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Virucidal activity of oral, hand, and surface disinfectants against respiratory syncytial virus

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SUMMARY

Background: Respiratory syncytial virus (RSV) is known as a major cause of respiratory tract infection in adults and children. Human-to-human transmission occurs via droplets as well as direct and indirect contact (e.g. contaminated surfaces or hands of medical staff). Therefore, applicable hygiene measures and knowledge about viral inactivation are of utmost importance.
Aim: To elucidate the disinfection profile of RSV.
Methods: The study evaluated the virucidal efficacy of oral rinses specifically designed for children, World Health Organization (WHO)-recommended hand-rub formulations, and ethanol, as well as 2-propanol against RSV in a quantitative suspension test (EN14476). The stability of RSV on stainless steel discs was assessed and its inactivation by different surface disinfectants (EN16777) investigated.
Findings: All tested oral rinses except one reduced infectious viral titres to the lower limit of quantification. The two WHO-recommended hand-rub formulations as well as 30% ethanol and 2-propanol completely abolished the detection of infectious virus. Infectious RSV was recovered after several days on stainless steel discs. However, RSV was efficiently inactivated by all tested surface disinfectants based on alcohol, aldehyde, or hydrogen peroxide.

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Conclusion: Oral rinses, all tested hand-rub formulations as well as surface inactivation reagents were sufficient for RSV inactivation *in vitro*.

Introduction

Respiratory syncytial virus (RSV) is a common viral infection that affects the respiratory system, particularly the lungs and airways. RSV infection can cause a range of symptoms, from mild cold-like symptoms to severe respiratory illness, especially in infants and young children, but also in older adults with weakened immune systems [1,2]. According to the US Centers for Disease Control and Prevention (CDC), RSV is the most common cause of bronchiolitis and pneumonia in children aged <1 year in the USA. Most children are believed to have experienced at least one RSV infection by the age of 2 years [3,4].

RSV is a seasonal virus, with outbreaks typically occurring in autumn, winter, and early spring [5,6]. However, the timing and severity of RSV outbreaks can vary from year to year [7,8]. In recent years, RSV outbreaks occurring outside of the typical season have been reported, which may be due to changes in climate or other environmental factors [9]. COVID-19-related non-pharmaceutical measures to prevent SARS-CoV-2 transmission simultaneously reduced exposure to other respiratory viruses, including RSV, despite sporadic epidemics observed in France and Iceland [10—12]. As these measures were relaxed, including lifted travel restrictions and the resumption of socioeconomic activities, there was a rebound effect leading to increased RSV transmission and substantial morbidity and mortality among children [13—15]. Consequently, detection and cumulative hospitalization rates were considerably higher compared with pre-COVID-19 years according to the Respiratory Syncytial Virus Hospitalization Surveillance Network (RSV-NET) launched by CDC and others [11,16].

Despite the large number of studies on RSV replication and pathogenesis, relatively little is known about infection prevention and treatment [17]. Despite positive developments regarding new vaccination strategies, RSV is likely to continue being a significant contributor to infant morbidity and mortality worldwide. Unfortunately, effective vaccination protocols for low-income countries, where RSV-related childhood deaths are most prevalent, have not yet been established [18]. Thus, hygiene measures such as frequent hand washing, avoiding close contact with sick individuals, covering the nose and mouth when coughing or sneezing, and disinfecting frequently touched surfaces such as doorknobs, toys, and electronic devices, and gargling to reduce transmission by respiratory droplets remain of utmost importance (https://www.cdc.gov/rsv/about/prevention.html). The present study analysed the potential of oral rinses specifically designed for children to inactivate RSV, and it assessed the virucidal efficacy of World Health Organization (WHO)-recommended hand-rub formulations as well as ethanol and 2-propanol against RSV in a quantitative suspension test. Furthermore, it assessed the stability of RSV on stainless steel discs and evaluated its inactivation by different surface disinfectants.

Methods

Cell culture and virus propagation

HEp-2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) fetal calf serum (FCS), 1% (vol/vol) non-essential amino acids, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine. For RSV (long, ATCC #VR-26) production, Hep-2 cells were seeded at 3×10^6 cells/cell culture flask in 1% FCS-containing DMEM. After 24 h the cells were inoculated with RSV (MOI 1) and incubated for 72 h at 37 °C with 5% CO_2. Upon visual cytopathic effect, the supernatant was collected and centrifuged at 1000 rpm for 5 min to remove any cell debris. The virus suspension was aliquoted and stored at −80 °C until further usage.

