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.

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Letter to the Editor

Interpretation of SARS-CoV-2 replication according to RT-PCR crossing threshold value

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To the Editor,

In this study we assessed viral factors of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) shedding, trying to prove that a RT-PCR crossing threshold (Ct) value superior to 33 on nasopharyngeal swab (NPS) samples could be a major criterion to enlighten infection prevention and control measures such as duration of eviction, contact tracing, or discharge from hospital wards. The SARS-CoV-2 replication cycle includes direct translation of genomic RNA into several viral proteins, synthesis of negative- and positive-stranded replicative intermediate RNAs (RIs), viral assembly, and release of mature virions [1].

To provide proof of active replication, we conducted real-time RT-PCR assays to detect specifically the presence of the viral E (envelope) subgenomic RNA and E negative-strand RNA in clinical samples. We also attempted to isolate virus from samples to associate the presence of RIs with the detection of viable virus. Data were obtained from 61 immunocompetent healthcare workers (HCWs) diagnosed with SARS-CoV-2 infection by RT-PCR on nasopharyngeal samples (12 from asymptomatic subjects and 49 from subjects with mild/moderate clinical disease). Detailed descriptions of the characteristics of the HCWs, clinical context, and sample processing methods are presented in the Supplementary Material.

Overall, the median age was 28 (interquartile range (IQR) 23–36), and 38 were females. SARS-CoV-2 viral loads ranged from 9.64 to 3.57 $\log_{10}$ copies/mL (cp/mL) (Supplementary Material Table S1). Virus isolation was successful for 41.0% of clinical samples. Our data showed that the likelihood of recovering infectious virus correlates with high viral loads and the presence of RIs (Fig. 1A). Strikingly, no isolate was recovered when viral load was below 5.83 $\log_{10}$ cp/mL (i.e. Ct >28), which is similar to cut-offs reported previously [2–4]. Moreover, no RIs were detectable in samples when the viral load was below 4.34 $\log_{10}$ cp/mL (i.e. Ct >33). Interestingly, the ratios of mean normalized RIs per genome indicate a high level of viral replication during the first 5 days after the onset of symptoms followed by a significant decline thereafter (Fig. 1B), as previously reported [4]. Among asymptomatic HCWs, high viral loads (>5 $\log_{10}$ cp/mL) were associated with detection of significant signals of RIs and virus isolation.

Although recent studies have expressed reservations [5], our findings confirm subgenomic viral RNAs as indicators of active replication in clinical samples [4]. Moreover, we demonstrated that detection of negative strands—never used hitherto—perfectly correlates with subgenomic viral RNA detection. With the accumulating evidence available thus far, our findings strengthen the possibility of using—in association with a symptom-based strategy—the RT-PCR Ct value cut-off of 33 as a value above which individuals would no longer be contagious.

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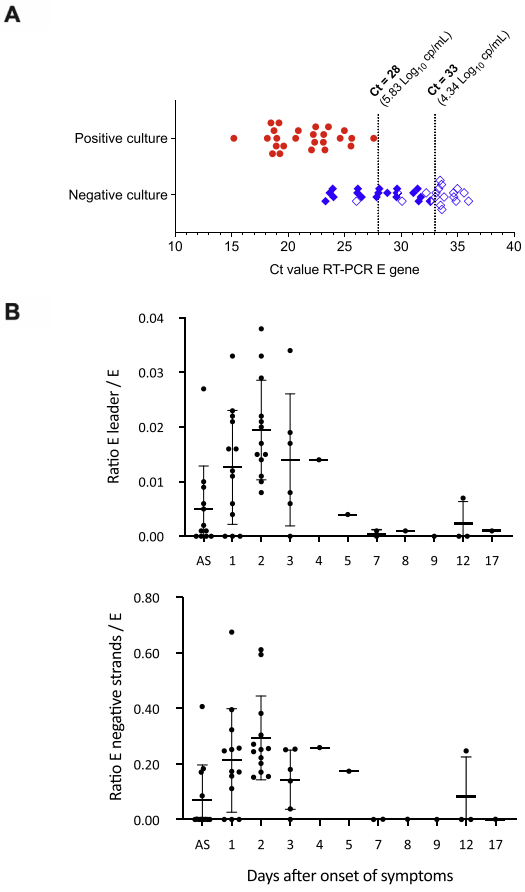

Fig. 1. (A) Virus isolation success in relation to Ct value of the RT-PCR targeting the E viral gene. Red circles and blue diamonds represent positive and negative cell culture assays, respectively. Empty and half-filled symbols showed clinical samples with no E subgenomic or E negative-strand RNA signal detected, respectively. Crossing threshold (Ct) values of 28 and 33 correspond to 5.83 and 4.34 $\log_{10}$ copies/mL (cp/mL), respectively. (B) Subgenomic viral and negative-strand RNAs in relation to viralore E genomic RNA. The ratios are depicted according to the number of days after the onset of symptoms. Dots represent mean values of RT-PCR data obtained from at least two independent experiments on samples from individual healthcare workers (HCWs). Plots show median values with interquartile ranges. Data concerning date of symptom onset were confidently available for 54 HCWs (88.5%); HCWs with no precise data were excluded from the analysis. E subgenomic ('E leader') and E negative-strand RNAs were never detected beyond 7 days after the onset of symptoms, except at 12 days after the onset of symptoms for HCW N°35 who experienced persistent clinical signs at the time of sampling. Moreover, no isolate was recovered from the clinical sample of this patient (viral load 5.83 $\log_{10}$ copies/mL). AS, asymptomatic HCWs.

Author contributions

SB initiated the study and coordinated all work carried out. SM did the targeted and strand-specific molecular assays, and cell culture isolation trials. ML provided clinical information about HCWs. SB, VC and A-GM drafted the initial manuscript with inputs. All authors contributed to the final submitted version. All authors have read and agreed to the final version of the manuscript.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.01.017.

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