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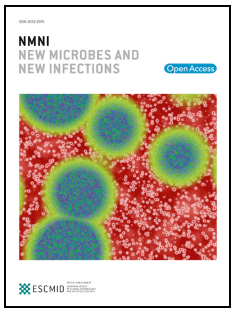

Re-positive COVID-19 PCR test: could it be a reinfection?

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Title: Re-positive COVID-19 PCR test: Could it be a reinfection?

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Re-positive COVID-19 PCR test: Could it be a reinfection?

Abstract

The coronavirus disease 2019 (COVID-19) outbreak started in December 2019 and rapidly spread around the globe as a major health threat. Several reports on re-positive cases subsequent to discharge from hospitals caught our attention. We aimed to highlight real-time polymerase chain reaction (RT-qPCR) positivity re-detection after discharge from the isolation, with special consideration on possible reasons behind it. We found that re-positive RT-qPCR assays for SARS-CoV-2 after prior negative results might be attributed to false-negative laboratory results and prolonged viral shedding, rather than re-infection. These findings are encouraging and should be validated in a larger cohort.

Keywords: COVID-19; false-negative; RT-qPCR, low viral load, SARS-CoV-2, CT scan

Introduction

The coronavirus disease 2019 (COVID-19) outbreak started in December 2019, spread around the globe, and has become an unprecedented major health issue. As of 3 July 2020, COVID-19 is responsible for 12,964,809 confirmed cases including 570,288 fatalities across 216 countries, and the number of cases is still increasing rapidly [1]. Symptoms of COVID-19 include fever, cough, shortness of breath, headache, sore throat, fatigue, loss of taste or smell, nausea, vomiting, and diarrhoea [2]. Most cases of COVID-19 are mild, whereas some patients (14%) develop more severe forms of disease

requiring oxygen therapy in hospital, and about 5% needing intensive care unit admission [3]. In severe cases of COVID-19, complications such as acute respiratory distress syndrome (ARDS), sepsis, septic shock, and multiorgan failure have been reported [4]. In the mild form of COVID-19, patients are usually admitted to the hospital to receive standard treatment, and if their conditions improve, they will be discharged according to the protocols and guidelines issued by local health authorities. According to the guidelines, they discharge patients with no fever for > 3 days and at least had two consecutive negative results of RT-qPCR testing, and no symptoms at the time of discharge from hospital [5]. Several reports on re-positive cases subsequent to discharge from hospitals in China and other countries caught our attention. Here, we report our review on these reports. We aimed to highlight RT-qPCR positivity re-detected after discharge from the isolation, with special consideration on possible reasons behind it.

Reports on re-positive PCR assay after discharge

The phenomenon of re-positive PCR for COVID-19 has been widely reported as an emerging global pandemic control challenge. One of the largest case series of re-positive COVID-19 was reported by Korea Centers for Disease Control and Prevention (KCDC), in which they conducted an extensive epidemiological investigation involving 285 re-positive cases and 790 contacts. During their routine screening on asymptomatic patients, KCDC reported a high detection of re-positive cases of 44.7% (126 out of 284) among the asymptomatic patients [6].

Yujian et al. in Guangdong, China, investigated the clinical and laboratory characteristics of seven patients who were readmitted due to re-positive PCR assays. While being

isolated in the hospital, four were positive for rectal swabs only, two were positive for throat swabs, and one had positive throat and rectal swabs [7]. Another study by Li and colleagues in Chongqing, China focused on identifying the 19 patients who had positive RT-qPCR results after being discharged [8]. In Guangzhou, China, Dabiao et al. reported that 41 women were tested positive after two consecutive negative results [9]. Anming from Wuhan, China, reported a case involving a woman aged 58 years with persistent fluctuating results for COVID-19 test [10]. Another report on fluctuating results was presented in a study by Yuanyuan et al. in Wuhan, China involving two cases [11]. From a study in Chongqing, China, Yan et al. reported the results of four patients, three of whom had positive results for nasopharyngeal swabs, and one had positive result for anal swab three days after discharge [12]. In Shenzhen, China, a study found that 20 out of 182 asymptomatic patients (10.99 %) were positive after initial negative results for SARS-CoV-2 RNA [13]. A case report involving a 41-year-old man from Chengdu, China, despite having recovered from COVID-19, was readmitted due to positive nasal swabs, sputum, and stool; however, the RT-qPCR results of throat swabs turned out to be negative [14]. Lifei et al. identified cases from Shenzhen, China, in which recurrent positive results accounted for 8.3% (35 out of 420) of cases [15]. Another study conducted in Shanghai, China, reported that 11 patients (16.7%) in the convalescent stage had persistent positive stool results for viral RNA [16]. A case report involving a 72-year-old woman from South Korea highlighted persistent positive RT-qPCR results six days after two negative results; though the patient completely recovered after the second positive test [17]. Another study from South Korea by Guangming et al. noted that five out of 55 (9%) had reactivation of SARS-CoV-2, in whom four had mild symptoms,

