Persistent viral infectivity after 27 days from COVID-19 symptoms onset

The persistence of SARS-CoV-2 in pharyngeal swabs belonging to patients affected by COVID-19 is not a rare occurrence. Indeed, the viral load determined through real-time reverse-transcriptase PCR (rRT-PCR) test peaks at the onset of symptoms and decreases to undetectable level within 1–3 weeks.  

Nonetheless, few studies investigated the potential infectivity of the clinical specimens, and to date virus culture isolation failed after some weeks from the first symptoms, being the 18th day the last timing in which virus replication in vitro was achieved in immunocompetent host. 2

Here we report the case of SARS-CoV-2 infection positivity at the molecular test after 42 days, describing successful viral replication in vitro obtained after 27 days from the first positive swab.

A 58-year-old man resulted positive to SARS-CoV-2 during routine screening for health workers; he was initially asymptomatic. He underwent isolation and was treated with luctoferrin, vitamin C and D. The subsequent day after testing positive, swab specimens from his wife and daughter yielded positive for SARS-CoV-2 infection; however, they returned negative after 15 and 10 days, respectively. After 4 days he developed fever (max 38.7°C) and cough and was treated with paracetamol and levolcomerastine. At the seventh day after the swab positivity he aggravated and presented dyspnoea exertional, although with 94%–97% saturation and no pulmonary abnormalities evidenced by direct lung auscultation; treatment with azithromycin (500 mg/day) for 6 days was started. After 3 days of treatment, he did not show fever. After 23 days he continued to have a cough that was successfully treated with prednisone (50 mg/day for 4 days, 25 mg/day for 2 days, 12.5 mg/day for 2 days). At the haematologic analysis the unique alteration regarded an increment of D-dimer (D-dimer=996 ng/mL), a characteristic associated with a hypercoagulative status often found in severe cases of COVID-19. 3

Treatment with fondaparinux (2.5 mg/day for 10 days) was initiated. The leucocytes number were in the normal level range with a slight decrement of lymphocytes (8.82 × 10^9/L lymphocytes, 6.56 × 10^9/L neutrophils, 1.39 × 10^9/L lymphocytes). After 42 days he showed diffuse respiratory wheezing in forced expiration with pulmonary obstructive features deduced through direct lung auscultation, and therapy with symbicort 160/45 (two inhalations every 12 hours) was started. After 48 days, the molecular test was negative. After 49 days D-dimer returned to normal level (420 ng/mL), leucocytes decremented (6.14 × 10^9/L leucocytes, 2.48 × 10^9/L neutrophils, 2.83 × 10^9/L lymphocytes) and antibody anti-SARS-CoV-2 S1/S2 (193 AU/mL) were detected (Figure 1).

Table 1 Molecular results from the swabs obtained during the COVID-19

<table>
<thead>
<tr>
<th>Days</th>
<th>E gene</th>
<th>RdPR gene</th>
<th>N gene</th>
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<tbody>
<tr>
<td>0</td>
<td>37.06</td>
<td>37.65</td>
<td>33.43</td>
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<td>20</td>
<td>37.68</td>
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<td>30</td>
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<td>36</td>
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The swabs were submitted to thermolysis (15 µL of samples mixed with 45 µL of distilled water protocol: 98°C for 3’ and 4°C for 5’). Then, 5 µL were tested with the Allplex SARS-CoV-2 assay (Seegene, Seoul, South Korea) on the CFX Connect Real-Time PCR Detection System (Bio-rad, Hercules, California, USA) following manufacturer’s instructions.

The cycle threshold for E, RdPR and N genes are displayed.

ND, not detected.

A total of seven swabs were collected during the course of the COVID-19 and tested for the viral E, RdR and N genes with the Allplex SARS-CoV-2 assay (Seegene, Seoul, South Korea) (table 1). The analysis showed a positivity with a cycle threshold above 35 for all the timings.

After 3 weeks of swab positivity, the Division of Laboratory Medicine (ASUGI, Trieste, Italy) decided to attempt the viral culture in order to unravel if the patient still presented infectious secretions.

Briefly, the third swab (27 days after the first positivity) was filtered and transferred to a monolayer of Vero E6 cells and then monitored for 7 days. The molecular detection of SARS-CoV-2 by RT-PCR showed a decrement of cycle threshold and a relative increment of the viral load at days 2, 5 and 7 after the virus inoculation (from E+4 copies/mL to E+10 copies/mL, figure 2), as well as an evident cytopathic effect at the seventh day (figure 2).

