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# Differences in SARS-CoV-2 viral RNA re-positivity in discharged COVID-19 patients

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## Abstract

***The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly infectious RNA coronavirus responsible for the pandemic of the coronavirus disease 2019 (COVID-19). Recent advances in virology, epidemiology, diagnosis, and clinical management of COVID-19 have contributed to the control and prevention of this disease, but re-positivity of SARS-CoV-2 in recovered COVID-19 patients has brought a new challenge for this worldwide anti-viral battle. Reverse transcription polymerase chain reaction (RT-PCR) tests of the SARS-CoV-2 pathogen is widely used in clinical diagnosis, but a positive RT-PCR result may be multifactorial, including false positive, SARS-CoV-2 RNA fragment shedding, re-infection of SARS-CoV-2, or re-activation of COVID-19. Re-infection of SARS-CoV-2 or re-activation of COVID-19 is an indicator of live viral carriers and isolation/treatment is needed, but SARS-CoV-2 RNA fragment shedding is not. SARS-CoV-2 RNA is recently reported to integrate into the host genome, but the far-reaching outcome is currently unclear. Therefore, it is critical for appropriate manipulation and prevention of COVID-19 to distinguish these causal factors of SARS-CoV-2 re-positivity. In this review article, we updated the current knowledge of SARS-CoV-2 re-positivity in discharged COVID-19 patients with a focus on re-infection and re-activation. We proposed a hypothetical flowchart for handling of the SARS-CoV-2 re-positive cases.*** ***(AIDS Rev. 2021;23:153-163)***

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## Key words

***SARS-CoV-2. COVID-19. Polymerase chain reaction re-positivity. Re-infection. Re-activation.***

## Introduction

A novel RNA coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in Wuhan, China in December 2019. To date, Coronavirus Disease 2019 (COVID-19) caused by SARS-CoV-2 has spread over 200 countries as a pandemic. SARS-CoV-2 viruses are transmitted primarily through respiratory droplets during close face-to-face

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contacts; and SARS-CoV-2 infection causes respiratory diseases and may be followed by multiple organ complications in severe cases, including myocarditis, cardiomyopathy, ventricular arrhythmias, hemodynamic instability, acute cerebrovascular disease, encephalitis, and kidney damage[1]. While scientists are engaged in the epidemiology, transmission, viral variants, clinical management, and vaccine development, the re-positivity of SARS-CoV-2 in discharged COVID-19 patients has emerged as a critical issue in the clinical management and prevention of COVID-19[2].

The WHO's guidelines on clinical management suggests that COVID-19 patients may be discharged from hospitals after two consecutive negative results of reverse transcription polymerase chain reaction (RT-PCR) at least 24 h apart. Millions of patients with confirmed SARS-CoV-2 infection have been released from hospitals under this guideline, but an event emerging is the re-positivity of RT-PCR tests of SARS-CoV-2 in some discharged COVID-19 patients. Most of SARS-CoV-2 re-positive cases were asymptomatic, but some were indeed symptomatic, even more severe. Re-positivity of SARS-CoV-2 brings complexity of clinical management and prevention of COVID-19 patients as it could be multifactorial, such as false positive RT-PCR, remnants of the virus RNA fragments, re-infection of SARS-CoV-2, or re-activation of COVID-19 (also named relapse or recurrence of COVID-19). In brief, re-infection of SARS-CoV-2 indicates that a person who was infected and recovered once on is infected again with a new strain of SARS-CoV-2 virus. Re-activation of COVID-19 denotes the re-replication of the delitescent SARS-CoV-2 viruses and recurrence of COVID-19 symptoms in discharged patients. Basically, there are no new origins of SARS-CoV-2 viruses in re-activation subjects. In the literature, re-activation is also referred to as relapse or recurrence of COVID-19. False positive of RT-PCR and remnants of the virus RNA fragments are non-harmful, re-infection and re-activation are harmful, in which subjects carry infectious live viruses.

In June 2020, a 25-year-old Nevada man was identified as re-infection with a new strain of the SARS-CoV-2 virus and appeared with more severe symptoms at the second infection[3]. Thereafter, a SARS-CoV-2 re-infection case was reported in Hong Kong, who was identified as an asymptomatic infection of a new SARS-CoV-2 strain in a screening of travelers at the airport[4]. This subject was discharged 4 months ago after recovery from COVID-19 and travelled in Spain and the United Kingdom. In August 2020, Svenja et al., reported two COVID-19 re-activation cases, who presented fever or non-productive cough and were re-positive of SARS-CoV-2 RT-PCR tests after discharge[5]. These reports indicate that re-infection of SARS-CoV-2 and re-activation of COVID-19 indeed occurs, which brings a new challenge in clinical management and prevention of COVID-19, particularly in the asymptomatic re-infection. For precise medicine of avoiding inappropriate management or over-treatment, SARS-CoV-2 re-infection or re-activation of COVID-19 occurring in discharged COVID-19 patients need to be firmly discriminated from remnants of the virus RNA fragments, and a practical standard of procedure (SOP) may be needed as a guideline for clinicians and epidemiologists. This is the focus to be discussed in the current article.

