

Stability of the COVID-19 virus under wet, dry and acidic conditions

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To the Editor,

An outbreak of pneumonia caused by a novel coronavirus was reported in December 2019 in Wuhan, Hubei Province of China [1-3]. The COVID-19 virus (SARS-CoV-2) has spread to every continent and WHO declared COVID-19 as a pandemic [4]. In this study, the presence of CPE was used as the main index for the detection of the infectivity of the COVID-19 virus. When CPE was not observed, immunofluorescence of intracellular viral N protein and RT-PCR of viral RNA in the cell culture supernatant were used for the detection of viral infection. The strain nCoV-SH01 (GenBank accession no. MT121215) used in this study was isolated and plaque -purified from the nasal-pharyngeal swab of a clinically confirmed COVID-19 patient in Shanghai [5].

Using the strain nCoV-SH01 and these criteria, we first investigated the infectivity of the COVID-19 virus. We found that as the virus titer decreases (2000, 1000, 500, 250, 100, 50, 10, 5, and 1 PFU), the time for CPE to appear is delayed. One PFU is able to cause infection of Vero-E6 cells, resulting in obvious CPE at 72 hours, whereas higher titer of the virus induced CPE at 24 hours post inoculation (Table 1). It indicates that the COVID-19 virus is highly infectious, and underscores the challenge to control the spread of the COVID-19 virus.

We then studied the stability of the COVID-19 virus in the wet (in 100 uL culture medium) and dry (10 uL supernatant on filter paper) environments at room temperature (22°C) for 1, 2, 3, 4, 5, 6, 7 days respectively. Our results show that the COVID-19 virus can survive for 3 days in the wet or dry environment investigated in this study. Although the virus maintained its infectivity within 3 days in the dry condition, CPE appeared later than that kept in the wet environment, indicating that the dry environment may be less favorable for the survival of the COVID-19 virus (Table 2 & Table 3). However, when the virus had been kept in the wet or dry condition for more than 4 days, no CPE was observed (Table 2 & Table 3), which was confirmed by immune fluorescence staining (data not shown) with the antibody against viral N protein as well as qRT-PCR.

We further investigated the stability of the COVID-19 virus at pH2.2 condition. It shows that the COVID-19 virus has a certain degree of tolerance to acidic environment. In the present study, when 1.2×10^3 PFU of the COVID-19 virus were treated with acidic saline of pH2.2 for 30 or 60 minutes, it still resulted in CPE in the cells, whereas 1.0×10^3 PFU of the COVID-19 virus treated with pH2.2 saline for 30 or 60 minute, no CPE were observed (Table 4). It suggests under the acidic condition the COVID-19

virus at a relatively high titer can survive under acidic condition for at least 1 hour.

In conclusion, our findings show that the COVID-19 virus is highly infectious that one PFU can results in cell infection in our *in vitro* system, and can survive for 3 days in the wet or dry environment. In addition, we for the first time demonstrated that the COVID-19 virus at relative high concentrations can survive under acidic condition that mimic the gastric environment. The study would also provide guidance on taking appropriate measures to control the spread of the COVID-19 virus and improve laboratory safety.

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Table 1. The cytopathic effect of Vero-E6 cell infected by different PFU of the COVID-19 virus

| Time (d.p.i.)# | Cell control | Viral inoculation (x 10 ² PFU)* | | | | | | | | | |
|-------------------|-----------------|--|------|------|------|------|------|------|------|--------|------|
| | | 20 | 10 | 5 | 2.5 | 1 | 0.5 | 0.1 | 0.05 | 0.01** | |
| 1 | - | ++++ | ++++ | ++ | + | - | - | - | - | - | - |
| 2 | - | / | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | - |
| 3 | - | / | / | / | / | / | / | ++++ | ++++ | ++++ | ++++ |

#, d.p.i. days post-inoculation. CPE of Vero E6 cells was checked under microscope. Degree of CPE, “++++”, >75% of cells; “+++”, 50%~75%; “++”, 25%~50%; “+”, 0~25%

* The experiments were carried out in triplicate wells for each dilution. The cell control was without the virus. The cytopathic effects were observed under a microscope daily for 3 days.

** In the triplicate wells inoculated with 0.01x10² PFU, CPE appeared at different time points in individual wells and was separately presented.

