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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Short Communication

Rapid detection of SARS-CoV-2, replicating or non-replicating, using RT-PCR

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ABSTRACT

To identify animals susceptible to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection or to determine whether SARS-CoV-2 contaminated meat is from a SARS-CoV-2-infected animal, a convenient and safe method was developed for rapid detection of SARS-CoV-2 in a replicating or non-replicating status in samples using reverse transcriptase–polymerase chain reaction (RT-PCR). This strategy can also be applied to develop assays for the detection of other viruses, either replicating or not.

Searching for animals susceptible to SARS-CoV-2 infection is one of the essential strategies to trace the origin of SARS-CoV-2 (Shi et al., 2020). It is also important to determine whether SARS-CoV-2-contaminated meat is from a SARS-CoV-2 infected animal or not. The established method to determine the status of SARS-CoV-2 replication in tissues or cells is through culturing these samples in a biosafety level 3 facility, however, this is very labor- and time-consuming, and unsafe for researchers/technicians.

SARS-CoV-2 is a single-stranded positive-sense RNA virus. The viral negative-sense RNA is produced only when it is replicating in cells (Baltimore, 1971) (Figure 1A). At present, most methods detect the total RNA of the virus, but the results do not indicate whether the virus is replicating, or not (Kim et al., 2020; WHO, 2020). Here, we designed and developed a simple RT-PCR assay to detect both viral positive- and negative-sense RNA simultaneously to determine whether the virus in tissues or cells is replicating, or not (Figure 1B).

In brief, Vero-E6 cells were infected with SARS-CoV-2 and incubated at 37 °C for 48 h. The infected cells were then collected. Another sample consisting of the medium obtained after washing SARS-CoV-2-attached salmon was collected as previously described and is also explained in the supplement (Dai et al., 2020). Then the total RNAs were extracted from each sample for reverse transcription with the strand-specific primers (Figure 1B) (Deer et al., 2010). The results indicated that both viral positive- and negative-sense RNA were detected from the virus-infected cells, while only positive-sense RNA was detected from the medium, as shown in Figure 1C (see Supplemental Materials).

In summary, this assay to detect replicating SARS-CoV-2 in cell or tissue samples is convenient, rapid and safe for researchers/technicians. This strategy can also be applied to develop assays for the detection of other viruses, either replicating or not.

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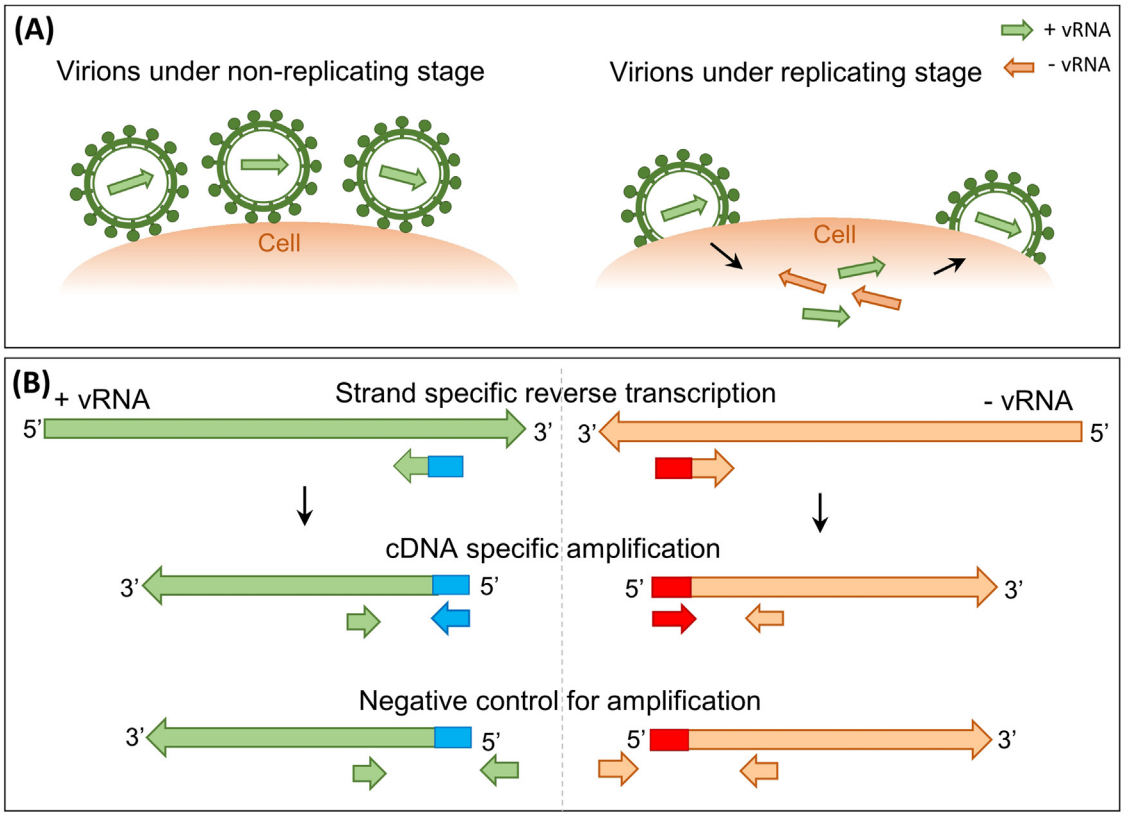