## SARS-CoV-2 virus, host responses, and diseases

SARS-CoV-2 is a single-stranded RNA-enveloped virus, and is the third coronavirus that has caused pandemic respiratory diseases (i.e., COVID-19) in humans in the past two decades. The other two pandemics were severe acute respiratory syndrome (SARS) caused by SARS-CoV (also named SARS-CoV-1) and Middle East respiratory syndrome (MERS) caused by MERS-CoV. SARS-CoV-2 infects hosts through mucosal epithelial cells, such as nasal and bronchial epithelial cells and pneumocytes. Spike (S) protein of SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor located on the membrane of host cells, and then the transmembrane serine protease 2 (TMPRSS2) modulates SARS-CoV-2 entry through cleavage of ACE2 and activation of the SARS-CoV-2 S protein. Hence, S protein is an important mediator in SARS-CoV-2 infection and is a core target in development of vaccines against SARS-CoV-2 viruses (Fig. 1)[1]. After entry into host cells, SARS-CoV-2 RNA is released, replicated using the host cell machinery and then packaged into new protein envelopes as infectious virus particles. An infected host cell can produce and release hundreds of new SARS-CoV-2 viruses, rapidly promoting progression of the infection.

SARS-CoV-2 is primarily transmitted from person to person through respiratory droplets. The R0 (Basic reproduction number) value of SARS-CoV-2 is 1.4-5.5, similar to SARS-CoV at 2-5[6]. Therefore, exposure to an infected person who is either symptomatic or asymptomatic is associated with a high risk of transmission; and controlling social distance, wearing masks in

