

EVALUATION OF SARS-COV-2 VIABILITY ON EXPERIMENTAL SURFACES OVER TIME

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Infected SARS-CoV-2 virus occurs not only through contact with an infected person, but also through surfaces with which the illness has contacted. The problem of preserving an infectious virus over time capable of infecting remains actual. We evaluated the SARS-CoV-2 viability preservation on different model surfaces over time. Ceramic tiles, metal (aluminum foil), wood (chipboard), plastic and cloth (towel) were used as model materials. Assessment of the presence of SARS-CoV-2 RNA was carried out by quantitative RT-PCR. Viable virus was determined by tissue culture assay on 293T/ACE2 cells. It was found that the SARS-CoV-2 RNA was detected on all studied surfaces for 360 minutes, but a significant decrease RNA by 1 log₁₀ copies/ml was detected after contact of the virus with cloth (towel). While the viability of the virus was completely lost after 120 minutes. Type of experimental surface significantly affects viability preservation.

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ОЦЕНКА ЖИЗНЕСПОСОБНОСТИ SARS-COV-2 НА ЭКСПЕРИМЕНТАЛЬНЫХ ПОВЕРХНОСТЯХ ВО ВРЕМЕНИ

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Заражение вирусом SARS-CoV-2 происходит не только при непосредственном контакте с инфицированным человеком, но и через поверхности, с которыми соприкасался больной. Вопрос сохранения инфекционного вируса, способного заразить спустя время, остается открытым. Целью исследования было изучить жизнеспособность SARS-CoV-2 на различных модельных поверхностях с течением времени. В качестве модельных материалов были использованы керамическая плитка, металл (алюминиевая фольга), дерево (ДСП), пластик и ткань (полотенце). В ходе исследования проводили оценку наличия РНК SARS-CoV-2 методом количественной ОТ-ПЦР. Жизнеспособность вируса определяли на клеточной линии 293T/ACE2. Показано, что РНК SARS-CoV-2 продолжала детектироваться спустя 360 мин, но значительное снижение РНК на 1 log₁₀ копий/мл обнаружено после контакта вируса с тканью (полотенцем). Жизнеспособный вирус практически полностью отсутствовал через 120 мин. На жизнеспособность вируса значительно влияет тип экспериментальной поверхности.

Ключевые слова: коронавирус, SARS-CoV-2, жизнеспособность, поверхность, ПЦР**Финансирование:** данное исследование было финансировано Министерством здравоохранения РФ в рамках государственного задания #056-00119-21-00**Благодарности:** авторы выражают благодарность И. В. Коробко за идею и обсуждение дизайна исследования.**Вклад авторов:** М. А. Никифорова — планирование эксперимента, работа с вирусом и определение его жизнеспособности, анализ данных, написание текста; А. Э. Синявин — работа с вирусом и определение его жизнеспособности, анализ данных, написание текста; Е. В. Шидловская — ПЦР-анализ, обработка данных, написание текста; Н. А. Кузнецова — ПЦР-анализ; В. А. Гушчин — планирование эксперимента, написание текста.✉ **Для корреспонденции:** Мария Андреевна Никифорова
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Environmental surfaces are suspected to be contaminated with the SARS-CoV-2 and are likely sources of COVID-19 transmission [1]. The World Health Organization (WHO) has found that there is still not enough scientific evidence of the viability of SARS-CoV-2 on inert surfaces. Scientific reports on the viability of SARS-CoV-2 report that the virus can persist differently according to the surface, from hours to days. For example, the SARS-CoV-2 is stable on glass, stainless steel, cardboard, and copper with durations detected up to 84, 72, 24, and 4 h, respectively [2].

However, the fact that the virus is present on the surface does not mean that the surface itself is dangerous and can

become a source of infection [3, 4]. At the same time, there is data showing that SARS-CoV-2 is transmitted between people by touching surfaces that have recently been in contact with COVID-19 patients (coughing or sneezing), and then directly touching the mouth, nose or eyes [5, 6].

Other studies show that after a 3-hour incubation the infectious virus is not detected on the paper for printer and napkins or on treated wood and cloth in one day. In contrast, SARS-CoV-2 was more stable on smooth surfaces. Thus 39 non-infectious samples were positive, which indicates that non-infectious viruses could still be detected [7].