whereas one patient was asymptomatic [18]. Svenja et al. conducted a study in Switzerland on the identification of two old women with underlying heart diseases. They had positive test results after 18 and 21 days of two consecutive negative results for nasopharyngeal swabs [19]. On 17 April 2020, a case report from South Korea highlighted that 163 out of 7,829 patients (2.1%) were tested positive and most of them (66.9%) were females [20]. Another case report from Italy identified a 48-year-old man who had a severe form of the disease. The patient recovered and was discharged after tested negative using RT-qPCR, but the presence of IgM and IgG anti-SARS-CoV-2 antibodies was detected. Over time, he developed dyspnoea and chest pain, and became positive when retested [21]. Zhongxiao et al. presented a case report from Jiangsu, China, in which a 56-year-old man and his daughter (21 years old) were diagnosed with COVID-19 and were discharged after negative results. However, 17 days later, both had positive results for nucleic acid swab test [22]. Lan and colleagues in Wuhan, China, presented a report of four medical professionals who had positive test results after two negative assay results. RT-qPCR tests were repeated 5–13 days later, and all were tested positive [23]. Kenneth et al. reported three cases of patients with improved COVID-19 and discharged one week later; were tested positive for nasopharyngeal and saliva swabs during first follow-up, but with mild symptoms [24]. A summary of the previous reports is shown in Table 1.

Timing of testing positive from discharge: Taken all these studies together, the median time of being tested positive from discharge was 12 days (range, 1-37 days) [6-10,14,18,19,21-23].

Symptoms of re-positive cases: Most patients had mild symptoms [20]. Some cases had cough, sore throat [6]; dyspnoea, chest pain [22]; and fever, cough, dyspnoea, sore throat, and fatigue [18].

Contact tracing of re-positive case: For all the reported re-positive cases, no studies have reported any evidence of contact with suspected or confirmed cases [7,23, 24]. KCDC investigated 285 re-positive cases and 790 contacts. Over a 14-day duration of contact tracing, 27 of the contacts were positive, of which 24 (88.9%) were previously confirmed cases, while the remaining three (11.1%) newly confirmed cases were contacts who had been exposed to the re-positive cases [6].

Results of the presence of anti-SARS-CoV-2 antibodies in re-positive cases: Several studies have investigated the presence of antibodies in re-positive cases. KCDC reported that 96% of the 23 re-positive cases were found to be positive for neutralising antibodies [6]. Another study reported that IgM and IgG anti-SARS-CoV-2 antibodies were detected [21].

Real-time RT-PCRs

Real-time reverse transcriptase-PCR (RT-PCR) has become a popular molecular tool employed to detect coronavirus. In principle, PCR is used to amplify specific target gene sequence into huge number of copies using sequence specific primers and a DNA polymerase enzyme [25].

Viral load and test results: Accurate detection and measurement of viral load is crucial for clinical practice and decision making. RT-qPCR could be used to directly quantify viral load by observing the fluorescence signal that proportionally increases with the amount of nucleic acid. This test serves to confirm the positivity of a case under investigation based on a specified threshold of detected fluorescence and a certain number of PCR cycles. A high cycle threshold (Ct) value indicate low viral load. A Ct value of 40 is a cut-off point commonly used in many laboratories.