Since the patient remained positive at the subsequent swab, the viral isolation was further tried but unsuccessfully. Concomitantly, the serologic test, performed with a chemiluminescent microparticle immunoassay (CMIA, SARS-CoV-2 IgG assay, Abbott, Chicago, Illinois, USA) displayed a signal to cut-off ratio of 7.74 for antinucleocapsid IgG. Therefore, the serum was tested against SARS-CoV-2 in vitro, showing the viral neutralisation even at a dilution of 1:100 (figure 3). At this time the patient still presented respiratory symptoms, but probably the immune system was now able to partially counteract the infection, since the viral isolation was not achieved. Nevertheless, the sixth swab (42 days) resulted in a new positivity, with a high Ct, however the virus replication in vitro failed again. Finally, the seventh swab (48 days) resulted as negative at the molecular test and the patient was released from isolation.

![Figure 1](http://jcp.bmj.com/) Schematic representation of the disease course of the patient.

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The cycle threshold for E, RdPR and N genes are displayed.

ND, not detected.
WHO criteria for patient discharge recommended the ending of isolation 10 days after symptom onset, plus at least three additional days without symptoms (including fever and respiratory features), nevertheless prolonged viral RNA presence can occur. In the present case report, viral infectivity was observed after 27 days from the first swab obtained in an asymptomatic phase. Noteworthy, the subsequent attempts to isolate the virus failed, probably due to a positive tail of viral RNA not linked with the presence of infectious virions, and therefore not able to establish infection in vitro and probably not contagious. Moreover, it can be speculated that initially the patient was not able to produce neutralising antibodies but after some weeks the immune response became efficient in counteracting the virus. An immunophenotypic characterisation (not available for this study), like the analysis of the CD8+ T cells could add more information about the cellular immune response activated by the patient. Possible issue related to technical aspects should be also considered, as the swab collection, storage and processing that may have not maintained the virus viable.

The duration of infectivity after the symptoms upset is still a matter of debate in the scientific community, indeed, the positivity at the molecular test does not imply that the virus is intact (the RT-PCR investigated a small viral genome fraction) and still infectious. A deep understanding of the COVID-19 infection course will help to interpret the virus shedding kinetics and the potential contagiousness of the affected individuals, indeed, in the case here described, the transmission of SARS-CoV-2 to patient’s cohabitants occurred, although the Ct at the rRT-PCR were high and the patient still asymptomatic at the beginning of the COVID-19.

Our results also showed that in this case there is no correlation between the Ct and the residual infectivity, since all the swabs yielded a weak positivity during the course of COVID-19. Moreover, very interestingly, although the specimen collected at the third analysis revealed a high Ct value at the rRT-PCR (above 35), it was still able to lead to viral isolation in vitro even after 27 days from the first positive molecular test. Being aware that we just described a single case, our findings suggested that it is necessary to not underestimate individuals with weak positivity and to consider all the patients affected with COVID-19 as potential spreaders.
Figure 3  Neutralisation assays. Pictures at 20× magnification and crystal violet staining are shown. SARS-CoV-2 was neutralised with serum diluted at 1:10 (A, G), 1:50 (B, H), 1:100 (C, I). Panels D, J showed the not treated well, meanwhile panel E, K and F, L the wells infected with SARS-CoV-2 (100 PFU). A signal to cut-off ratio of 1.5 of antinucleocapsid IgG was detected by using the SARS-CoV-2 IgG chemiluminescent microparticle immunoassay (CMIA, Abbott, Chicago, Illinois, USA) on the architect i2000SR instrument (Abbott, Chicago, Illinois, USA). Therefore, neutralisation assay was performed. Briefly, serum was inactivated for 30′ at 56°C and then diluted at 1:10, 1:50 and 1:100 with MEM (Minimum Essential Medium with Earle’s Salts, supplemented with 2% fetal bovine serum, 8 mM glutamine, 100 U/mL penicillin, 100 mg/L streptomycin and 2.5 µg/mL amphotericin B, Euroclone, Pero, Italy). About 200 µL of diluted serum was incubated with 200 µL of SARS-CoV-2 (200 PFU) for 1 hour at 37°C, then 200 µL were transferred to Vero E cells (200 000 Vero cells in 12 multiwell plates) for 1 hour at 37°C. At the end of the experimental procedures, the supernatants were removed and replaced with a new one composed by MEM (Minimum Essential Medium with Earle’s Salts, 2% fetal bovine serum, 8 mM glutamine, 100 U/mL penicillin, 100 mg/L streptomycin and 2.5 µg/mL amphotericin B, Euroclone, Pero, Italy): carboxy methylcellulose (C5678, Sigma Aldrich, Saint Louis, Missouri, USA) (1:1) for 3 days. At the end of the 3 days, the supernatants were removed and the cells were fixed in paraformaldehyde 4% in phosphate buffer saline for 20′ and stained with crystal violet for 30′.

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