# Isolation or De-isolation: Measuring the Infectivity of SARS-CoV-2?

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To control the ongoing pandemic of COVID-19, rapid identification and appropriate isolation of individuals infected by SARS-CoV-2 is considered critical to restrict the widespread of highly transmissible COVID-19[1, 2]. Detection of the SARS-CoV-2 RNA by real-time reverse transcription polymerase chain reaction (rRT-PCR) in appropriately collected specimens is a sensitive and reliable assay to diagnose SARS-CoV-2 infection. Detailed analysis of viral kinetics and distribution in infected patients with COVID-19 found high-titer viral RNA genomes in nasopharyngeal, lung-derived and stool specimens, and the titer declined quickly within the first week from the onset of symptoms. Infectious SARS-CoV-2 could only be successfully isolated from nasopharyngeal and lung-derived specimens in the first few days from onset of symptoms, usually also within one week [3, 4]. However, persistent viral RNA could be detected for a long time, up to 3 months, from the symptom onset [5, 6]. This may lead to prolonged isolation of infected individuals, which will pose a huge impact on the whole society and economy, and place a heavy burden on limited medical resources under COVID-19 pandemic. Therefore, the U.S. Centers for Disease Control and Prevention recommends the symptom-based strategy rather than test-based strategy to determine the timing to end isolation. It is recommended to release patients from isolation 10 days after symptom onset for most patients, and the duration of isolation may be extended up to 20 days after symptom onset for those with severe illness. This recommendation is based on accumulative evidence showing that infectious SARS-CoV-2 cannot be isolated from most COVID-19 patients 3 weeks after symptoms appear [3, 4]. In addition, a large contact tracing study showed that no viral transmission occurred if the exposure to a case patient started 6 or more days after the COVID-19 illness onset[7].

Although practical and reasonable, such a policy may cause unnecessary quarantine in some patients who actually clear their virus earlier. In this regard, measuring the viral infectivity may help identify these patients. The problem for using the PCR assay in this scenario is that it fails to distinguish viable viruses from residual viral nucleic acids [8, 9]. The latter is thought the main reason causing the prolonged virus shedding in some COVID-19 patients [5, 6]. In contrast, viral culture is able to measure the viral infectivity. Thus, the viral culture assay may complement the PCR assay, and help to distinguish truly infectious virus from persistent residual viral nucleic acids. However, currently there is no sufficient data to clarify the relationship between PCR assay and culture assay, particularly in a daily clinical practice.

In this issue of *Clinical Infectious Disease*, Basile et al [10] measured the SARS-CoV-2 infectivity by the virus culture assay and intended to determine its utility for safe de-isolation. Among the 234 collected sample in this cohort, the majority were collected from outpatients, and the remaining from inpatients and ICU patients. They quantified the viral titer by rRT-PCR, and also simultaneously performed cell-based viral culture using Vero C1008 cells (Vero-E6). To increase the sensitivity of viral culture, they defined positive cultures not only by inspection of the cytopathic effect (CPE) of infected cells but also by the difference between the cycle threshold (Ct) of the day 4 sample PCR and original sample PCR ($Ct_{sample} - Ct_{culture} \geq 3$, approximately 10-fold increase after viral culture for 4 days). Consistent with previous studies [3], they showed that the yield rate of virus culture was correlated with high viral titer, indicated by the low Ct value of rRT-PCR. Successful viral culture was only achieved in samples with the rRT-PCR Ct value $\leq 32$. The successful culture rate was highest in samples from ICU patients (9/11, 82%), followed by samples from inpatients (19/42, 45%), and lowest in samples from outpatients (28/181, 15%). Interestingly, they found a few samples had positive virus culture without CPE, but showed active viral replication ($Ct_{sample} - Ct_{culture} \geq 3$). In addition, all patients except for one had positive viral culture samples collected before 10 days post

symptom onset. This observation also justifies the current symptom-based strategy for releasing patients from isolation. This assay is also useful to help shorten the unnecessary isolation of patients post SARS-CoV-2 infection in hospitals, and allows for safe de-isolation.

Although the study from Basile *et al* provided useful information, several caveats need to be further investigated. First, the virus culture assay should be standardized, so it can be established by different laboratories and yield reproducible and comparable results across them. However, currently, no standardized protocols or procedures have been established for SARS-CoV-2 culture in the daily practice. Besides, variation in the sample collection and transportation procedures and culture systems may affect the sensitivity and yield rate of the viral culture assay. Furthermore, the role of viral culture negativity in de-isolation of patients should be cautiously examined. The thresholds of infectious viral particles for in vitro and in vivo viral infection may be very different. How can the in vitro viral culture results be correlated with the risk of the SARS-CoV-2 transmission between humans remains to be determined. To solve this issue, the patients who are de-isolated by this protocol should be carefully followed up to investigate the risk of viral transmission between humans by epidemiological and contact tracing study. Unfortunately, this issue is not addressed by this study.

Another practical issue is the requirement of biosafety level 3 (BSL3) laboratory facility for carrying out the virus culture assay. BSL3 laboratory is not routinely available in most of healthcare institutions. This limitation makes the virus culture assay for SARS-CoV-2 somewhat impractical in this COVID-19 pandemic. In this study, Basile *et al* observed a good correlation between high viral titer and infectivity. Virus particles with the copy number below a certain threshold, they will fail to infect target cells. This result suggests the existence of a significant proportion of defective virions, which have been observed in other viruses, like HBV and HIV. Therefore, an alternative strategy is to use viral titer as an indirect marker for estimating viral infectivity. To fulfill this purpose, it requires development of the standardized viral quantification rRT-PCR assay, and determine the threshold of viral titer or Ct value in appropriately collected specimens, like nasopharyngeal and throat swabs, that allows for effective viral infection in vitro. If the viral titer measured by rRT-PCR is below the pre-defined threshold, like Ct value $\leq$ 32 in this study, the risk for transmission of COVID-19 is considered negligible and the patients can be safely de-isolated.

Isolation of individuals who continuously shed infectious SARS-CoV-2 is critical to curb the spread of COVID-19, whereas unnecessary prolonged quarantine of patients with persistent residual viral RNAs should be avoided. A convenient and standardized viral assay that can measure the viral infectivity may assist the clinicians to make a better decision on this issue.



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