported polymerase chain reaction (PCR) positivity in about one quarter of stool samples from patients with acute gastroenteritis symptoms. Milliere et al. in their letter to the editor in response to Fumian's study also reported positive agreement of 44.4% between stool and nasopharyngeal specimens for the initial detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in patients with suspected coronavirus disease 2019 (COVID-19) using PCR.[2] There has been growing interest in discussion on the clinical utility of stool specimens for the diagnosis of SARS-CoV-2 since the early reports on SARS-CoV-2 RNA shedding in fecal specimens.[3,4] The persistence of SARS-CoV-2 RNA in fecal specimens after respiratory tract samples turned negative in COVID-19 patients[5,6] also raised concerns about infection control although PCR positivity is not equivalent to infectivity.[7] World Health Organization currently recommends nucleic acid amplification testing for fecal specimens only for those in the second week of symptoms where upper and lower respiratory tracts specimens are negative, and clinical suspicion remains.[8]

Milliere et al.[2] underscore the preanalytical challenges, which may cause false negative results in nasopharyngeal swabs in their letter. From the perspective of a molecular diagnostic laboratory, detection of SARS-CoV-2 RNA in fecal specimens is more technically challenging than in respiratory swabs due to additional specimen preparation steps before nucleic acid extraction. The presence of impurities in the fecal specimens is known to exert different degrees of inhibitory effect on PCR.[9,10] Different pretreatment methods with varied efficiency on removing impurities can also affect the detection of SARS-CoV-2 in fecal samples.[11] Fecal samples with low amounts of viral RNA may be reported falsely as negative due to potential PCR inhibition. With a dilution of the nucleic acid extract, potential inhibitory effect may be reduced or totally removed should there be a presence of PCR inhibitors in the purified nucleic acids. As a precautionary step, the laboratory usually performs PCR on the diluted nucleic acid alongside the "neat" extracts. In laboratory practice, validity of PCR results relies on the presence and performance of internal controls, which are assay dependent. These controls may be human gene targets found endogenously in the specimens, or exogenous controls, either spiked into PCR master mix, or during nucleic acid extraction to also control for extraction efficiency. Although dilution to remove PCR inhibitors may reduce false negativity in samples with a high viral titer, it is of limited usefulness in samples with low amounts of viral RNA because dilution reduces the amount of target for detection in the PCR assay. In the study by Fumian et al.,[1] the cycle threshold cut-off was set at 38 cycles for viral targets. Assuming a similar setting, a 10-fold dilution of nucleic acid is expected to delay the cycle threshold by a value of 3.3, which will significantly reduce the assay's ability to detect the target. In view of these caveats, we would recommend an "inconclusive result" to avoid issuing a potentially false-negative report, if a negative result is obtained after sample dilution for a fecal sample showing initial signs of PCR inhibition.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

## AUTHOR CONTRIBUTIONS

Kok-Siong Poon conceptualized the ideas and wrote the original draft. Nancy Wen-Sim Tee reviewed and edited the final draft.

## PEER REVIEW

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