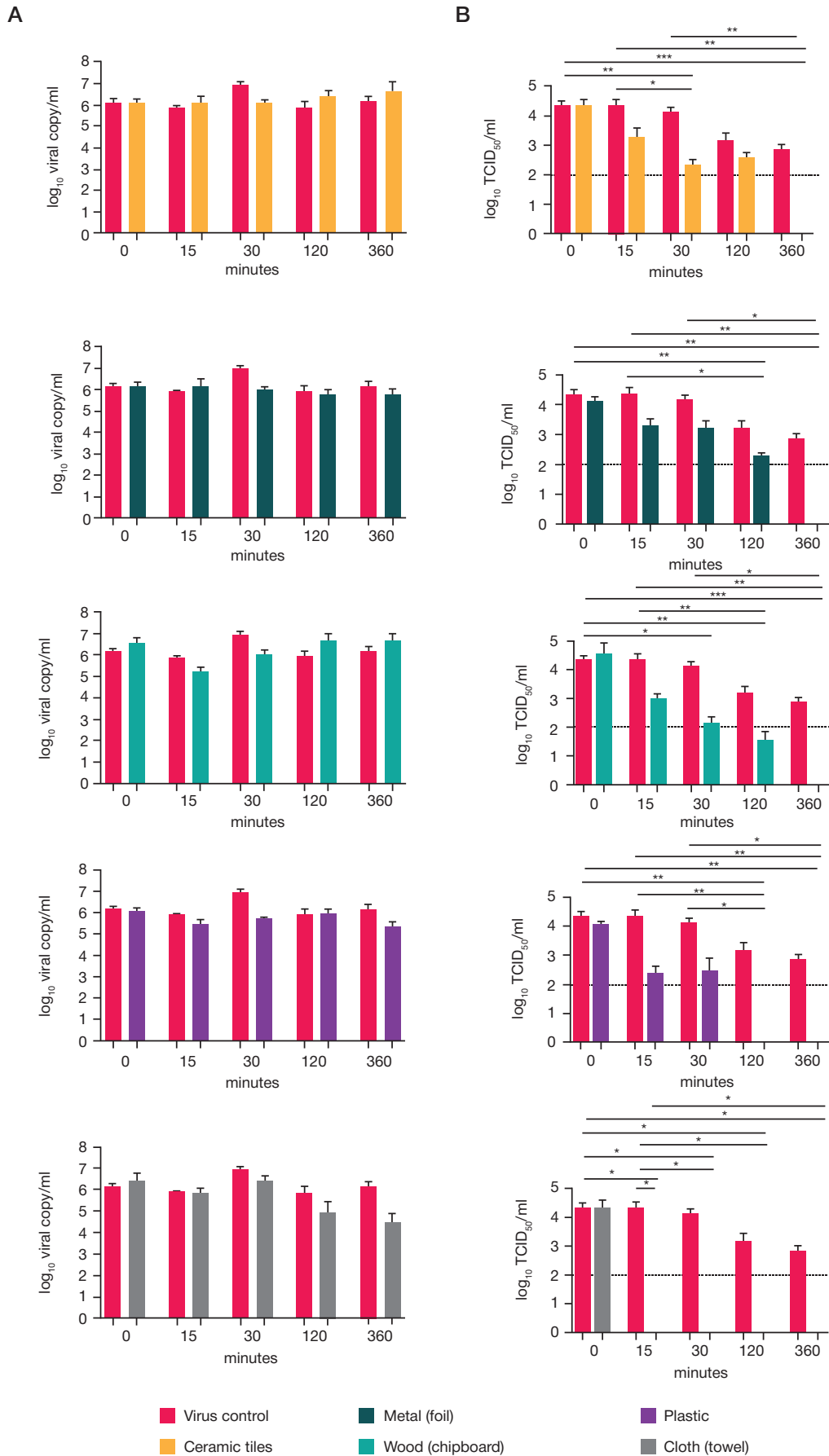


Fig. 1. Stability of SARS-CoV-2 on model surfaces under different conditions. Various experimental surfaces were inoculated with $0,4 \times 10^5$ TCID₅₀/ml SARS-CoV-2 and incubated at room temperature. At indicating time points the virus were eluted and residual virus was detected by (A) qRT-PCR or (B) viable virus titer was determined by tissue culture assay on 293T/ACE2 cells.

Therefore, our study is paid to the types of surfaces that are most common around us and can pose a risk in terms of transmission of the SARS-CoV-2. We also compare the viability of the virus on different surfaces over time using quantitative RT-PCR and cell culture.