RSV inactivation by WHO-recommended hand-rub formulations, ethanol, 2-propanol, or oral rinses

Virucidal activity of three oral rinses for children (Supplementary Table S1) and WHO-recommended hand-rub formulation I and II (Supplementary Table S2), as well as ethanol, and 2-propanol were assessed based on European guideline EN14476 as described previously [19]. Specifically, eight parts of disinfectant/oral rinse or cell culture medium for the untreated control were mixed with one part interfering substance (bovine serum albumin (BSA), final concentration 0.3 g/L, clean condition) and one-part RSV and incubated for 30 s at room temperature. An endpoint dilution assay was performed on HEp-2 cells to determine remaining infectious viral titres. WHO formulations I and II as well as ethanol and 2-propanol were tested for final concentrations of 20%, 30%, 40%, 60%, and 80%. Oral rinses were tested for a final concentration of 80%. An endpoint dilution assay was performed on HEp-2 cells to determine the remaining infectious viral titres. After seven days, cytopathic effects were evaluated microscopically and used to calculate the 50% tissue culture infectious dose ($TCID_{50}$)/mL.

RSV stability testing

Stainless steel discs (2 cm diameter discs, article no. 4174-3000; GK Formblech GmbH, Berlin, Germany) were decontaminated in 70% (vol/vol) ethanol for 15 min. Subsequently, the stainless steel discs were contaminated with 50 μL virus solution containing nine parts RSV and one-part interfering substance (BSA, final concentration 0.3 g/L, clean condition). All specimens were stored at room temperature. Virus was recovered at different timepoints (0, 15, 30, 60, 120, and 360 min, and every 24 h between one and seven days) post contamination by transferring the specimens into a 25 mL container harbouring 2 mL cell culture medium (without FCS)

and subsequent vortexing. For each timepoint three specimens were collected. An endpoint dilution assay was performed on HEp-2 cells as described above. Simultaneously, humidity and temperature were measured over the course of the experiment.

RSV inactivation by surface disinfectants

Stainless steel discs were decontaminated and spiked with virus solution as described above. The steel discs were incubated until the virus solution was desiccated completely. Subsequently, 100 µL of surface disinfectant (Supplementary Table S3) at indicated concentrations was applied on to the carrier and incubated according to the manufacturer's instructions. Cell culture medium was used for the untreated control. Thereafter, the specimens were transferred into a 25 mL container harbouring 2 mL cell culture medium (without FCS) and subsequent vortexing. An endpoint dilution assay was performed on HEp-2 cells as described above.

Results

Virucidal activity of oral rinses against RSV

Since RSV is predominantly transmitted by respiratory droplets and aerosols, oral rinses possess the ability to reduce the risk of viral transmission by temporarily reducing the viral load in the oral cavity. Thus, we tested the potential of commercially available oral rinses for adults and children (aged >6 years) to inactivate RSV within 30 s of exposure (Figure 1). RSV was highly susceptible to various oral rinses. All three tested oral rinses exclusively designed for adults inactivated RSV and reduced viral infectivity up to 4.5 $\log_{10}$ $TCID_{50}$/mL (Figure 1A). In the case of oral rinses for children, three oral rinses, namely Dontodent Junior, Elmex Junior, and Listerine – Smart Rinse, efficiently reduced infectious viral titres to the lower limit of quantification. After exposure of RSV to Odol med 3 Junior the amount of infectious virus was reduced but could still be recovered, suggesting inefficient inactivation. In conclusion, the majority of the tested oral rinses efficiently inactivated RSV.

Inactivation of RSV by WHO-recommended hand-rub formulations and ethanol or 2-propanol

WHO-recommended hand-rub formulations have been shown to be effective against a variety of viruses. The present study analysed the virucidal efficacy of WHO formulations I and II and their two major ingredients ethanol and 2-propanol (Figure 2). The solutions were tested at 20%, 30%, 40%, 60% and 80% final concentration in a quantitative suspension test according to EN14476. For the major ingredients of the WHO-recommended hand-rub formulations 30% of both ethanol and 2-propanol efficiently inactivated RSV (Figure 2A). It was found that 30% WHO formulation I significantly decreased infectious viral titres compared with the untreated control (UTC);