Table 2. Stability of the COVID-19 virus under wet condition

| Time (d.p.i.)# | Infectivity of 1.2×10^3 PFU virus treated at room temperature under wet condition** | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|--|------------------|------|-------|------------------|------|-------|------------------|-----|-------|------------------|-----|-------|----|---|-------|----|---|-------|----|---|-------|---|
| | Day 0 | | | Day 1 | | | Day 2 | | | Day 3 | | | Day 4 | | | Day 5 | | | Day 6 | | | Day 7 | |
| 1 | ++ | ++ | ++ | ++ | ++ | ++ | - | - | - | ± | ± | ± | - | - | - | - | - | - | - | - | - | - | - |
| 2 | CPE | ++++ | ++++ | ++++ | ++++ | ++++ | + | + | +++ | + | ++ | ++ | - | - | - | - | - | - | - | - | - | - | - |
| | ORF1ab* | 15.61 ± 0.28 | | | 15.64 ± 0.09 | | | 15.74 ± 1.13 | | | 33.36 ± 1.05 | | | UD | | | UD | | | UD | | | |
| | N* | 15.99 ± 0.10 | | | 15.66 ± 0.04 | | | 16.27 ± 0.06 | | | 32.92 ± 1.33 | | | UD | | | UD | | | UD | | | |
| 3 | / | / | / | / | / | / | +++ | +++ | +++ | ++ | ++ | +++ | - | - | - | - | - | - | - | - | - | - | - |
| | | | | | | | + | + | + | | | | | | | | | | | | | | |
| 4 | / | / | / | / | / | / | / | / | / | +++ | ++ | +++ | ± | ± | ± | ± | ± | ± | - | - | - | - | - |
| 5 | CPE | / | / | / | / | / | / | / | / | +++ | +++ | +++ | ± | ± | ± | ± | ± | ± | - | - | - | - | - |
| | ORF1ab* | ND | | | ND | | | ND | | | ND | | | UD | | | UD | | | UD | | | |
| | N* | ND | | | ND | | | ND | | | ND | | | UD | | | UD | | | UD | | | |
| 6 | / | / | / | / | / | / | / | / | / | / | / | / | - | - | - | - | - | - | - | - | - | - | - |
| 7 | / | / | / | / | / | / | / | / | / | / | / | / | - | - | - | - | - | - | - | - | - | - | - |
| 8 | / | / | / | / | / | / | / | / | / | / | / | / | - | - | - | - | - | - | - | - | - | - | - |

#, d.p.i. days post-inoculation. CPE of Vero E6 cells was checked under microscope. Degree of CPE, “++++”, >75% of cells; “+++”, 50%~75%; “++”, 25%~50%; “+”, 0~25%; “±”, not clear-cut; “-”, no CPE.

* The Viral RNA in the supernatant after 2-day and 5-day incubation was extracted. Quantitative RT-PCR was performed using the primers and probes for viral ORF1ab and N (listed in Table 1). Ct values were presented as Mean \pm SD. Cutoff value: Ct value $>$ 38. UD: under detectable level. ND: Not determined.

** The experiments were carried out in triplicate wells for each dilution. The cytopathic effects were observed under a microscope daily for 8 days.

Table 3. Stability of the COVID-19 virus under dry condition.

