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## Case Report

## A case of extremely prolonged viral shedding: Could cell cultures be a diagnostic tool to drive COVID-19 patient discharge?



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## ABSTRACT

This study addressed the case of a patient with prolonged COVID-19 viral shedding, reported by Real-Time PCR, until 71 days from symptom onset. However, viral culture received negative results after 30 days from symptom onset. Therefore, viral culture may be a worthwhile test for patients requiring discharge, in particular for those presenting prolonged viral shedding.

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As of 09 November 2020 the World Health Organization (WHO) reported a total of 50,232,068 confirmed cases of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection and 1,254,567 deaths, of which 960,373 and 41,750, respectively, were registered in Italy (Italian Ministry of Civil Protection Department and Italy, 2020; World Health Organization. WHO Coronavirus Disease COVID-19 Dashboard, 2020). This virus can cause a wide spectrum of clinical manifestations ranging from asymptomatic cases to alveolar-interstitial pneumonia with severe respiratory failure and acute respiratory distress syndrome (ARDS). Social distancing and quarantine are the most effective measures against virus spread. Therefore, it is of paramount importance to estimate the persistence of competent SARS-CoV-2 virions. Numerous studies have been published on the matter so far, showing variable duration of SARS-CoV-2 viral shedding in humans, ranging 4–34 days; three patients have been reported to have had persistent viral shedding for 37, 45 and 60 days (Qian et al., 2020; Zhou et al., 2020; Zhang et al., 2020; Li et al., 2020). Xu et al. found that male gender, delayed hospitalisation and mechanical ventilation are factors

independently associated with prolonged viral shedding (Xu et al., 2020). It is unclear whether patients with prolonged viral shedding may infect other people, since data on in vitro replication of the virus in this population are lacking.

This paper reports the case of a patient with persistently positive SARS-CoV-2 nasopharyngeal swabs (NPS), which were all used for viral isolation in cell culture. The patient was an 84-year-old man with a history of COPD, valvulopathy, atrial fibrillation being treated with acenocoumarol and prostatic cancer being treated with bicalutamide; he had been vaccinated against influenza.

He presented at the Emergency Room Department on the 26 February 2020 complaining of dyspnoea and dry cough since the 20 February 2020. After the diagnosis of SARS-CoV-2-related pneumonia, he was admitted to the Infectious Disease ward. Upon admission, lung auscultation revealed diminished vesicular breath sounds with rales on the lower left lung. Low-flow oxygen was administered due to poor peripheral oxygen saturation levels. No deterioration of neurological status was observed. After obtaining informed consent, antiviral therapy was commenced with lopinavir/ritonavir and hydroxychloroquine (Chakraborty et al., 2020). The patient also received a seven-day course of ceftriaxone and a five-day course of azithromycin, since community-acquired pneumonia could not be ruled out. Acenocoumarol was changed to

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**Table 1**  
Data of the 14 nasopharyngeal swabs collected from 02 March to 10 May 2020.

Collection date (dd/mm/year)	Real-time PCR UTM						Viral culture CPE
	RdRp	Ct	N	Ct	E	Ct	
02/03/2020	Detected	20	Detected	20	Detected	20	Positive
12/03/2020	Detected	17	Detected	17	Detected	17	Positive
17/03/2020	Detected	23	Detected	23	Detected	23	Positive
20/03/2020	Detected	24	Detected	24	Detected	25	Positive
23/03/2020	Detected	19	Detected	18	Detected	19	Positive
26/03/2020	Detected	24	Detected	24	Detected	25	Positive
30/03/2020	Detected	28	Detected	27	Detected	29	Negative
06/04/2020	N/D	N/D	Detected	30	N/D	N/D	Negative
13/04/2020	N/D	N/D	Detected	37	N/D	N/D	Negative
17/04/2020	N/D	N/D	Detected	40	N/D	N/D	Negative
24/04/2020	N/D	N/D	Detected	38	N/D	N/D	Negative
01/05/2020	Detected	28	Detected	27	Detected	29	Negative
08/05/2020	N/D	N/D	N/D	N/D	N/D	N/D	N/A
10/05/2020	N/D	N/D	N/D	N/D	N/D	N/D	N/A

The molecular analysis was performed using a three target gene Real-Time PCR (RdRp, N, E); the Cycle threshold value is reported for each detected target.

The presence of cytopathic effect on cell culture (Positive) provided evidence of viable virus.

The two last swabs were not tested because of negative Real-Time PCR results.