Sensitivity and accuracy of real-time RT PCR: Many researchers reported that sensitivity and specificity of the real-time RT-PCR test are greatly varied and lack of consistency. A systematic review has revealed rates of false negative between 2% and 29% (sensitivity of 71-98%) [26], possibly due to differences in personnel competency level, standards of laboratory practice, nucleic acid extraction method used, targeted DNA sequence, probe and primer design, sampling procedures, timing for peak viral load in the patient, and sampling site during specimen collection. Some researchers reported that sputum is the most accurate specimen, followed by nasal swabs, and throat swabs are least suitable for the diagnosis of COVID-19 [27]. Another study found that the sensitivity of bronchoalveolar lavage samples was 93%, sputum samples 72%, nasal swabs 63%, and throat swabs were the least suitable, at 32% [28].

Validation of different PCR techniques: There are different real-time RT-PCR assays commonly used for targeting on different SARS-CoV-2 genomic regions, including

ORF8 regions, ORF1b, spike (S), nucleocapsid (N), envelope (E) genes, or RNA-dependent RNA polymerase (RdRP) [29]. These gene-specific primers may also affect the results of the tests due to the variation in targeted viral RNA sequences. Limit of detection (LOD) of COVID-19 tests can be validated by applying intact virus to yield better detection of actual samples compared to using nucleotide sequence. Therefore, improved PCR techniques with higher amplification efficiency are now routinely used, such as the addition of a second primer pair or a multiple-target gene amplification, and the use of probing primer sets that are designed to minimise misdetection.

Limitations of RT-PCR: RT-PCR test detects the genetic material of the virus, but it does not differentiate between live and dead virus. Therefore, the gold standard for detection of live virus is viral culture. Another limitation of the test is the false negative result which may be attributed to low level of viral RNA that does not reach the LOD of the test. Hence, despite a negative result, there remains a possibility of undetected infection.

Possible explanations for positive SARS-CoV-2 RT-qPCR after negative results

Reactivation of the virus: Guangming et al. suggested the possibility of viral reactivation [18] and proposed three categories of risk factors: host immunity status, virologic factors, and type and degree of immunosuppression [18]. Another study suggested that some patients could be virus carriers after recovery [23]. Additionally, Jiajun et al. found that most of the investigated cases were asymptomatic, and with low viral loads. Therefore, they attributed this phenomenon to low viral load rather than the reactivation of SARS-CoV-2 [8]. In the study conducted by KCDC, 108 re-positive cases were found to have

negative results for viral cell culture. Further investigation on 76 re-positive cases using RT-qPCR revealed that most patients (89.5%) were positive at cycle threshold values above 30, indicating low viral loads which were undetected. However, these findings were limited in interpretation since it could not explain the actual viral load in either the patients or the collected samples. They also found that 23 (96%) were tested positive for neutralising antibodies [6]. Another study found evidence of positive IgM and IgG in 8 of 16 patients [8], indicating the presence of active immunity and ongoing infection.

Persistent infection: Peipei et al. confirmed the presence of significant lesions detected on serial CT images that were not resolved in re-positive cases [22]. Prolonged viral shedding was detected using respiratory swabs in a 71-year-old woman 60 days after the onset of symptoms, and 36 days after symptoms had subsided [30]. Researchers have suggested certain factors that may be associated with protracted viral shedding, including gender, delayed admission, and cases requiring mechanical ventilation [31]. Therefore, prolonged viral shedding may explain persistent infection in re-positive cases.

New infection with the same strain: This hypothesis seems to be unwarranted because all investigated patients were self-quarantined at home and were not exposed or in contact with confirmed cases, as stated in a previous study [22].

New infection with another strain: Some evidence suggest that the virus is evolving. Some strains might coexist, such as the European, North American, and Asian strains, with the possibility of different mutation patterns [32].

185

186 *Laboratory errors (false-negative/positive, or sample contamination):* Early diagnosis
187 and treatment of COVID-19 is the fundamental approach for the prevention and control
188 of this health crisis. Hence, clinical manifestations alone cannot accurately diagnose
189 COVID-19, as many patients are asymptomatic or have mild or clear respiratory
190 symptoms. Nucleic acid assays have the ability to detect viruses using rapid and validated
191 methods. Particularly, PCR assay is considered the 'gold standard' for the investigation
192 of viruses. RT-qPCR is considered one of the most commonly used methods to detect
193 SARS-CoV-2 [33,34,35]. However, RT-qPCR method could not differentiate between
194 infectious and non-infectious RNA [19] and it has a certain risk of false-negative results
195 due to low levels of viral load. After false-negative results identified in a case report in
196 China, investigators performed re-testing using RT-qPCR for throat swab specimens,
197 which yield positive results [36]. Xingzhi et al. reported five symptomatic patients with
198 false-negative RT-qPCR but typical findings of ground-glass appearance were detected
199 using computed tomography (CT) scans [37]. Remaining three patients had negative
200 throat swabs but positive rectal swabs, so they needed to continue their quarantine [7]. A
201 case report from China involving a woman aged 58 years with COVID-19 indicated
202 fluctuations in her results from positive to negative [10]. Another case of fluctuating
203 results involved a patient in whom test results changed from negative to positive
204 repeatedly [11]. Another study investigated patients using RT-qPCR for SARS-CoV-2
205 and found a high false-negative rate of 12.5% (48 out of 384 assays) [38]. Differences in
206 results from different sample sites have been reported. Some evidence suggests the
207 possibility of viral shedding in faeces for long durations, extending into fifth week after

