HEALTH AND MEDICAL RESEARCH FUND COMMISSIONED RESEARCH ON THE NOVEL CORONAVIRUS DISEASE

Stability and transmissibility of SARS-CoV-2: abridged secondary publication

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KEY MESSAGES

- 1. The stability of SARS-CoV-2 under various environmental conditions, including temperature and pH, is demonstrated. Droplet transmission of SARS-CoV-2 is efficient.
- 2. SARS-CoV-2 remains stable on smooth surfaces for days but becomes inactive relatively quickly on porous surfaces.
- 3. Copper-containing stainless steel, metal oxidecontaining, and porous surfaces effectively inactivate SARS-CoV-2.
- 4. SARS-CoV-2 is sensitive to commonly used disinfectants.

5. Contaminated N95 masks can be disinfected by heating at 70°C for 1 hour in an oven.

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Introduction

SARS-CoV-2 is the causative agent of the COVID-19 pandemic.¹⁻³ Epidemiological studies indicate that COVID-19 is primarily transmitted through droplets between individuals in close contact. However, indirect contact transmission is also possible (touching the eyes, nose, or mouth after contact with a virus-contaminated surface or object).⁴⁻⁶ Additionally, facilities occupied by patients are heavily contaminated with SARS-CoV-2.^{7,8} We conducted a series of in vitro and in vivo studies to examine the virus' stability and infectivity under various environmental conditions, to identify effective methods for decontamination, and to determine the potential role of droplets or droplet nuclei in COVID-19 transmission.

Methods

SARS-CoV-2 (BetaCoV/Hong Kong/VM20001061/2020) was isolated using Vero E6 cells, and virus stock was prepared and stored. The virus was exposed to various environmental conditions for specific durations. After incubation, infectious viral titre was quantified using the 50% tissue culture infectious dose assay. The efficiency of droplet transmission was determined using an established hamster model.⁹ At 2 days post-infection, an infected hamster and contact hamsters were placed

in separate containers within the same chamber. The contact hamsters were then housed separately and monitored for signs of infection.

Results

Effect of temperature on SARS-CoV-2 stability

SARS-CoV-2 in infection medium was incubated at various temperatures. At 4°C, the virus remained stable for at least 14 days. At room temperature (22°C), the infectious titre was reduced by at least 3 logs after approximately 7 days. Infectivity decreased by at least 99.9% after 30 minutes of incubation at 56°C, and infectious virus was not detected by the 50% tissue culture infectious dose assay after 5 minutes of incubation at 70°C. These results indicate that SARS-CoV-2 exhibits reduced stability at higher temperatures.

Effect of pH on SARS-CoV-2 stability

To determine the stability of SARS-CoV-2 at various pH levels ranging from 3 to 10, the virus was spiked into viral transport medium (VTM) adjusted to pH levels. After incubation for 1 hour at room temperature, viral titre decreased by <1 log across all tested pH levels. These findings suggest that SARS-CoV-2 remains stable and retains infectivity between pH 3 and pH 10.

SARS-CoV-2 stability in various clinical samples

The stability of the virus in respiratory specimens—including nasal/throat swabs, nasopharyngeal aspirates, and sputum—was comparable to that observed in the VTM control at both room temperature and 37°C. In contrast, viral stability in faecal samples was significantly reduced at both temperatures.

Stability of SARS-CoV-2 viral RNA in various transport media

Comparable quantities of viral RNA were detected when the virus was stored in VTM, phosphate-buffered saline (PBS), or ethanol for up to 7 days at both room temperature and 37°C. These results indicate that PBS or ethanol can be used instead of VTM for the transport of clinical specimens.

SARS-CoV-2 stability on different surfaces

The virus remained relatively stable on smooth surfaces such as stainless steel, glass, and plastic. A reduction of 99.9% infectivity required 1 to 4 days. Surprisingly, infectious virus was still detectable on the surface of a surgical mask—composed of polypropylene fibre—after incubation for 7 days. On porous surfaces such as tissue paper and printing paper, infectivity decreased much more rapidly. Infectious viral titre decreased by >99.9% within 6 hours on paper and within 30 minutes on wooden surfaces. These results indicate that SARS-CoV-2 can remain infectious on smooth surfaces including stainless steel, plastic, and glass, whereas infectivity rapidly decreases on porous surfaces.

