Review Article

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Dynamics of viral RNA load, virus culture, seroconversion & infectivity in COVID-19 patients: Implications on isolation policy

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The ongoing SARS-CoV-2 pandemic has spread all over the world due to rapid person-to-person transmission. More information about viral load dynamics and replication is needed for clarity on duration of infectiousness of an individual, along with its implications on transmission. This is important to healthcare facilities and public health authorities in formulating guidance on the duration of isolation for patients and return to work criteria for healthcare workers. The duration of detection of viral RNA by molecular methods in the upper respiratory tract has ranged from 2 to 12 wk. Viral RNA detection by reverse transcription polymerase chain reaction (RT-PCR) does not necessarily mean that the individual is infectious to others, as the detected virus may not be replication competent. Infectious virus is generally not shed beyond 20 days of the onset of symptoms in most patients, including severely ill and immunocompromised, as indicated by failure to isolate replication-competent virus beyond this timeline in available studies. Further, detection of neutralizing antibodies in the serum, although associated with positive RT-PCR, is generally not associated with infectious virus shedding as indicated by negative viral cultures beyond this period. In this review, we analyze the current literature on the dynamics of viral load, culture, seroconversion and their implications on infectivity and the duration of isolation precautions for COVID-19 patients.

Key words Antibody - COVID-19 - culture - infectivity - seroconversion - viral load

The ongoing SARS-CoV-2 pandemic has spread all over the world, the WHO has since referred to this disease as COVID-19 and globally there have been 172,694,184 laboratory-confirmed cases, including 3,723,155 deaths as reported by the WHO as on May 31, 2021[1]. The infection can be particularly severe in the elderly and those with comorbidities such as hypertension, cardiovascular and cerebrovascular conditions, diabetes followed by cancer, renal disease and immunodeficiencies[2].

SARS-CoV-2 spreads primarily via droplets and aerosols from one person to another during coughing, sneezing or talking and when the individuals are in close and prolonged contact with each other[3,4]. Incubation period ranges from 2-12 days, more commonly 5-7 days. While patients with symptoms can transmit the infection, pre-symptomatic and asymptomatic people (who may constitute up to 40-45% of cases) can also transmit the infection[5-7].

Due to the serious implications of rapid spread of the virus to other people, it is important to identify and isolate the infected individual and also quarantine the high-risk contacts. Information about the viral load dynamics and replication is of paramount importance to healthcare facilities and public health authorities in formulating a guidance on the duration of isolation for patients and return to work criteria for healthcare workers (HCWs) as well as non-HCWs. In this review, we attempted to correlate the dynamics of viral RNA load, virus culture, seroconversion and infectivity in COVID-19 patients, based on the existing literature, and derive implications on isolation policy.

Dynamics of viral load, replication, infectivity and antibody detection

The important parameters affecting infectivity over the course of infection include the duration of viral RNA detection by reverse transcription polymerase chain reaction (RT-PCR), viral load the duration of viral culture positivity and the time taken to mount a neutralizing antibody response (seroconversion) (Table).

Viral load, virus culture and infectivity

The duration of detection of viral RNA by molecular methods such as RT-PCR in the upper respiratory tract has ranged from 2 to 12 wk[8,9], more commonly between 3 and 4 wk. In one study, even after symptoms had resolved, prolonged viral RNA shedding was seen in many patients, with a median duration of 53.5 days[17]. It is now understood that viral RNA detection by RT-PCR does not necessarily mean that the individual is infectious to others, as the detected RNA may not be of replication-competent virus. Wölfel *et al*[9] found that during the first week after symptom onset, pharyngeal viral shedding was maximum and showed a peak of 7.11×10^8 RNA copies/swab on day four. Their findings showed that viral cultures were negative eight days after onset of symptoms. They suggested that early discharge and home isolation could be followed for patients who completed 10 days after onset of symptoms and showed the presence of <100,000 viral RNA copies per ml of sputum. The study by Woodruff *et al*[18] showed that 53 per cent of the patients eligible as per the then CDC interim guidelines for removal from isolation continued to test positive for viral RNA and the mean time for the RT-PCR to become negative was 21.5 days after onset of symptoms.

