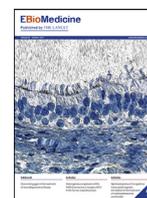




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## Commentary

## Positive, again! What to make of “re-positive” SARS-CoV-2 molecular test results

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## ARTICLE INFO

## Article History:

Received 3 September 2020

Accepted 3 September 2020

Available online 22 September 2020

Nine months after the first reports describing a novel corona virus (Severe Acute Respiratory Syndrome coronavirus 2, SARS-CoV-2) causing severe disease in humans (coronavirus Disease 2019, COVID-19) [1], and with over 25 million infected individuals worldwide, questions regarding the clinical relevance and public health implications of SARS-CoV-2 viral shedding remain unanswered. In this article of *EBioMedicine*, Dr. Changwen Ke and colleagues report that, in a subset of COVID-19 patients, SARS-CoV-2 nucleic acid can be detected intermittently in nasopharyngeal specimens despite symptom resolution [2]. In this specific study setting, hospitalised COVID-19 patients who were discharged after symptom improvement and whose respiratory or digestive tract specimens tested negative twice using nucleic acid amplification testing (NAAT), were monitored in a hotel setting for an additional two-week period under strict quarantine and social distancing measures. During this post-hospital discharge but continued isolation period, 14% of the COVID-19 survivors, all of whom had mounted neutralizing SARS-CoV-2 antibody responses, again tested positive by NAAT. Younger patients with milder initial COVID-19 manifestations were more likely to experience such recurrences, even though the antibody titers were comparable between patient groups with and without late detection of SARS-CoV-2 genetic material. Thus, in some individuals, neutralizing immune responses did not eliminate intermittent detection of viral nucleic acids in the upper respiratory tract. Importantly, attempts to grow infectious virus or obtain complete viral genome sequences from these “re-positive” patients failed, suggesting that

the NAAT positivity may represent degraded genetic material rather than intact viruses.

SARS-CoV-2 is a positive strand RNA virus that replicates in the cytoplasm of cells. Molecular diagnostic tests using oropharyngeal or nasopharyngeal swabs provide sensitive and specific detection of virus RNA and have been authorised for the diagnosis of acute SARS-CoV-2 infection [3]. Using these molecular testing tools, viral nucleic acids have also been detected in many bodily fluids including, but not limited to, saliva/sputum, stool, gut biopsies, and ocular fluids [4-6]. Most immune competent patients clear the virus within a few weeks, but several reports indicate that prolonged NAAT positivity (often referred to as “viral shedding”) can persist weeks after the resolution of the clinical symptoms [7,8]. Of note, in a subgroup of COVID-19 survivors, NAAT positivity is associated with lingering sequelae, often in the presence of a humoral immune response, but it remains unclear whether these long-term medical conditions are due to the presence of the (replication-competent) virus or caused by dysregulation and tissue damage triggered by the initial acute viral infection [8-10]. Considerable uncertainty remains whether or not such findings translate into infectiousness and transmissibility, but the findings presented in this article show that intermittent NAAT positivity may be observed without detectable production of infectious virus. It is conceivable that in the absence of an efficient immune response (e.g., chemotherapy, post-transplant, or other immunodeficiency), prolonged viral shedding may, indeed, reflect intact, infectious virus. Given the grave public health implications, longitudinal comprehensive studies to confirm this possibility, including virus cultures and complete viral genome sequencing, are urgently needed in these specific patient populations.

Another topic of increasing public concern is the question of how to distinguish between intermittent shedding of SARS-CoV-2 genomic materials from the initial infection and an independent, second re-infection. In the report by Ke, et al., the study patients were in isolation in a monitored setting when the NAAT- “re-positive” specimens were collected, thus the likelihood of new, second SARS-CoV-2 re-infection is very low. However, re-infection could not be absolutely excluded, since viral sequences were not available from the initial infections and sequencing of the re-positive specimen yielded incomplete viral genomes in all cases, underscoring the

DOI of original article: <http://dx.doi.org/10.1016/j.ebiom.2020.102960>.

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importance as well as the difficulties of confirming NAAT positivity with sequencing.

The report by Ke and colleagues contributes to the understanding of the likelihood and clinical significance of positive SARS-CoV-2 NAAT results after symptom resolution, which is of major importance for those regions of the world where the first waves of the pandemic have been contained. Since public health measures often include broad SARS-CoV-2 molecular testing and contact tracing, repeat positive tests may be detected with increasing frequency in COVID-19 survivors. In some cases, this may, indeed, represent a true new, independent infection, as suggested in very recent preliminary reports (preprints not yet peer-reviewed and several news outlets) of individuals who were shown to be re-infected by SARS-CoV-2 strains that differed from the strain that caused their previously documented infections. Conclusions regarding re-infection can, however, only be drawn if supported by complete genome information for virus from specimens collected during both the initial and the subsequent infectious episodes, e.g. indicating that the disease-causing viral strains belong to different clades. Currently, difficulties in recovering biospecimens pertinent to an earlier acute infection, and the possibility of being re-infected with a viral strain too genetically similar to distinguish with certainty remain hurdles that need to be overcome when investigating putative re-infection cases.

The finding that viruses could not be sequenced from patients who tested re-positive using NAAT in the study by Ke and colleagues points to the urgent need for new diagnostic tools to distinguish replication competent SARS-CoV-2 from defective ones. In addition, it is of great public health importance to implement comprehensive surveillance programs to enhance capacity for banking of diagnostic specimen for future comparative testing.

### Contributors

VS, HvB and EMS wrote the manuscript, edited the manuscript and approved the final version.

### Declaration of Competing Interest

The authors declare no conflict of interest. Mount Sinai has licensed SARS-CoV-2 serological assays to commercial entities and has filed for patent protection for serological assays (VS; patent number pending).

### Acknowledgments

The knowledge on COVID-19 is a rapidly evolving and we apologize to the many researchers whose contributions have not been discussed due to space limitations.

The authors are supported, in part, by federal grants (Collaborative Influenza Vaccine Innovation Centers, [CIVIC] contract [75N93019C00051](#) and Centers of Excellence for Influenza Research and Surveillance contract [HHSN272201400008C](#)) as well as generous philanthropic donations (JPB foundation, the Open Philanthropy Project [#2020-215611](#)). The funders had no role in writing this commentary.

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