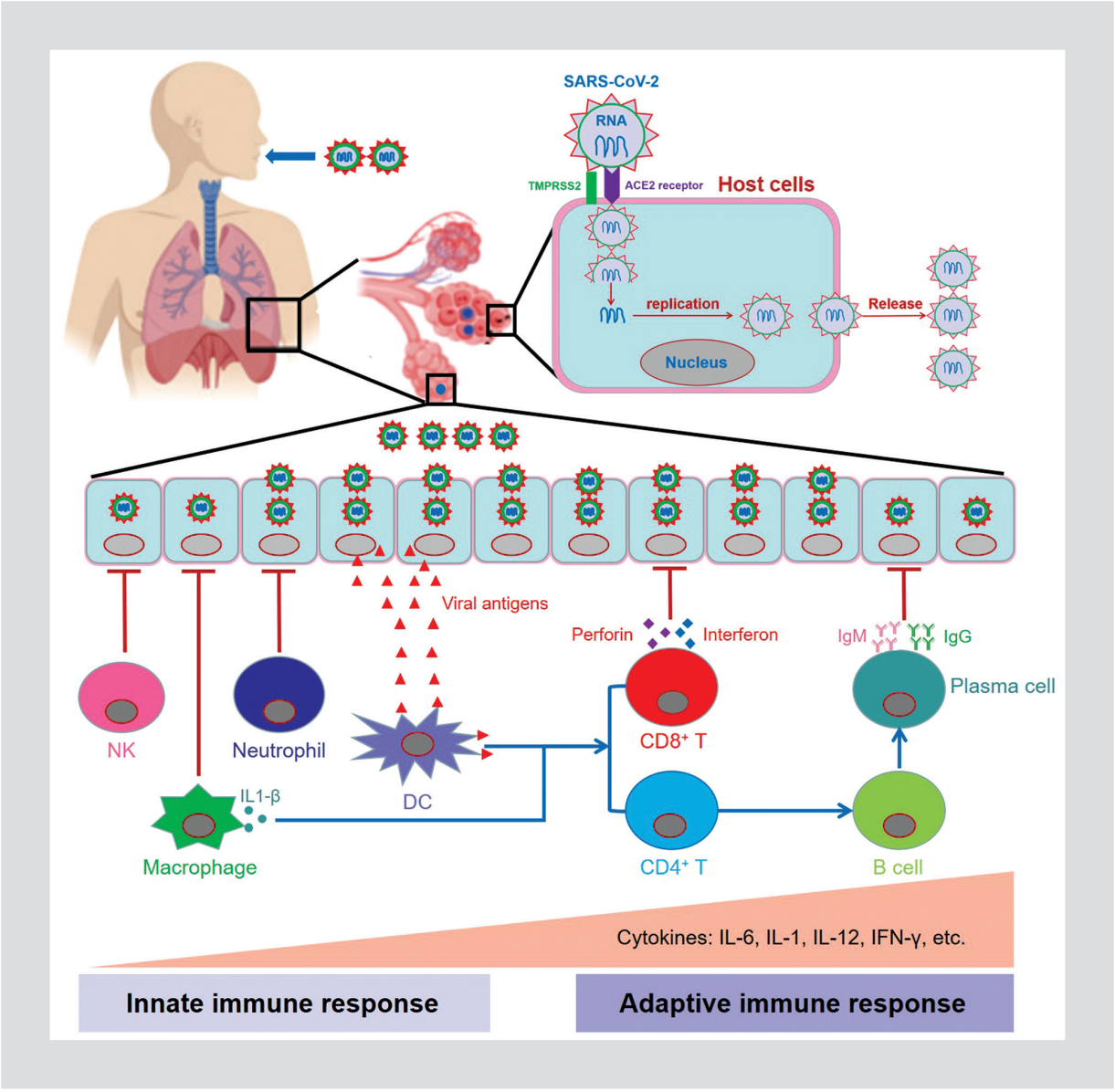


**Figure 1.** *SARS-CoV-2 viral infection and host immune response. SARS-CoV-2 targets cells through the S protein that binds to the ACE2 receptor. The TMPRSS2 on the host cell membrane promotes viral uptake by cleaving the ACE2 and activating the S protein. After endocytosis and subsequent uncoating, the SARS-CoV-2 produces new viruses using the machinery of host cells, followed by release through exocytosis. The recruitment of macrophages, NK cells, DCs, and neutrophils results in production of pro-inflammatory cytokines. Subsequently, helper T cells are activated and promote activation of CD8⁺ T cells and B cells. CD8+ T cells are effective in killing cells infected by viruses. Plasma cells derived from active B cells produce SARS-CoV-2 specific antibodies that may neutralize viruses. S protein: viral structural spike (S) protein, ACE2: angiotensin I-converting enzyme 2, TMPRSS2: transmembrane protease serine 2, NK: natural killer, DC: dendritic cells.*

public areas, and quarantining infected persons are strongly recommended to prevent viral transmission. Tracking and isolating the close contacts of a diagnosed patient are an important strategy to cutoff the transmission chain.

The immune response induced by SARS-CoV-2 includes both innate and adaptive immunity[7]. SARS-CoV-2 infection in lung leads to recruitment of T lymphocytes, monocytes, and neutrophils, which release chemokines and cytokines triggering inflammation. Subsequently, helper T cells are activated by viral antigens presented by antigen presenting cells (APC), and the activated helper T cells promote cytotoxic activity of CD8⁺ T cells and differentiation of B cells through release of cytokines, including IL-2, IL6, and IFN-γ. CD8⁺ T cells are effectors killing viral infected

cells. Plasma cells differentiated from B cells produce anti-SARS-CoV-2 antibodies, including IgG, IgM, and secretory IgA[7]. The antibodies play a protective role against re-infection after recovery from COVID-19. Anti-SARS-CoV-2 IgG antibody may be produced in most patients in 14 days after infection and could last several months after recovery from COVID-19. Thus, an effective and safe vaccine may protect subjects from infection of SARS-CoV-2.

Similar to other respiratory viral diseases, SARS-CoV-2 may trigger inflammatory response and local inflammation. In severe situations, SARS-CoV-2 may induce thrombotic complications, such as deep venous thrombosis, pulmonary embolism, and thrombotic arterial complications[1]. According to a study of 44,672 patients with SARS-CoV-2 infection, 81% of patients had no or mild symptoms, 14% had moderate to severe manifestations, and 5% had critical manifestations[8]. COVID-19 patients with mild symptoms were usually recovered at home without clinical management required, while patients with moderate or severe symptoms were usually monitored in the hospital. The most common symptoms in hospitalized patients were fever, dry cough, shortness of breath, fatigue, nausea/vomiting, diarrhea, and myalgia. Some patients also presented with non-classical symptoms, such as isolated gastrointestinal symptoms, olfactory, and/or gustatory dysfunctions[1]. More than 75% of hospitalized COVID-19 patients required supplemental oxygen therapy[1]. Drugs targeting viruses may be effective at the early stage of infection, while immunomodulatory agents may be helpful to prevent disease progression[1,9,10]. Anticoagulants may work to prevent thromboembolic complications[1].