| Time (d.p.i.)# | Infectivity of 1.2×10^3 PFU virus treated at room temperature under dry condition** | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|--|------------------|------|-------|------------------|-----|-------|------------------|----|-------|------------------|----|-------|----|---|-------|----|---|-------|----|---|-------|---|
| | Day 0 | | | Day 1 | | | Day 2 | | | Day 3 | | | Day 4 | | | Day 5 | | | Day 6 | | | Day 7 | |
| 1 | - | - | - | ± | ± | ± | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | CPE | ++++ | ++++ | ++++ | + | + | + | + | - | + | ± | ± | ± | - | - | - | - | - | - | - | - | - | - |
| | ORF1ab* | 15.25 ± 0.22 | | | 29.05 ± 0.45 | | | 35.09 ± 0.18 | | | 35.74 ± 0.09 | | | UD | | | UD | | | UD | | | |
| | N* | 15.06 ± 0.59 | | | 28.60 ± 0.15 | | | 35.12 ± 0.42 | | | 35.98 ± 0.19 | | | UD | | | UD | | | UD | | | |
| 3 | / | / | / | + | + | + | + | + | + | ± | ± | ± | - | - | - | - | - | - | - | - | - | - | - |
| 4 | / | / | / | ++++ | ++ | + | + | + | + | ± | ± | ± | ± | ± | - | - | - | - | - | - | - | - | - |
| 5 | CPE | / | / | / | ++++ | ++ | ++ | ++ | ++ | + | + | + | + | ± | ± | ± | ± | ± | - | - | - | - | - |
| | ORF1ab* | ND | | | ND | | | ND | | | ND | | | UD | | | UD | | | UD | | | |
| | N* | ND | | | ND | | | ND | | | ND | | | UD | | | UD | | | UD | | | |
| 6 | / | / | / | / | +++ | +++ | +++ | +++ | + | ++ | ++ | ++ | - | - | - | - | - | - | - | - | - | - | - |
| 7 | / | / | / | / | +++ | +++ | +++ | +++ | + | ++ | ++ | ++ | - | - | - | - | - | - | - | - | - | - | - |
| 8 | / | / | / | / | / | / | / | / | + | ++ | ++ | ++ | - | - | - | - | - | - | - | - | - | - | - |

#, d.p.i. days post-inoculation. CPE of Vero E6 cells was checked under microscope. Degree of CPE, “++++”, >75% of cells; “+++”, 50%~75%; “++”, 25%~50%; “+”, 0~25%; “±”, not clear-cut; “-”, no CPE.

* The Viral RNA in the supernatant after 2-day and 5-day incubation was extracted. Quantitative RT-PCR was performed using the primers and probes for viral ORF1ab and N (listed in Table 1). Ct values were presented as Mean \pm SD. Cutoff value: Ct value $>$ 38. UD: under detectable level. ND: Not determined.

** The experiments were carried out in triplicate wells for each dilution. The cytopathic effects were observed under a microscope daily for 8 days.

Table 4. Stability of the COVID-19 virus under acidic condition

| Time (d.p.i) [#] | <u>Treatment under acidic condition*</u> | | | | | | | | | | | | | | | Virus control | Cell control | | | | | | | |
|------------------------------|--|------|------|------|------|------|---|------|----|---|---|-----|---|------|------|---------------|-----------------|---|-----|------|------|------|------|----------|
| | 30 s (pH 2.2) | | | | | | 30 min (pH 2.2) | | | | | | 60 min (pH 2.2) | | | | | | | | | | | |
| | Inoculated virus (x10 ² PFU) | | | | | | Inoculated virus (x10 ² PFU) | | | | | | Inoculated virus (x10 ² PFU) | | | | | | | | | | | |
| | 12 | 10 | 5 | 1 | 0.2 | 0.05 | 0.01 | 12 | 10 | 5 | 1 | 0.2 | 0.05 | 0.01 | 12 | 10 | 5 | 1 | 0.2 | 0.05 | 0.01 | 12 | 0.01 | No virus |
| 1 | +++ | ++ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++++ | - | - | |
| 2 | ++++ | ++++ | ++++ | - | - | - | - | ++++ | - | - | - | - | - | - | ++++ | - | - | - | - | - | ++++ | - | - | |
| 3 | / | / | / | ++++ | ++++ | ++++ | ** | - | / | - | - | - | - | - | / | - | - | - | - | - | / | ++++ | - | |
| 4 | / | / | / | / | / | ++++ | - | / | - | - | - | - | - | - | / | - | - | - | - | - | / | / | - | |
| 5 | / | / | / | / | / | / | - | / | - | - | - | - | - | - | / | - | - | - | - | - | / | / | - | |

#, d.p.i. days post-inoculation. CPE of Vero E6 cells was checked under microscope. Degree of CPE, “++++”, >75% of cells; “+++”, 50%~75%; “++”, 25%~50%; “+”, 0~25%; “±”, not clear-cut; “-”, no CPE.

* The experiments were carried out in triplicate wells for each dilution. The virus control was treated with physiological saline (final pH = 7.12) for 60 min. Physiological saline (pH = 7.0) was used as a blank control. The cytopathic effects were observed under a microscope daily for 5 days.

** The 0.05 x10² PFU group (30 seconds) showed CPE (++++) in 1 well at 72 hrs, and all become ++++ at 96 hrs.