Effect of copper-containing stainless steel on SARS-CoV-2 inactivation

Copper has been shown to inactivate SARS-CoV- $2.^{10}$ We fabricated copper-containing stainless steel samples with varying copper content using powder metallurgy to investigate their virus-inactivating properties. Samples containing $\geq 10\%$ copper inactivated SARS-CoV-2 more rapidly than the standard stainless steel control. These materials may be applied to frequently touched surfaces to reduce the risk of fomite-mediated transmission.

Effect of metal oxide-containing surface coatings on SARS-CoV-2 inactivation

Several surface coatings containing copper oxide or zinc oxide were tested. These coatings substantially reduced viral infectivity within 1 to 3 hours.

Additional advantages include hydrophilicity, which enables absorption of larger droplets, and the availability of sprayable, transparent formulations suitable for application on touch-screen devices. Overall, we demonstrated that multiple surface coatings could inactivate SARS-CoV-2. Their use may help to reduce the risk of COVID-19 transmission via the fomite route.

Effect of different disinfectants on SARS-CoV-2

The virus was mixed with various commonly used disinfectants at their working concentrations for 5 to 15 minutes. SARS-CoV-2 demonstrated sensitivity to all tested disinfectants, including household bleach, ethanol, povidone-iodine surgical scrub, chloroxylenol, chlorhexidine, benzalkonium chloride, and a 1:49 hand soap solution.

Decontamination of SARS-CoV-2-contaminated N95 masks with heat

We developed an evidence-based method to disinfect used masks. SARS-CoV-2 applied to various 3M N95 masks was fully inactivated after 1 hour of heating in an oven at 70°C. Repeated heat treatment did not compromise mask fit on wearers.

Droplet transmission in hamsters

There was droplet transmission in hamsters under various conditions, including different humidity levels.

Discussion

SARS-CoV-2 showed rapid inactivation at 70°C, and we developed a heat-based disinfection protocol using an oven for contaminated or used N95 masks. This protocol may help to alleviate supply shortage, particularly during the early stages of an outbreak. Additionally, SARS-CoV-2 remained stable at 4°C, retaining infectivity for up to 2 weeks. This result implies that transmission via cold-chain transport, such as the packaging of refrigerated food, is possible. Indeed, reports from China have documented cases of fomite transmission via cold-chain transport. 11,12 Our findings may support implementation of precautionary measures when handling items transported under cold-chain conditions. The virus remined stable across a range of pH levels and was susceptible to commonly used disinfectants. Given that rapid and accurate diagnosis is critical for controlling the spread of COVID-19, our results indicate that PBS or ethanol can be used as alternative

transport media for clinical samples from the point SARS-CoV-2 inactivation and respirator function. of collection to laboratories for nucleic acid testing. In countries with limited resources where VTM may not be readily available, PBS and ethanol are more readily available and economical reagents, thus strengthening diagnostic capacity for COVID-19 control. The virus can remain infectious on smooth surfaces for 1 to 2 days, highlighting the potential for fomite transmission. Furthermore, SARS-CoV-2 exhibits high stability on surgical masks, underscoring the importance of hand hygiene after touching potentially contaminated surfaces and removal of personal protective equipment. Copper-containing stainless steel and anti-COVID-19 surface coatings may be applied to frequently touched surfaces in daily life to reduce the risk of transmission. Further research is warranted to evaluate the effectiveness of these materials in healthcare settings and community environments.

Conclusions

We demonstrated multiple properties of SARS-CoV-2, including its sensitivity to heat, pH, and a range of disinfectants. These findings support evidence-based recommendations for disinfection in laboratories, healthcare settings, and the community. Several materials and surface coatings developed in this study efficiently inactivated the virus, contributing to the reduction of transmission risk via the fomite route.

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Disclosure

The results of this research have been previously published in:

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