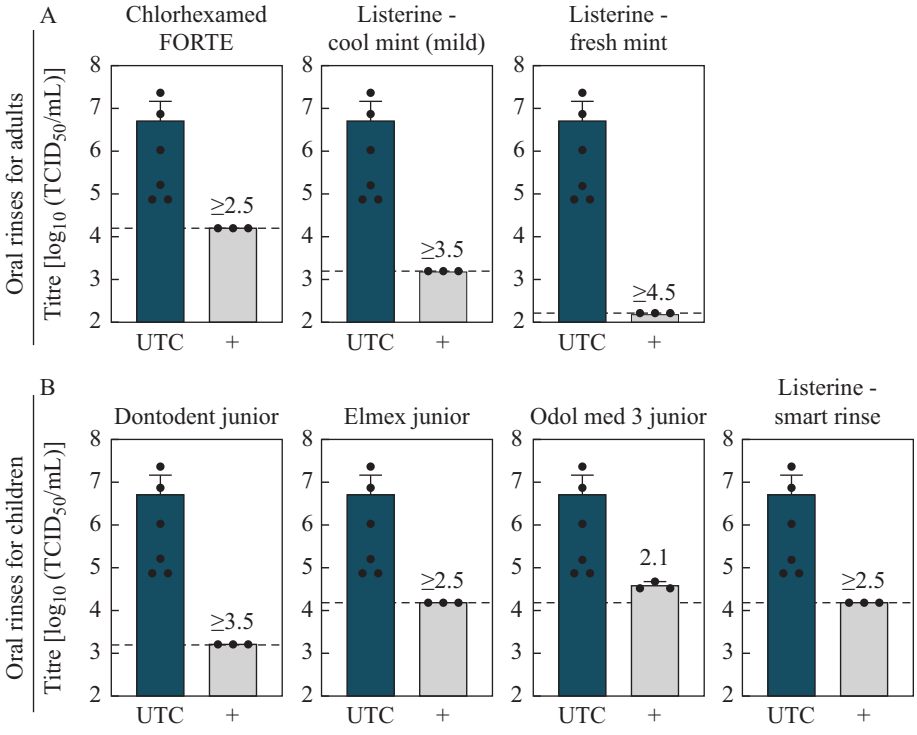

Figure 1. Inactivation of respiratory syncytial virus (RSV) by oral rinses. Oral rinses for (A) adults and (B) children were tested regarding their potential to inactivate RSV in a quantitative suspension test according to EN14476. Eight parts of oral rinse were mixed with one part interfering substance (bovine serum albumin, 0.3 g/L final concentration) and one part RSV for 30 s. Remaining infectious viral titres were determined in an endpoint dilution assay and are displayed as 50% tissue culture infectious dose (TCID50)/mL. The untreated control (UTC) is displayed as the dark blue bar. The light grey bar represents viral titres recovered after exposure to the oral rinses. Reduction factors are shown above the grey bars. Dotted line indicates the lower limit of detection.