respiratory samples became negative [16,39,40]. Differences in respiratory swab results were observed in a 49-year-old man. His sputum was tested positive for much longer than throat swab detection [41]. Another case report involved a 41-year-old man from Chengdu, China, who was readmitted after recovery from COVID-19. His nasal swabs, sputum, and stool samples tested positive, while his throat swabs were negative [14]. Therefore, it is possible for re-positive results to be persistent infections, as patients could be tested falsely negative at discharge.

Infection with other respiratory viruses: When a patient develops symptoms again after being discharged and tested negative, there is a possibility of new infection with other types of influenzas or corona species. A study of 93 patients identified new infections in two cases with adenovirus (2.2%) and one case of bocavirus (1.1%) [6].

Conclusions

We conclude that re-positive RT-qPCR assays for SARS-CoV-2 after prior negative results might be attributed to false-negative laboratory results and prolonged viral shedding, rather than re-infection. Considering the significance of this ongoing global public health emergency, it is necessary to carry out large scale and multicentre studies to better understand the issue of potential SARS-CoV-2 recurrence in patients with COVID-19. Prevention of re-positive testing is a fundamental measure in containing the outbreak, in addition to proper diagnosis and treatment. We would suggest that health authorities need to consider the importance of maintaining social distancing, even after treating the infection and discharging the patient, and to encourage patients to comply

with strict post recovery home isolation for at least two weeks. Moreover, they should consider adding RT-qPCR testing for rectal swabs and low-dose CT to the criteria for patient discharge. Finally, there is a need to re-assess the guidelines for patient discharge.

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Conflict of Interest

The authors have disclosed there is no potential conflicts of interest, financial or otherwise.

CRediT Author Statement

Author 1: Conceptualization, Methodology, Data curation, Writing- Original draft preparation. **Author 2**: Data curation, Writing- Original draft preparation. **Author 3**: Data curation, Writing-Reviewing and Editing.

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References:

[1] World Health Organization. Coronavirus disease (COVID-19) Pandemic. Coronavirus Disease (COVID-19) Pandemic 2020. https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed 14 July 2020).

[2] Centers for Disease Control and Prevention. Coronavirus disease 2019 (COVID-

19)– Symptoms. CDC 2020. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html (accessed 3 July 2020).

[3] World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: interim guidance. WHO 2020;2019:12.

[4] Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020;8:475–81. https://doi.org/10.1016/S2213-2600(20)30079-5.

[5] European Centre for Disease Prevention and Control. Novel coronavirus (SARS-CoV-2): Discharge criteria for confirmed COVID-19 cases. ECDC Tech Rep 2020:1–5.

[6] Korea Centers for Disease Control and Prevention. Findings from investigation and analysis of re-positive cases | Press Release | News Room: KCDC 2020. https://www.cdc.go.kr/board/board.es?mid=a30402000000&bid=0030&act=view&list_no=367267&nPage=8 (accessed 3 July 2020).

[7] Zhang B, Liu S, Dong Y, Zhang L, Zhong Q, Zou Y, et al. Positive rectal swabs in young patients recovered from coronavirus disease 2019 (COVID-19). J Infect 2020:10–3. https://doi.org/10.1016/j.jinf.2020.04.023.

[8] Li J, Wei X, Tian W, Zou J, Wang Y, Xue W, et al. Clinical features of discharged COVID-19 patients with an extended SARS-CoV-2 RNA positive signal in respiratory samples. Virus Res 2020:198047. https://doi.org/10.1016/j.virusres.2020. 198047.