(C)

RNA extracted from	+ vRNA (Mean ± SD)	Negative Control for + vRNA	- vRNA (Mean ± SD)	Negative Control for - vRNA
SARS-CoV-2-infected cells	17.9 ± 0.2	NA	32 ± 0.9	NA
Medium from washing virus-attached salmon	24.1 ± 0.2	NA	NA	NA
Mock infected cells	NA	NA	NA	NA
Plasmids control	17.6 ± 0.3	NA	16.6 ± 0.4	NA

Figure 1. Detection of SARS-CoV-2 positive- and negative-sense RNA in a sample. (a) Illustration of replicating and non-replicating SARS-CoV-2. The viral negative-sense RNA was produced during the virus replicating stage in cells. (b) A schematic of the methodology of strand-specific RT-PCR. The blue and red fragments represent two different internal amplification controls. (c) The results of RT-PCR detection in different samples. NA means no specific PCR products amplified.

Author contributions

CP and ML conceived the idea and supervised the study; CP, JW, MD, HL, YN, and RY designed and performed the experiments; CP and ML wrote the manuscript.

Conflictof interest

The authors have applied for a Chinese patent based on the methods described in this study (Application No. CN202010994889.5).

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Ethical approval

This work was approved by the National Health Commission of the People's Republic of China and performed in biosafety level 3 laboratory in South China Agricultural University.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.01.043.

References

Baltimore D. Expression of animal virus genomes. Bacteriol Rev 1971;35(3)235–41 PMID: 4329869; PMCID: PMC378387.

Dai M, Li H, Yan N, Huang J, Zhao L, Xu S, et al. Long-term survival of SARS-CoV-2 on salmon as a source for international transmission. J Infect Dis 2020;(November), doi:http://dx.doi.org/10.1093/infdis/jiaa712 jiaa712.

Deer DM, Lampel KA, González-Escalona NG. A versatile internal control for use as DNA in real-time PCR and as RNA in real-time reverse transcription PCR assays. Lett Appl Microbiol 2010;50(4):366–72, doi:http://dx.doi.org/10.1111/j.1472-765X.2010.02804.x.

Kim JM, Kim HM, Lee EJ, Jo HJ, Yoon Y, Lee NJ, et al. Detection and isolation of SARS-CoV-2 in serum, urine, and stool specimens of COVID-19 patients from the Republic of Korea. Osong Public Health Res Perspect 2020;11(3):112–7, doi: http://dx.doi.org/10.24171/j.phrp.2020.11.3.02.

Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science 2020;368 (6494):1016–20, doi:http://dx.doi.org/10.1126/science.abb7015.

WHO. Coronavirus Disease (COVID-19) Pandemic–Emergency Use Listing Procedure (EUL) Open for in Vitro Diagnostics. URL:. 2020. https://www.who.int/diagnostics_laboratory/EUL/en/.