## SARS-CoV-2 laboratory tests and diagnosis

RT-PCR based SARS-CoV-2 RNA detection from samples of the upper respiratory tract or stool is the gold-standard of COVID-19 diagnosis[1]. The SARS-CoV-2 RT-PCR is a PCR-based method which uses fluorescent probes and specific primers to detect specific regions of SARS-CoV-2. In RT-PCR tests, the term cycle threshold (Ct) refers to the number of cycles needed for a sample to amplify and cross a threshold to be considered positive. A Ct value is inversely proportional to abundance of the detected viral sequences in a sample. In re-infection and re-activation, live SARS-CoV-2 viruses are present and replicated, and thus the Ct value would be low. In contrast, in viral RNA fragment shedding, the copy number of detected viral sequences is low, and the Ct value would be high. In literature, a Ct > 35 is suggested to discriminate viral RNA fragment shedding from re-infection and re-activation[11]. However, the false-negative or false-positive RT-PCR results of SARS-CoV-2 occur and are potential concerns of SARS-CoV-2 diagnosis. Xie et al. reported that 13.66% of patients had a positive test result of RT-PCR after two consecutive negative tests in 161 discharged COVID-19 patients[12], and Xiao et al. reported 21.4% of false-negative RT-PCR tests in 70 discharged COVID-19 patients[13]. These reports indicate the frequency of false-positive and false-negative results in the SARS-CoV-2 RT-PCR tests. The false-positive RT-PCR tests of SARS-CoV-2 may stem from technical artifacts, non-specific cross reaction, and contamination[14], while the false-negative SARS-CoV-2 RT-PCR tests may be ascribed to technical artifacts, specimen collection techniques, time from exposure, and specimen sources[13]. The false negative/positive issues were usually solved through repetitive RT-PCR assays. Shedding of SARS-CoV-2 RNA fragments may be a more sophisticated concern in clinical diagnosis. The shed RNA fragments yield a true positive RT-PCR result, but are not live and not infectious. In literature reports, shed RNA fragments of SARS-CoV-2 may occur up to 92 days after discharge[15]. Several factors may link to the duration of SARS-CoV-2 RNA fragment shedding, including less active T-cell response, corticosteroid therapy, time from onset to hospitalization, fever, and lack of lopinavir/ritonavir therapy. Very recently, SARS-CoV-2 RNA was found to be reverse-transcribed and integrated into the human genome, which may lead to life-time shedding of SARS-CoV-2 RNA fragments[16].

Serological tests of anti-SARS-CoV-2 antibodies may be used as an auxiliary diagnostic method for suspected patients with a negative RT-PCR and for the identification of asymptomatic infections. In SARS-CoV-2, the S protein (SP) and N protein (NP) are the major immunogens, and most immunological serological diagnostics are based on the detection of antibodies against these antigens[7]. IgM antibody is presented earlier and detectable at a median of 5 days after onset while IgG antibody is detectable after 14 days, and thus IgM antibody is often used as an indicator of early infection. However, the positive rate of IgG was higher than IgM in reported data from SARS-CoV-2 infections. Moreover, the titers of IgG and IgM against SARS-CoV-2 in the severe patients were higher than those in the mild or asymptomatic cases. Other laboratory tests of COVID-19

include serum C-reactive protein, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, albumin, and lymphocyte and platelet counts[1]. These tests are mainly indicators of inflammation or inflammatory tissue injuries, but not specific to SARS-CoV-2. Instrumental chest computed tomographic imaging is an important clinical diagnostic method of COVID-19 patients and suspected patients[1].

Viral culture provides solid evidence of viral infection. SARS-CoV-2 viruses have been isolated and cultured by several groups in the world[17]. Whole genome sequencing (WGS) is a gold standard of identification and characterization of novel viral strains and/or variants. To date, several novel variants of the SARS-CoV-2 have been discovered by WGS, such as B.1.1.7 strain, B.1.429 strain, B.1.351 strain, and B.1.1.28.1 strain et al.[18]. Some of these variants contribute to the re-infection of SARS-CoV-2.

Yet viral culture and WGS are critical for pathogen diagnosis and identification of novel viral strains/variants, but they may not be a free access in clinical practice due to the technical and facility limitations. In contrast, serological tests of SARS-CoV-2 antibody IgM, which is present at a median of 5 days after onset, may not be as informative in pathogenic diagnosis as viral culture and WGS, but are more realistic in general clinical practice and thus are involved in our differential protocol.

## SARS-CoV-2 re-positivity in discharged COVID-19 patients: false positivity, viral RNA fragment shedding, re-infection, and re-activation

The WHO' s guideline on clinical management of COVID-19 states that patients with no clinical symptoms can be discharged from the hospital after two consecutive negative RT-PCR of SARS-CoV-2 at least 24 h apart. Discharged COVID-19 patients who become RT-PCR positive of SARS-CoV-2 are identified as re-positive of SARS-CoV-2. Lan et al. first reported four patients with re-positive RT-PCR of SARS-CoV-2 after recovery from COVID-19. Thereafter, a mega cohort study was reported by the Korea Center for Disease Control and Prevention, in which 292 (3.3%) of 8,922 recovered COVID-19 patients were re-positive of SARS-CoV-2[19]. To date, re-positivity of SARS-CoV-2 in discharged COVID-19 patients has been reported from different laboratories over the world.

SARS-CoV-2 re-positivity in discharged COVID-19 patients could stem from false positivity, viral RNA fragment shedding, re-infection, or re-activation. False positivity may be ruled out by repetitive tests, but viral RNA fragment shedding is more complicated. In literature reports, the duration of RNA fragment shedding was up to 92 days[15]. With the recent finding of SARS-CoV-2 integration into the host genome, the RNA fragment shedding may last for a life time. Nevertheless, false positivity and viral RNA fragment shedding are both clinically non- harmful and no action of prevention needs to be taken.