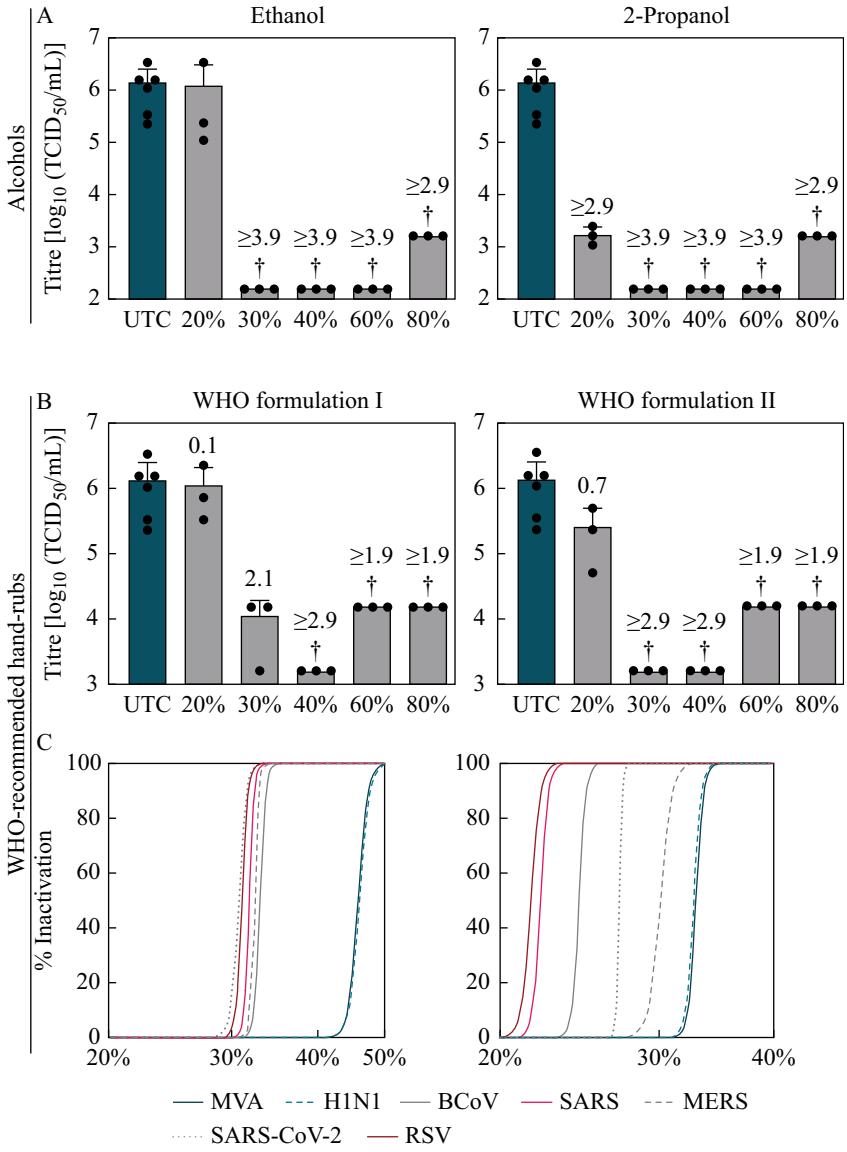

Figure 2. Inactivation of respiratory syncytial virus (RSV) by World Health Organization (WHO) recommended hand-rub formulations and ethanol or 2-propanol. All four disinfectants were tested regarding their potential to inactivate RSV in a quantitative suspension test according to EN14476. Ethanol and 2-propanol (A) as well as WHO formulations I and II (B) were diluted to 20%, 30%, 40%, 60%, and 80% final concentration and mixed with one part interfering substance (bovine serum albumin, 0.3 g/L final concentration) and one part RSV for 30 s. Remaining infectious viral titres were determined in an endpoint dilution assay and are displayed as 50% tissue culture infectious dose (TCID50)/mL. The untreated control (UTC) is displayed as the dark blue bar. The light grey bar shows viral titres recovered after exposure to the disinfectants. The cross (†) indicates a reduction of infectious viral titres to the lower limit of detection. Inactivation by WHO formulations I and II was compared with other respiratory viruses such as H1N1 (subtype of influenza A virus), SARS (severe acute respiratory syndrome coronavirus) MERS (Middle East respiratory syndrome coronavirus), SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and BCoV (bovine coronavirus) as well as the reference virus MVA (modified vaccinia Ankara) using robust Hill non-linear dose–response fit (C).

however, viable virus was still detectable. Increasing the concentration to 40% completely abolished detection of infectious virus. For WHO formulation II a final concentration of 30% was sufficient to reduce infectious viral titres to the lower limit of quantification (Figure 2B). Comparing the inactivation capacity of WHO formulations I and II to other respiratory and/or emerging viruses such as bovine coronavirus, SARS-CoV, MERS-CoV, SARS-CoV-2, influenza virus H1N1 as well as modified vaccinia Ankara (MVA), we found that RSV showed the highest susceptibility towards inactivation by both formulations. Among all tested viruses, MVA and H1N1 required the highest concentration of WHO formulations I and II (Figure 2C).

Surface stability of RSV on stainless steel

Viruses are known to remain infectious on inanimate surfaces for a certain period depending on the virus itself as well as on environmental factors or the texture of the fomite considering porosity and its material composition. Stainless steel discs were contaminated with infectious RSV and remaining

virus was retrieved from the specimens at different timepoints (0, 15, 30, 60, 120, and 360 min, and 1, 2, 3, 4, 5, 6, and 7 days post contamination). Infectious viral titres were determined by an endpoint dilution assay. Simultaneously environmental data regarding temperature and humidity were collected (Figure 3). The temperature varied between 20—22 °C, whereas humidity ranged from 25% to 35% (Figure 3A). After 7 days at room temperature protected from UV light, infectious RSV was recovered from stainless steel discs, although titres dropped from 2.32×10^5 to 1.54×10^3 $TCID_{50}$/mL, resulting in a half-life time of 17.36 h post contamination (Figure 3B). These results demonstrated that RSV is viable on inanimate surfaces such as stainless steel for several days.

Inactivation of RSV by surface disinfectants

Given the long-term detection of infectious RSV on fomites, surface disinfectant can be utilized to reduce the potential risk of transmission. Therefore, five different surface disinfectants based on alcohol (Bacillol AF and Antifect N liquid, Figure 4A), aldehyde (Kohrsolin FF and Incidin Rapid, Figure 4B), and hydrogen peroxide (Incidin OxyFoam, Figure 4C) were evaluated regarding their potential to inactivate RSV. The experiment was performed according to the European guideline EN16777. We found that all disinfectants reduced infectious viral titres to the lower limit of quantification within the exposure time and concentration implicated by the manufacturers (Figure 4).