A study by van Kampen *et al*[10], which included hospitalized patients (mostly immunocompromised and intensive care), found that the duration of shedding of infectious virus was up to 20 days (median 8 days) with a ≤5 per cent chance of isolating live virus after ≥15 days since the onset of symptoms. They also showed that the probability of positive virus culture increased with higher viral loads in terms of $\log_{10}$ RNA copies/ml (especially with viral loads of 7 $\log_{10}$ RNA copies/ml or more). This correlates with the United States' Centers for Disease Control (US-CDC) recommendation for isolation of COVID-19 patients with critical disease or severely immunocompromised patients[19], as the above study demonstrated high viral loads and culture positivity in critically ill/immunocompromised patients. Another point of interest suggested in the above study was that the probability of isolating infectious virus was ≤5 per cent when the viral load was less than 6 $\log_{10}$ RNA copies/ml[10]. Replication-competent virus was not found 21 days after symptom onset, and shedding of infectious virus ceased with development of neutralizing antibody. It is now known that viral shedding may continue up to a maximum of 8-12 wk after symptom onset; however, replication-competent virus may be not be present after 20 days in most individuals. It is not clear whether viable virus may still be present beyond this time period in some severely immunocompromised hosts.

The phenomenon of persistently positive or re-positive patients has been highlighted in the Korean CDC report[8] in which monitoring of 790 contacts of 245 re-positive patients showed that none of the contacts became positive for COVID-19 after exposure to the re-positive cases. Further, replication-competent virus was not detected in samples from COVID-19 recovered patients who developed fresh symptoms and were re-tested RT-PCR positive. However, re-positive patients who were tested for viral cultures were negative. Another study showed that 21.4 per cent patients had a positive test result of RT-PCR after two consecutive negative results, highlighting the possibility of prolonged viral shedding in some patients[20]. Testing re-positive after testing negative may also be related to the quality of specimen collected, in terms of lesser viral load in poor-quality specimens. This phenomenon of re-positive RT-PCR has important implications in contact tracing and prevention of infection. There is still a dilemma to define the criteria for a patient treated for SARS-CoV-2 to be declared as non-infective or

Table. Summary of selected published studies on duration of reverse transcription polymerase chain reaction (RT-PCR) positivity, viral culture positivity and time to seroconversion in COVID-19-positive patients

Study	Setting	Maximum duration of RT-PCR positivity in days (from onset of symptoms)	Maximum duration of viral culture positivity in days (from onset of symptoms)	Seroconversion in days (from onset of symptoms)
Wölfel *et al*[9]	Patients with mild or prodromal symptoms (n=9) Germany	28	8	14
van Kampen *et al*[10]	Majority of patients in intensive care and immune-compromised patients, the Netherlands (n=129)	-	20 (median eight days)	-
Korea CDC Report[8]	Korean CDC COVID Epidemiology Report - symptomatic patients, Korea (n=285)	82 (mean 44.9 days)	-	14
Liu *et al*[11]	Case report - Acute-onset fever (Taiwan)	63	18	
Bullard *et al*[12]	Public Health Laboratory, Canada; 90 samples		8	
Kujawski *et al*[13]	Mild to moderately severe illness, hospitalized and non-hospitalized, USA (n=12)	36 (median 19 days)	-	-
Hu *et al*[14]	Hospitalized patients diagnosed with COVID-19, Qingdao, China (n=59)	25 (median 20 days)	-	-
Arons *et al*[5]	Nursing facility/symptomatic and symptomatic; Washington (n=76)	-	9	-
To *et al*[15]	Hospitalized patients with mild-to-severe disease diagnosed with COVID-19 Hong Kong Hospital (n=23)	25	-	14
Qi *et al*[16]	Public Health Treatment Centre, Changsa, China (n=147), symptomatic patients	47 (median 17 days)		

CDC, Centers for Disease Control and Prevention

non-contagious. One way to decide this is to study samples that tested positive in RT-PCR for the virus and correlate these with the presence of virus in viral cultures. The RT-PCR detects any SARS-CoV-2 viral RNA (infectious whole-virus, unpackaged viral genome, even minus-strand or sub-genomic fragments and fragments that are generated by the destruction of viral infected cells by host immune cells), while culture detects properly packaged and viable virus particles. La Scola *et al*[21] studied 183 samples from 155 patients who tested positive by RT-PCR. Virus could be isolated in 129 samples. A significant relationship was found between the rate of positivity of culture and cycle threshold (Ct) values. They found that Ct values greater than 34 in the RT-PCR assays for *E* gene were consistently associated with negative viral cultures and suggested that such patients might not be contagious and considered for discharge from hospital care.