Since the first report of SARS-CoV-2 re-infection, several re-infection cases have been reported in the world[3,4,20-53]. The oldest case of SARS-CoV-2 re-infection was 92 years old, and the youngest was under 1 year old. Male patients accounted for 53.92% of all re-infective patients. Among all SARS-CoV-2 re-infection cases, five patients were asymptomatic and one patient was died at the second admission. SARS-CoV-2 specific IgG was positive in 24 cases of 30 tested patients with re-infection of SARS-CoV-2, while SARS-CoV-2 specific IgM was positive in 14 out of 17. Table 1 summarizes the reported SARS-CoV-2 re-infection cases.

Re-activation of COVID-19 following recovery and discharge from the hospitals has also been reported. Re-activation of COVID-19 indicates the re-activation of delitescent SARS-CoV-2 viruses in discharged patients. Table 2 summarizes the re-activation cases of COVID-19 reported thus far[2,5,6,54-69]. Gousseff et al. reported 11 cases of re-activation of COVID-19 in France[64]. All 11 patients presented mild to server symptoms during the second episode, and three cases were died. Another group in France reported three cases of re-activation of COVID-19, who all had severe symptoms at the second episode[59]. In two COVID-19 re-activation cases reported in Switzerland, one had moderate symptoms, and the other died[5]. Thus, re-activation of COVID-19 often had severe clinical symptoms, even death. Serological tests revealed that only one case was IgG negative in 18 re-activation patients tested while eight cases were negative for IgM in these patients. The duration from the first discharge to re-activation of COVID-19 ranged from 4 to 91 days. The age of patients with re-activation of COVID-19 ranged from 3 to 90 years old, and 66.67% of patients were male.

In summary, the multifactorial causes of SARS-CoV-2 re-positivity in discharged COVID-19 patients raise a sophisticated issue in the clinical management and prevention of the disease. The SARS-CoV-2 re-positivity caused by RNA fragment shedding is risk-free; the subjects are not SARS-CoV-2 carriers and not

**Table 1. Reported re-infection cases of SARS-CoV-2**

| Num | Case | Sex | Age | Days from Di to re-p | Sym in 1st | Sym in 2nd | Serology in 1st | | | Serology in 2nd | | | Sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | IgA | IgG | IgM | IgA | IgG | IgM | |
| 1 | 1 | M | 33 | 123 | Mild | Asy | N/A | - | N/A | N/A | + | N/A | Oro/Thro |
| 2 | 1 | F | 46 | 71 | Mild | Mild | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 3 | 1 | F | 48 | 59 | Mild | Asy | N/A | + | N/A | N/A | + | N/A | Naso |
| 4 | 1 | F | 36 | 84 | Mild | Mod | N/A | + | N/A | + | + | N/A | Nasal |
| 5 | 1 | F | 51 | 90 | Mild | Mild | N/A | N/A | N/A | + | + | + | Naso |
| 6 | 1 | M | 46 | 49 | Mild | Mod | N/A | N/A | N/A | N/A | + | + | Oro |
| 7 | 1 | M | 70 | 119 | Mild | Asy | N/A | + | N/A | N/A | N/A | N/A | Naso |
| 8 | 1 | M | 25 | 40 | Mild | Mild | N/A | N/A | N/A | N/A | + | + | Naso |
| 9 | 1 | F | 60s | 140 | Sev | Mild | + | + | + | + | + | + | Naso |
| 10 | 1 | M | 25 | 180 | Sev | Mild | + | + | + | N/A | N/A | N/A | Naso |
| 11 | 1 | M | 57 | 72 | Asy | Mild | N/A | N/A | N/A | N/A | + | + | Naso |
| 12 | 1 | F | 21 | 60 | Mild | Mod | N/A | N/A | N/A | N/A | N/A | N/A | Naso |
| 13 | 1 | F | 24 | 49 | Mild | Mild | N/A | N/A | N/A | N/A | + | + | Naso/Oro |
| 14 | 1 | F | 23 | 99 | Mild | Mild | N/A | N/A | N/A | N/A | + | + | Naso |
| 15 | 1 | M | 42 | 66 | Mod | Mild | N/A | + | N/A | N/A | + | N/A | Naso |
| 16 | 1 | F | 38 | 140 | Mod | Mod | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 17 | 1 | M | 58 | 60 | Mod | Mild | N/A | N/A | N/A | N/A | N/A | N/A | Naso |
| 18 | 1 | F | 89 | 59 | Sev | Died | N/A | N/A | N/A | N/A | - | - | Naso |
| 19 | 1 | F | 39 | 185 | Mild | Mild | - | + | - | + | + | + | Naso |
| 20 | 1 | M | 43 | 51 | Mild | Mod | N/A | N/A | N/A | N/A | + | N/A | N/A |
| 21 | 1 | M | 82 | 10 | Sev | Sev | N/A | N/A | N/A | N/A | N/A | N/A | Focal |
| 22 | 1 | F | 21 | 28 | Mild | Mild | N/A | + | N/A | N/A | + | N/A | Naso/Oro |