METHODS

Modeling of the SARS-CoV-2 in time viability preservation upon contact with five model materials was carried out in laboratory controlled experimental conditions, steady relative humidity (55–60%) and temperature (22–24°C). The most common materials including ceramic tile, metal (aluminum foil), wood (chipboard), plastic, and cloth (towel) had been used. SARS-CoV-2 strain PMVL-3 (GISAID: EPI_ISL_470897) was isolated from naso/oropharyngeal swab and propagated on Vero E6 cells (ATCC CRL-1586). A 15 µl of viral culture the SARS-CoV-2 (containing $0,4 \times 10^5$ TCID₅₀/ml) was pipetted on a surface (~ 1.5–2 cm²) of each material in quintuplicate. Groups of sample material and virus control were incubated for 0 min, 15 min and 30 min (wet surface) or 120 min and 360 min (dried at room temperature). After virus exposure, the virus was eluted from the experimental surface with 200 µL of PBS.

Assessment of the presence of SARS-CoV-2 RNA was carried out by quantitative RT-PCR. Viable virus was determined by tissue culture assay on 293T/ACE2 cells and virus titer was calculated using the Reed and Muench method. The data was processed in the GraphPad Prism 7 software and analyzed using the ANOVA Kruskal-Wallis test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

According to the results of the experiments, it was found that SARS-CoV-2 RNA is detected on all experimental surfaces. Significant reduction of 0.5 log₁₀ copies/ml SARS-CoV-2 RNA was observed upon contact of the virus with wood (chipboard) for 15 min, as well as on 1 log₁₀ copies/ml SARS-CoV-2 RNA on contact with metal and plastic, after 15 min and 30 min respectively. However, in all eluates from experimental materials at both 120- and 360-minutes exposure were detected a high level of SARS-CoV-2 RNA (Fig. 1A). A significant reduction by 1 log₁₀ copies/ml of SARS-CoV-2 was noted after exposure for 6 h on a cloth (towel) sample. But, in general, the amount of SARS-CoV-2 RNA was stably high in all kinds of surface and did not differ from virus control (sample not in contact with the material).

Determination of the infectivity of SARS-CoV-2 after contact with model materials on 293T/ACE2 cells showed a sharp decreased viability of SARS-CoV-2 after 120 min (Fig. 1B). The

virus titer gradually decreased depending on the material in the following order: ceramic tile → metal → wood (chipboard) → plastic → cloth (towel). After 120 min exposure of virus on materials such as plastic and a cloth (towel), the infectious virus was not detected while SARS-CoV-2 RNA was still there.

During the assessment of the infectivity of the virus upon contact with model materials it was shown that SARS-CoV-2 RNA is detected on all experimental surfaces, regardless of the conditions and time of exposure to the virus. Even after 360 min the amount of virus on the surface, measured by quantitative RT-PCR, varies insignificant (within the order). However, the detection of SARS-CoV-2 RNA is not indicating the presence of a viable virus. Most significantly reduce the infectivity of the virus when the virus contacts with cloth (towel) samples, as well as plastic. Longer persistence of the infectious virus has been observed on surfaces such as metal, wood (chipboard) and ceramic tile. The decrease in the infectivity of SARS-CoV-2 occurs 120 min after contact with model materials and is completely lost for 360 min of exposure, when drying is achieved. It can be assumed that the complete loss of viability and the infectivity of the virus occurs at an earlier point in time (between 120–360 minutes) for all investigated materials

DISCUSSION

Our research is not without its flaws. We used culture fluid to simulate contamination. Its composition will be significantly different from human excreta formed because of natural contact with surfaces. In addition, the method itself for viable virus isolating using a cell culture may differ significantly in susceptibility to infection [8, 9]. Individual strains circulating in people with different genetic backgrounds may also differ in their ability to persist on surfaces. Nevertheless, accepting these limitations, the results can be useful for planning further research and preparing practical recommendations.

CONCLUSIONS

The results obtained in the framework of this study show that positive RT-PCR data do not allow us to speak about the viability of the virus. For at least 360 minutes on the surface, the amount of RNA SARS-CoV-2 on the surface practically does not change, while the virus viability drops by many orders of magnitude in 120 minutes and after 360 minutes is not detected for any of the experimental surfaces. Thus, in the context of environmental safety assessment, the use of RT-PCR alone can lead to highly distorted judgments. At the very least, the interpretation of RT-PCR results in the context of potential surface contamination with SARS-CoV-2 should be done with great care.

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