Abbreviations: NPS, nasopharyngeal swab; RdRp, RNA-dependent RNA polymerase; N, nucleocapsid; E, envelope; Ct, cycle threshold; CPE, cytopathic effect; N/D, not detected; N/A, not available.

fondaparinux to avoid potential drug-drug interactions. C-reactive protein (CRP) gradually decreased (from 183 to 29 mg/L) and chest X-ray improved during hospitalisation. As of 15 March, the patient was asymptomatic, did not need oxygen therapy and had a respiratory rate <22 breaths/minute. Therefore, he was considered clinically recovered.

Given the patient's advanced age, many comorbidities and need of assistance, and since nursing homes could not accept people infected with SARS-CoV-2, it was decided that he should stay in hospital until he could be declared not infective (i.e. after 14 days from clinical recovery and two negative NPSs performed 24 h apart, according to the Italian Ministry of Health dispositions at that time). However, the first negative NPS was reported on 08 May, after more than 11 weeks from symptom onset and several consecutive positive NPSs during the 74 days of hospitalisation. The patient was discharged on 10 May. It is noteworthy that serological tests performed with SARS-CoV-2 IgG and IgM chemiluminescence immunoassay (CLIA) kits on iFlash1800 analyzer (Shenzen YHLO Biotech Co., Ltd., Shenzhen, China) were negative on 24 April and 04 May for both IgG and IgM (cut-off, 10 AU/mL). In addition, a Real-Time PCR (RT-PCR) was performed on the same sera, employing the automated ELITE InGenius® system with the GeneFinder™ COVID-19 Plus RealAmp Kit assay (ELITechGroup, France) also used for swab processing: none of the three target genes (*E*, *N*, *RdRP*) had a Ct value <43, which is the limit of detection of the assay.

Considering the unusual long-lasting positivity, the presence of a replication-competent virus in all 12 available positive NPSs was investigated by means of virus culture: briefly, 1 mL of each sample plus 5 mL of growth medium were added to Vero E6 cells in 25 cm<sup>2</sup> cell culture flasks, which were incubated at 37 °C and 5% CO<sub>2</sub> and daily checked for cytopathic effect (CPE). After 72 h, CPE was detected in six of 12 flasks and confirmed by GeneFinder™ RT-PCR on supernatant, corresponding to the first six swabs; interestingly, a relationship between Ct-value and culture positivity was observed (Table 1).

Most studies have demonstrated that viable virus can be successfully isolated and cultured from nasal, throat or sputum samples up to 9 days after symptom onset in patients with positive SARS-CoV-2 PCR on NPS (Wölfel et al., 2020; Arons et al., 2020). A group from Taiwan was able to isolate SARS-CoV-2 from cultures of sputum samples in one patient up to 18 days after symptom onset

(Liu et al., 2020). It is believed that the current patient had the longest viral shedding on PCR reported so far: 71 days from symptom onset. Furthermore, viable virus from nasopharyngeal samples was isolated up to 30 days after symptom onset, which is the longest period of time compared with previously available studies. Viral shedding is often used as a marker of infectivity to determine quarantine duration. However, RT-PCR test can only detect part of the viral genome and cannot differentiate between vital and non-vital virus. A common and relevant issue in clinical practice is the duration of quarantine in patients with prolonged viral shedding and no symptoms. The current patient was considered clinically recovered on 15 March but had persistently positive NPS. When viral cultures were retrospectively performed, no viable virus could be detected after 30 March, indicating that he was not infective and could have been discharged much earlier, reducing the risk of hospital-acquired infection and healthcare expenses. Interestingly, this patient did not seroconvert; this event usually occurs by day 7 in half of patients and by day 15 in most of them. However, it is still unclear whether antibody production is sufficient to stop viral transmission to other individuals, since viral shedding has been shown after seroconversion, with successful isolation of viable SARS-CoV-2 in cultures (Widders et al., 2020). Undetected serum SARS-CoV-2 RNA also confirms that a patient is not in a critical clinical condition, at least at serum sampling time points, as already reported (Chen et al., 2020).

In conclusion, these findings are in agreement with previous studies suggesting that patients with prolonged viral shedding on NPS are able to transmit the virus only in the early phases of infection. Moreover, considering the relationship between the RT-PCR and cell culture positivity, the evaluation of Ct-value may be useful to drive a patient's discharge (La Scola et al., 2020). Further studies on the relationship between RT-PCR, antibody production, virus viability, and infection rates are needed to estimate timing and criteria for non-infectivity.

#### Conflict of interest

The authors declare no conflict of interest.

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None declare.

## Ethical approval

This study did not require ethical approval. The original data are anonymous.

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