*(Continues)*

**Table 1. Reported re-infection cases of SARS-CoV-2 *(Continued)***

| Num | Case | Sex | Age | Days from Di to re-p | Sym in 1st | Sym in 2nd | Serology in 1st | | | Serology in 2nd | | | Sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | IgA | IgG | IgM | IgA | IgG | IgM | |
| 23 | 1 | F | 36 | 180 | Mild | Mild | N/A | + | N/A | N/A | N/A | N/A | Naso |
| 24 | 1 | N/A | 60s | 140 | Sev | Mod | N/A | N/A | N/A | + | + | + | Naso |
| 25 | 1 | M | 60s | 210 | Sev | Mild | N/A | + | + | N/A | + | - | Sali/Naso |
| 26 | 1 | F | 51 | 91 | Mild | Asy | N/A | N/A | N/A | N/A | N/A | N/A | Naso |
| 27 | 1 | F | 21 | 50 | Mild | Mild | N/A | + | N/A | N/A | + | N/A | Naso/Oro |
| 28 | 1 | M | 42 | 51 | Mild | Sev | N/A | N/A | N/A | N/A | + | N/A | N/A |
| 29 | 1 | M | 78 | 256 | Mild | Sev | - | - | - | - | - | - | Nos/ thro |
| 30 | 1 | F | 1 | 44 | Mild | Mild | N/A | N/A | N/A | N/A | N/A | N/A | Naso |
| 31 | 1 | M | 25 | 180 | Sev | Mild | N/A | N/A | N/A | N/A | N/A | N/A | Naso |
| 32 | 1 | M | 41 | 148 | Sev | Mod | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 33 | 1 | M | 28 | 267 | Mild | Sev | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 34 | 1 | M | 32 | 44 | Mild | Mild | N/A | + | N/A | N/A | + | N/A | N/A |
| 35 | 2 | M, F | 25, 28 | 100, 101 | Asy | Asy | N/A | N/A | N/A | N/A | N/A | N/A | Naso/Oro |
| 36 | 4 | 2 M, 2 F | 34-57 | 60 | Mild | Sev | 4- | 3-,1+ | 4- | 4+ | 4+ | 4+ | N/A |
| 37 | 54 | 48 M, 6 F | 16-57 | 45-129 | Mild | Mild | N/A | N/A | N/A | N/A | N/A | N/A | Naso/Oro |
| 38 | 258 | 119 M, 139 F | 20-70s | 40-81 | N/A | 220 No seve, 38 Sev | N/A | N/A | N/A | N/A | N/A | N/A | Naso |
| 39 | 32 | 18 M, 14 F | 0-69 | 17-126 | N/A | 30 Asy, 9 Sym | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 40 | 2 | 2 M | 61, 38 | 75, 18 | Asy, Mild | Mild | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 41 | 4 | 3 M, 1 F | 24-31 | 19-66 | 2 Asy, 2 Mild | Mild | N/A | N/A | N/A | N/A | - | N/A | Naso/Oro |
| 42 | 3 | 3 F | 29-50 | 92-282 | 2 Mild, Mod | Mod, 2 Mild | N/A | 1- | 1- | N/A | 1+ | 1+ | Naso |
| 43 | 3 | 3 M | 59-92 | 214-217 | 3 Mild | Sev, 2 Mild | N/A | N/A | N/A | N/A | N/A | N/A | Nasal/Naso |

Num: number; M: male; F: female; 1st: first episode; 2nd: second episode; Di: discharge; re-p: re-positivity; Sym: symptom; Asy: asymptotic; Mod: moderate; Sev: severe; Oro: oropharyngeal; Naso: nasopharyngeal.