277 [9] Chen D, Xu W, Lei Z, Huang Z, Liu J, Gao Z, et al. Recurrence of positive SARS-
278 CoV-2 RNA in COVID-19: A case report. Int J Infect Dis 2020;93:297–9.
279 https://doi.org/10.1016/j.ijid.2020.03.003.
280 [10] Luo A. Positive SARS-Cov-2 test in a woman with COVID-19 at 22 days after
281 hospital discharge: A case report. J Tradit Chinese Med Sci 2020:1–5.
282 https://doi.org/10.1016/j.jtcms.2020.04.001.
283 [11] Xing Y, Mo P, Xiao Y, Zhao O, Zhang Y, Wang F. Post-discharge surveillance
284 and positive virus detection in two medical staff recovered from coronavirus
285 disease 2019 (COVID-19), China, January to February 2020. Eurosurveillance
286 2020;25:12–6. https://doi.org/10.2807/1560-7917.ES.2020.25.10.2000191.
287 [12] Chen Y, Bai W, Liu B, Huang J, Laurent I, Chen F, et al. Re-evaluation of retested
288 nucleic acid-positive cases in recovered COVID-19 patients: Report from a
289 designated transfer hospital in Chongqing, China. J Infect Public Health 2020:8–
290 10. https://doi.org/10.1016/j.jiph.2020.06.008.
291 [13] Yuan B, Chen Y, Zhang K, Wang C. Recurrence of positive SARS-CoV-2 viral
292 RNA in recovered COVID-19 patients during medical isolation observation
293 *Current status: under review*. Nat Res n.d.:1–12. https://doi.org/10.21203/rs.3.rs-
294 22829/v1.
295 [14] Li XJ, Zhang ZW, Zong ZY. A case of a readmitted patient who recovered from
296 COVID-19 in Chengdu, China. Crit Care 2020;24:8–10.
297 https://doi.org/10.1186/s13054-020-02877-8.
298 [15] Wang L-F, Yan H, Zhang D, Yun Y, Zhang C, An C, et al. Clinical characteristics
299 and imaging findings of 35 patients recovered from COVID-19 with recurrent

positive RT-PCR test results. SSRN Electron J 2020. https://doi.org/10.2139/ssrn.3564400.

[16] Ling Y, Xu SB, Lin YX, Tian D, Zhu ZQ, Dai FH, et al. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. Chin Med J (Engl) 2020;133:1039–43. https://doi.org/10.1097/CM9.0000000000000774.

[17] Chae KJ, Jin GY, Lee CS, Lee HB, Lee JH, Kwon KS. Positive conversion of COVID-19 after two consecutive negative RT-PCR results: A role of low-dose CT. Eur J Radiol 2020;129:109122. https://doi.org/10.1016/j.ejrad.2020.109122.

[18] Ye G, Pan Z, Pan Y, Deng Q, Chen L, Li J, et al. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. J Infect 2020;80:e14–7. https://doi.org/10.1016/j.jinf.2020.03.001.

[19] Ravioli S, Ochsner H, Lindner G. Reactivation of COVID-19 pneumonia: A report of two cases. J Infect 2020:12–3. https://doi.org/10.1016/j.jinf.2020.05.008.

[20] Kang Y-J, Joo H. South Korea's COVID-19 infection status: From the perspective of reconfirmation after complete recovery. J Pure Appl Microbiol 2020;14:1073–5. https://doi.org/10.22207/jpam.14.spl1.46.

[21] Loconsole D, Passerini F, Palmieri VO, Centrone F, Sallustio A, Pugliese S, et al. Recurrence of COVID-19 after recovery: a case report from Italy. Infection 2020:3–5. https://doi.org/10.1007/s15010-020-01444-1.

[22] Dou P, Zhang S, Wang C, Cai L, Liu Z, Xu Q, et al. Serial CT features in discharged COVID-19 patients with positive RT-PCR re-test. Eur J Radiol 2020;127:24–7. https://doi.org/10.1016/j.ejrad.2020.109010.

[23] Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR test results in

patients recovered from COVID-19. JAMA - J Am Med Assoc 2020;323:1502–3. https://doi.org/10.1001/jama.2020.2783.