The risk of silent transmission with asymptomatic infection, and its role in rapid spread of SARS-CoV-2 has been highlighted in some studies. In the study by Arons *et al*[5], 56 per cent of residents with positive COVID-19 results were found to be asymptomatic at the time of testing. One-third of these were viral culture positive, whereas 17 of 24 symptomatic residents were positive for viral culture up to a maximum of nine days of onset of symptoms, all of whom had low Ct values (<23.1). To summarize, though the mean time to become RT-PCR negative is approximately 3-4 wk post-infection, the shedding of

infectious virus is limited to 10-20 days and correlates with lower cycle threshold or higher viral loads. The mean time for the RT-PCR tests to become negative is approximately 3-4 wk with the available systems, while the shedding of infectious virus may occur up to a maximum of 10-20 days, which is associated with a positive viral culture that is generally related to higher viral loads (7 $\log_{10}$ RNA copies/ml or more) and lower Ct values (Table).

Seroconversion and infectivity

The appearance of neutralizing antibodies is also associated with reduced risk of transmission. Once a host is infected with the virus, B cells produce neutralizing antibodies. These antibodies can defend against viral infection by blocking the entry of the virus in the host cells[22]. SARS-CoV-2 has four major structural proteins. These are spike (S), membrane (M), envelop and nucleic capsid (N) proteins. The coronavirus attacks the host cell through receptor binding domain (RBD) on the spike protein by initiating the cell fusion. The RBD on the spike protein situated on the S1 unit interacts directly with the host receptors[23]. The antibodies, especially IgG3 and IgG1, against these exhibit various functional characteristics that deliver an efficient response against viruses. This systemic response against viruses by these antibodies results in neutralization of virus in the host[24]. The studies by To *et al*[15] and Wölfel *et al*[9] showed that patients demonstrated highest viral load at the presentation of symptoms; however, it declined steadily thereafter. About a third of patients showed persistence of virus up to 20 days[10]. There was a significant correlation between anti-SARS-CoV-2-RBD IgG antibodies which are neutralizing antibodies and the virus neutralization titre[10]. This finding is important as it supports the need to use RBD antibodies to evaluate the infectiousness in patients with SARS-CoV-2 infection. The Korea CDC report[8] also showed that the re-positive patients who were negative for virus culture, were found to be positive for the antibodies. A serum neutralization antibody titre of 1:80 or more was also shown to be associated with non-infectiousness[10]. Wölfel *et al*[9] found that seroconversion occurred in all patients in 14 days and was associated with negative cultures but not with rapid decline in viral load. In summary, the findings showed that a positive serological neutralizing antibody response for the virus was associated with reduced infectivity, which can guide earlier disposition of infected patients from quarantine (Table).

The US-FDA has recently authorized new tests that give an estimate of the RBD antibodies in patients recovered from SARS-CoV-2 infection; these are semi-quantitative tests that do not give exact titre of the antibodies present but give an estimate of the amount of antibodies present in the patients[25].

Current guidelines on isolation for COVID-19 patients

The Ministry of Health and Family Welfare (MoHFW)[26], Government of India, has advised patients with mild-to-moderate disease be discharged after 10 days of onset of symptoms and three days of being symptom free. Patients who are not able to maintain their oxygen saturation are to be discharged upon resolution of symptoms and maintaining oxygen saturation for three days. All patients are advised to quarantine at home for further seven days after discharge. These patients do not need to be tested again for RT-PCR. Those with severe illness including immunocompromised patients such as those suffering from HIV and malignancy and post-transplant patients should be discharged after resolution of symptoms and testing negative for RT-PCR once.