**Table 2. Reported re-activation cases of COVID-19**

| Num | Case | Sex | Age | Days from Di to re-p | Sym in 1st | Sym in 2nd | Serology in 1st | | | Serology in 2nd | | | Sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | IgA | IgG | IgM | IgA | IgG | IgM | |
| 1 | 6 | 5M, 1F | 29-67 | 53-70 | Mild-Sev | Mild-Sev | N/A | N/A | N/A | N/A | N/A | N/A | Naso/Oro |
| 2 | 11 | 8M, 3F | 33-72 | 6-27 | Asy-Mod | Asy-Mod | N/A | N/A | N/A | N/A | 9+ | 6+, 3- | Oro |
| 3 | 1 | M | 68 | 23 | Mild | Mild | N/A | + | + | N/A | + | + | Naso |
| 4 | 2 | 2F | 77, 81 | 20, 26 | Sev, Mild | Mild, Mild | N/A | N/A | N/A | N/A | N/A | N/A | Naso |
| 5 | 1 | M | 41 | 9 | Mod | Mod | N/A | N/A | N/A | + | - | - | Naso/Oro |
| 6 | 1 | M | 26 | 31 | Mild | Sev | - | - | - | + | + | + | Naso/Oro |
| 7 | 1 | F | 57 | 4 | Mild | Mild | N/A | + | - | N/A | + | - | Naso |
| 8 | 1 | M | 40 | 5 | Sev | Sev | N/A | N/A | N/A | N/A | + | + | Oro |
| 9 | 1 | F | 69 | 23 | Mod | Asy | N/A | N/A | N/A | N/A | + | - | Naso |
| 10 | 3 | 1M, 2F | 84-90 | N/A | Sev | Died | N/A | N/A | N/A | N/A | N/A | N/A | Naso/Spu |
| 11 | 1 | M | 35 | 14 | Mild | Mild | N/A | N/A | N/A | N/A | + | - | Naso/Spu |
| 12 | 1 | M | 70 | 13 | Mod | Asy | N/A | N/A | N/A | N/A | + | + | Naso |
| 13 | 1 | M | 8 | 4 | Mild | Mild | N/A | N/A | N/A | N/A | N/A | N/A | Resp |
| 14 | 1 | M | 8 | 14 | Mild | Mild | N/A | N/A | N/A | N/A | N/A | N/A | Resp |
| 15 | 1 | M | 48 | 30 | Sev | Mod | N/A | + | + | N/A | + | - | Naso |
| 16 | 1 | M | 3 | 42 | Asy | Asy | N/A | N/A | N/A | N/A | + | N/A | Naso/Thr |
| 17 | 3 | 3M | 26-39 | 60-91 | 2Mild, 1Sev | 2Sev, 1Mod | N/A | N/A | N/A | N/A | N/A | N/A | Nasa/Naso |
| 18 | 1 | M | 69 | 18 | Mod | Mod | N/A | N/A | N/A | N/A | N/A | N/A | Naso |
| 19 | 5 | 2M, 3F | 27-42 | 4-17 | N/A | Mild | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 20 | 2 | 2F | 81, 77 | 36, 30 | Mild | Died, Sev | N/A | N/A | N/A | N/A | N/A | N/A | Naso |

Num: number; M: male; F: female; 1st: first episode; 2nd: second episode; Di: discharge; re-p: re-positivity; Sym: symptom; Asy: asymptotic; Mod: moderate; Sev: severe; Oro: oropharyngeal; Naso: nasopharyngeal; Spu: sputum; Thr: throat; Resp: respiratory.