[24] Zheng KI, Wang XB, Jin XH, Liu WY, Gao F, Chen YP, et al. A Case series of recurrent viral RNA positivity in recovered COVID-19 Chinese patients. J Gen Intern Med 2020:7–8. https://doi.org/10.1007/s11606-020-05822-1.

[25] Shen M, Zhou Y, Ye J, Abdullah AL-Maskri AA, Kang Y, Zeng S, et al. Recent advances and perspectives of nucleic acid detection for coronavirus. J Pharm Anal 2020;10:97–101. https://doi.org/10.1016/j.jpha.2020.02.010.

[26] Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, Zambrano-Achig P, del Campo R, Ciapponi A, et al. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. MedRxiv 2020:2020.04.16.20066787. https://doi.org/10.1101/2020.04.16.20066787.

[27] Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J, et al. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. MedRxiv 2020:2020.02.11.20021493. https://doi.org/10.1101/2020.02.11.20021493.

[28] Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020;323:1843–4. https://doi.org/10.1001/jama.2020.3786.

[29] Carter LJ, Garner L V, Smoot JW, Li Y, Zhou Q, Saveson CJ, et al. Assay techniques and test development for COVID-19 diagnosis. ACS Cent Sci 2020;6:591–605. https://doi.org/10.1021/acscentsci.0c00501.

[30] Li J, Zhang L, Liu B, Song D. Case Report: Viral shedding for 60 days in a woman

with COVID-19. Am J Trop Med Hyg 2020;102:1210–3. https://doi.org/10.4269/ajtmh.20-0275.

[31] Xu K, Chen Y, Yuan J, Yi P, Ding C, Wu W, et al. Factors associated with prolonged viral RNA shedding in patients with COVID-19. Clin Infect Dis 2020;2019:1–8. https://doi.org/10.1093/cid/ciaa351.

[32] Pachetti M, Marini B, Benedetti F, Giudici F, Mauro E, Storici P, et al. Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. J Transl Med 2020;18:1–9. https://doi.org/10.1186/s12967-020-02344-6.

[33] Shen M, Zhou Y, Ye J, Abdullah AL-maskri AA, Kang Y, Zeng S, et al. Recent advances and perspectives of nucleic acid detection for coronavirus. J Pharm Anal 2020;10:97–101. https://doi.org/10.1016/j.jpha.2020.02.010.

[34] Wan Z, Zhang Y, He Z, Liu J, Lan K, Hu Y, et al. A melting curve-based multiplex RT-qPCR assay for simultaneous detection of four human coronaviruses. Int J Mol Sci 2016;17:1–11. https://doi.org/10.3390/ijms17111880.

[35] Noh JY, Yoon SW, Kim DJ, Lee MS, Kim JH, Na W, et al. Simultaneous detection of severe acute respiratory syndrome, Middle East respiratory syndrome, and related bat coronaviruses by real-time reverse transcription PCR. Arch Virol 2017;162:1617–23. https://doi.org/10.1007/s00705-017-3281-9.

[36] Li D, Wang D, Dong J, Wang N, Huang H, Xu H, et al. False-negative results of real-time reverse-transcriptase polymerase chain reaction for severe acute respiratory syndrome coronavirus 2: Role of deep-learning-based ct diagnosis and insights from two cases. Korean J Radiol 2020;21:505–8.

https://doi.org/10.3348/kjr.2020.0146.

[37] Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for typical 2019-nCoV pneumonia: Relationship to negative RT-PCR testing. Radiology 2020:200343. https://doi.org/10.1148/radiol.2020200343.

[38] Li Y, Yao L, Li J, Chen L, Song Y, Cai Z, et al. Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19. J Med Virol 2020;92:903–8. https://doi.org/10.1002/jmv.25786.

[39] Chen L, Lou J, Bai Y, Wang M. COVID-19 disease with positive fecal and negative pharyngeal and sputum viral tests. Am J Gastroenterol 2020;115:790. https://doi.org/10.14309/ajg.0000000000000610.

[40] Wu Y, Guo C, Tang L, Hong Z, Zhou J, Dong X, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol Hepatol 2020;5:434–5. https://doi.org/10.1016/S2468-1253(20)30083-2.

[41] Qu YM, Kang EM, Cong HY. Positive result of SARS-CoV-2 in sputum from a cured patient with COVID-19. Travel Med Infect Dis 2020;34:101619. https://doi.org/10.1016/j.tmaid.2020.101619.