The US-CDC[19,27], in the guidance on the discontinuation of transmission related precautions for COVID-19 positive patients in healthcare settings, have proposed two strategies: the symptom-based strategy and the test-based strategy. In the symptom-based strategy, for a patient to be discharged, at least 10 days (for mild/moderate illness) or 10 to 20 days (for severe/critical illness/severely immunocompromised) must have passed since the onset of symptoms along with resolution of symptoms such as cough, breathlessness and fever for at least 24 h. Examples of severely immunocompromised include patients on cancer chemotherapy, HIV positives with CD4 count <200/ml prednisolone >20 mg/day, solid organ or haematopoietic stem cell-transplant patients. In the test-based strategy, there should be complete resolution of fever and improvement of symptoms and two consecutive molecular viral RNA PCR assays collected ≥24 h apart must be negative. The time period after which a HCW can return to work after COVID-19 infection is important, considering the fact that a HCW may still be carrying live virus and may potentially infect patients while providing care. The Korean CDC report[8] recommends that, for individuals who develop new COVID-19–related symptoms within three months after the first onset of symptoms, re-testing for

COVID-19 may be done if no other aetiology can be ascribed to the illness. Isolation may be considered, if required, especially if there was high-risk contact.

The viral RNA RT-PCR may be persistently positive for prolonged periods as shown in some studies[8,11], but without the patient being infectious to others. This sets an important limitation in advocating the test-based strategy for deciding when to discontinue isolation precautions for COVID-19 patients.

Guidance on isolation

In view of the above, the symptom-based strategy for isolation proposed by the MoHFW and US-CDC of at least 10 days for mild/moderate illness from the onset of symptoms with resolution of symptoms followed by home isolation of seven days, appears worth implementing. These guidelines are supported by the findings that replication competent virus was not detected (as indicated by negative virus cultures) after 10 days of symptom onset in mild/moderate illness, thereby not posing an infection risk to others after that period (Figure). For severe/critical illness/severely immunocompromised patients, at least 10 and up to 20 days must have passed since the onset of symptoms and all symptoms such as cough and breathlessness must have resolved with the patient being afebrile without fever reducing medications for at least three days.

Some studies have reported detection of virus in blood (viraemia) after 10 days of symptom onset, especially in critically ill patients with poor outcomes[28,29]. In addition to this, testing for the presence of neutralizing antibodies (RBD antibodies) may be used to safeguard against spread of infection from persistently RT-PCR–positive individuals beyond the isolation period. Presence of neutralizing antibodies can also be used as an indicator for non-infectivity in those individuals retesting positive by RT-PCR after testing negative.

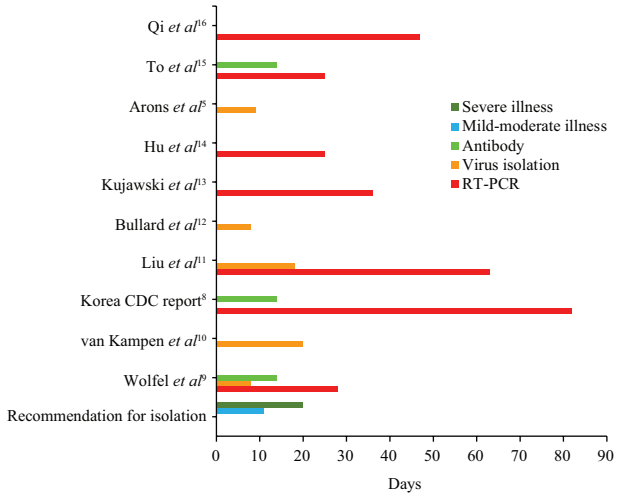

Figure. Graphical summary of timeline of reverse transcription polymerase chain reaction positivity, virus isolation and antibody production from selected studies.

Conclusion

While our understanding of the dynamics and kinetics of viral load, culture positivity, neutralizing antibody response and infectiousness in COVID-19 patients is continuously evolving, the status of actual infectivity as represented by viral culture positivity suggest that: (*i*) patients can be discharged from healthcare facility earlier without waiting for RT-PCR negative results, (*ii*) serology can indirectly suggest anticipated non-infectivity, and (*iii*) RT-PCR screening after infectivity window may not be necessary for quarantine guidance.

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