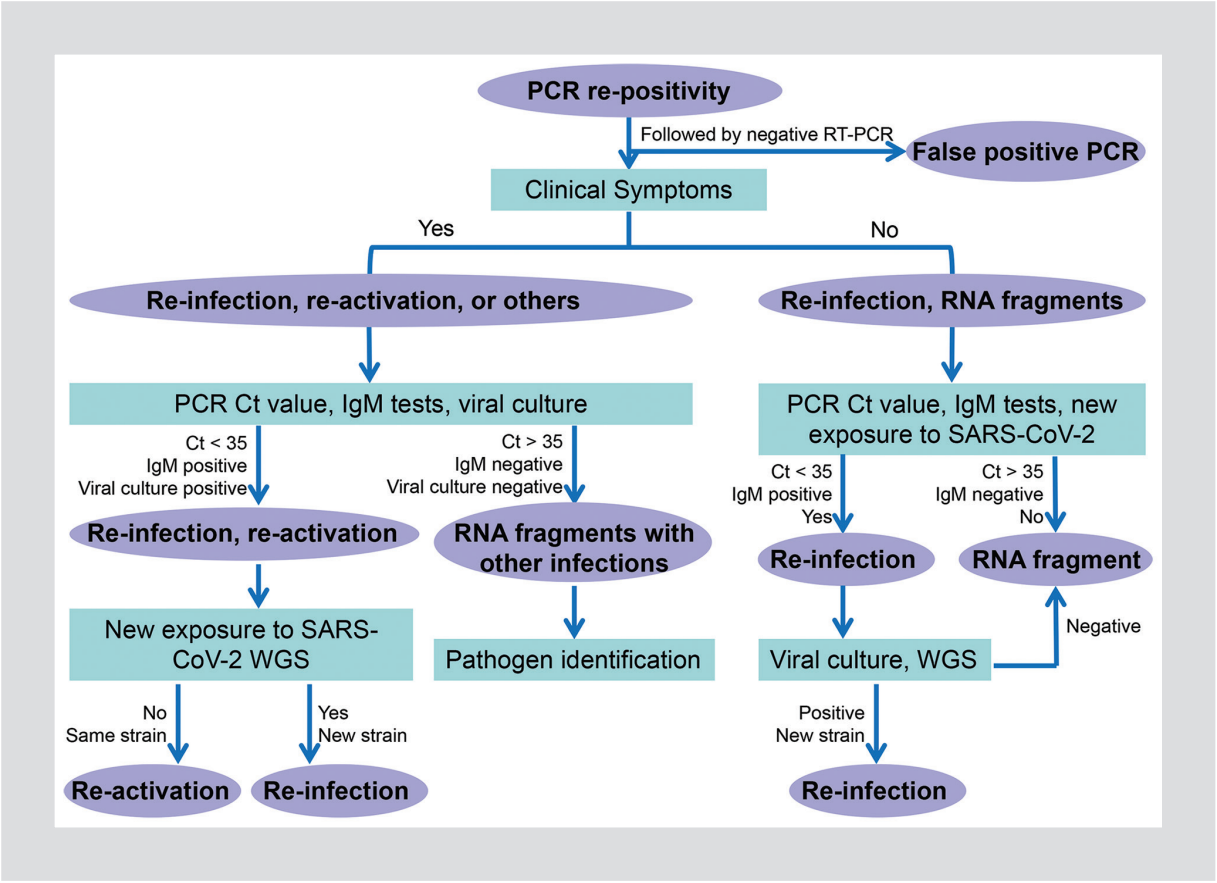


**Figure 2.** *A hypothetic flowchart for handling of SARS-CoV-2 re-positive cases. At first, false-positive RT-PCR is ruled out by a repetitive test. The true re-positive cases are then grouped by symptoms of COVID-19. Symptomatic group (left) includes re-infection, re-activation, or other infections with SARS-CoV-2 RNA fragment shedding (e.g., influenza), while asymptomatic group (right) includes asymptomatic re-infection and RNA fragment shedding. Then Ct cycles (35 cycles as a threshold), IgM tests, viral culture, and new exposure history would be used for further stratification of sub-groups. Viral WGS and genetic analysis is the gold standard of definition of re-infection and re-activation. RT-PCR: reverse transcription polymerase chain reaction, WGS: whole genome sequencing.*

infectious, and thus no clinical treatment and isolation are needed; in sharp contrast, re-infection of SARS-CoV-2, or re-activation of COVID-19 indicates presence of live viruses; the subjects are viral carriers and infectious, and thus clinical management or close monitoring is required. Close contacts may need to be tracked and appropriately isolated. However, practical criteria/guidelines of SARS-CoV-2 re-infection or COVID-19 re-activation are currently lacking. How to discriminate harmful SARS-CoV-2 re-positivity from non-harmful one is currently a top challenge in the clinical management and prevention of COVID-19 pandemic.

## A tentative proposal for handling of SARS-CoV-2 re-positivity

In view of the harmful nature of re-infection of SARS-CoV-2 and re-activation of COVID-19, scientists tried to set up criteria for identification, but as increasing knowledge of this disease, the limitations and confusion of these criteria emerges. For instance, in the SARS-CoV-2 re-infection criteria proposed by Sara et al., the time of patients with a confirmed re-positive SARS-CoV-2 PCR was set at 28 days after recovery from COVID-19[70]. However, prolonged SARS-CoV-2 RNA fragment shedding was up to 92 days[15]. With the integration of SARS-CoV-2 into the host genome, the shedding of RNA fragments may even last for lifetime[16]. A novel SOP for manipulation of SARS-CoV-2 re-positive cases in discharged COVID-19 patients may be urgently needed. After deep evaluation of literature, we proposed a flowchart of handling SARS-CoV-2 re-positive tests in discharged COVID-19 patients (Fig. 2).

A positive SARS-CoV-2 RT-PCR in discharged COVID-19 patients would be repeated first to rule out

false positivity. Re-activation or relapse of COVID-19 by definition indicates the presence of clinical symptoms; the patients with re-infection of SARS-CoV-2 may or may not have symptoms while RNA fragment shedding is asymptomatic. Therefore, clinical symptoms of COVID-19 would group the SARS-CoV-2 re-positive cases into two groups. Symptomatic group may include re-infection, re-activation and others, such as RNA fragment shedding with non- SARS-CoV-2 infection (e.g., influenza); and asymptomatic groups group would include RNA fragment shedding and asymptomatic re-infection. Re-infection and re-activation imply the existence of live SARS-CoV-2 viruses with viral replication, and thus may have more viral RNA copies compared to RNA shedding cases. By literature, SARS-CoV-2 RT-PCR of re-infection and re-activation may be strong-positive, usually < 35 Ct, while RNA fragment shedding is weak-positive, > 35 Ct. Viral culture may provide solid evidence of live viruses. Serum IgM usually indicates recent viral infection or replication, and thus is also indicative of re-infection and re-activation. In addition, a re-exposure history of SARS-CoV-2 may be informative to re-infection. Finally, WGS of SARS-CoV-2 viruses to identify the viral genetics would provide solid evidence of re-infection or re-activation, if the case had sequencing data available in the first episode.

## Conclusions

The SARS-CoV-2 re-positivity in discharged COVID-19 patients has been challenging the current post-treatment management and prevention. Proper stratification of non-harmful false positivity and viral RNA fragment shedding from harmful re-infection of SARS-CoV-2 and re-activation of COVID-19 is critical in the efforts to eventually control this disaster pandemic and minimize the waste of exhausting medical resources. The tentative flowchart of handling SARS-CoV-2 re-positive cases proposed herein may provide a practical guideline for the management of recovered COVID-19 patients. We understand this guideline may need to be perfected as the increased of knowledge of this pandemic, but we hope that our effort could intrigue a constructive debate.

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