388 **Table 1. Summary of the reports on PCR re-positive COVID-19 cases**
389

No.	First Author	Country/ Date	N	Male/%	Age/year	Type of sample	Timing of re-positive from discharge	Symptomatic/ asymptomatic	Severity	Ct value: below/ Above 30	Main findings and/or conclusions
1	KCDC [6]	Korea, May 2020	285	31 (33.3%) of viral culture	-	-	1-37 (14.3)	126/158	-	8/68	No infectivity
2	Zhang et al. [7]	China, Jan-Feb 2020	7	6 (85.7%)	10 months – 35 years	Throat, rectal swabs	7-11	4/3	Mild (85.7%)	-	Recovered patients may still be virus carriers, longer positive rectal swab
3	Li et al. [8]	China, Feb 2020	19	12 (63.2)	48 (18-71)	Throat	1-10 (4.4)	0/19	Mild (78.9%)	2/17	Longer positive throat swabs represent non-infectious virus
4	Chen et al. [9]	China, Feb 2020	1	1 female	46	Oropharyngeal	2	0/1	Mild	-	False negative
5	Luo A. [10]	China, Mar 2020	1	1 female	58	Throat	22	0/1	No symptoms	-	Incomplete clearance of the virus, false negative
6	Xing et al. [11]	China, Feb 2020	2	1(50%)	20, 40	Throat	2-3	0/2	No symptoms	-	Recovered patients may had a small amount of virus
7	Chen et al. [12]	China, Jan - Feb 2020	4	2 (50%)	12, 29, 38, 49	Nasopharyngeal, anal swabs	3	0/4	No symptoms	-	False negative or positive results do not mean there is

											live virus
8	Yuan et al. [13]	China, Jan - Feb 2020	20	7 (35%)	41.5 (1-72)	Nasopharyngeal, anal swabs	7, 14	0/20	No symptoms	-	Recovered patients might still carry virus
9	Li et al. [14]	China, Feb 2020	1	1 (100%)	41	Nasal swabs, sputum, and stool	18	1/0	Mild symptoms	-	Some patients may have a long repeatable process
10	Wang et al. [15]	China, Jan – Mar 2020	35	15 (42%)	32 (21 - 45)	Nasopharyngeal, anal swabs	10 (7-16)	0/35	No symptoms	-	Persistent virus in the body, patients still in a recovery process
11	Ling et al. [16]	China, Feb 2020	11	28 (42.4%) from all investigated patients	44 (34 – 62)	Stool	2-22	-	-	-	Virus may be transmitted through the digestive tract or re-transmitted through aerosols
12	Chae et al. [17]	South Korea	1	1 female	72	Nasopharyngeal,	6	-	-	-	Reconsidered discharging patients based on mismatched radiologic and PCR results
13	Ye et al. [18]	China, Feb 2020	5	2 (40%)	27 - 42	Respiratory tract	4-17	4/1	Mild symptoms	-	Reactivation
14	Ravioli et al. [19]	Switzerland	2	2 females	77, 81	Nasopharyngeal	18, 25	2/0	Severe symptoms	-	Reactivation assumed. Re-infection unlikely

15	Kang et al. [20]	South Korea, Apr 2020	163	53 (33.1%)	(20- 29) most of them	Nasopharyngeal	13.5 (1-35)	61	Mild symptoms	-	Reactivation
16	Loconsole et al. [21]	Italy, May 2020	1	1 (100%)	48	Nasopharyngeal	30	1	Moderate symptoms	-	Reactivation
17	Dou et al. [22]	China, Jan - Feb 2020	2	1 (50%)	21, 56	Throat, anal swabs	17	-	-	-	False negative
18	Lan et al. [23]	China, Jan - Feb 2020	4	2 (50%)	31 - 36	Throat swabs	5-13	0/4	No symptoms	-	Some of the recovered patients may be virus carriers and positivity is unlikely due to reinfection
19	Zheng et al. [24]	China, Jan - Feb 2020	3	-	-	Salivary and faecal	7	0/3	No symptoms	-	

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Highlights:

- Re-positive RT-qPCR attributed by false-negative and prolonged viral shedding.
- RT-qPCR for rectal swabs and low-dose CT as criteria for patient discharge.
- Re-infection of SARS-CoV-2 not warranted.