Discussion

In the post-pandemic years, RSV infections have been on the rise. Due to the limited adoption of preventive measures, the corresponding hygiene practices continue to play a significant role in virus transmission prevention. Since RSV is predominantly transmitted by respiratory droplets, oral rinses possess the ability to reduce the risk of transmission by temporarily reducing the viral load in the oral cavity. Therefore, we tested the virucidal efficacy of different oral rinses which had been found to effectively inactivate RSV or reduce infectious viral titres (Figure 1). This data is in line with findings for other enveloped viruses such as SARS-CoV-2, which was efficiently inactivated by a broad range of oral rinses and some nasal sprays [20,21]. However, this effect was directly affected by the type of active ingredient and its concentration. Ingredients such as chlorhexidine, essential oils, povidone-iodine, benzalkonium chloride, cetylpyridinium chloride, octenidine dihydrochloride and surfactants but not hydrogen peroxide or dequalinium chloride have been shown to inactivate SARS-CoV-2 in a dose-dependent manner [22]. Of note, hydrogen peroxide might also exhibit inefficacy, due to compound stability concerns.

In addition, we showed that ethanol and 2-propanol diluted to as low as 30% reduced infectious viral titres of RSV to the lower limit of quantification (Figure 2A). By comparison, higher concentrations were needed to inactivate hepatitis C virus (HCV) or Ebola virus [23,24]. These two alcohols are the major ingredients of WHO-recommended hand-rub formulations I and II, which also efficiently inactivated RSV (Figure 2B). Recently, we and others showed that SARS-CoV-2, MERS, SARS, monkeypox virus (MPXV), and other viruses were also inactivated by these hand-rub formulations and alcohols [19,25,26]. However, RSV was shown to have the highest susceptibility when exposed to WHO formulations I and II compared with other respiratory viruses as well as MVA (Figure 2C).

Respiratory viruses have been proven stable on a variety of inanimate surfaces that differ in material composition and porosity [27—30]. For example, SARS-CoV-2 and influenza virus remained viable on different types of cash for hours to days [31,32]. Furthermore infectious SARS-CoV-2 was recovered from face masks, plastic, glass, metal, paper, fabric, skin, and silver-coated fomites upon experimental contamination [27,33—37]. SARS-CoV-2 was also found to remain infectious on stainless steel discs for five days, whereas even longer stability

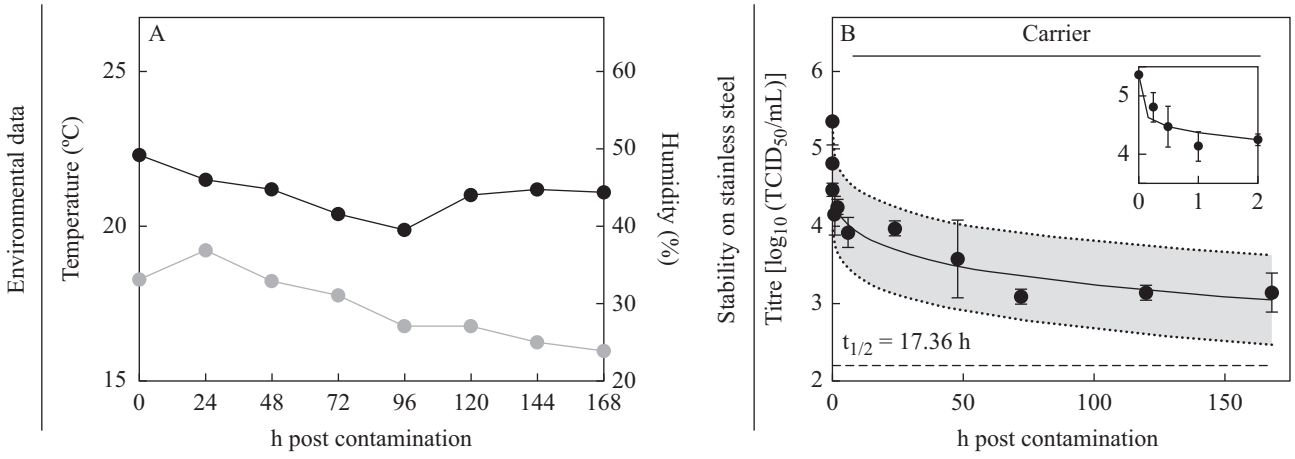

Figure 3. Stability of respiratory syncytial virus (RSV) on stainless steel. Nine parts RSV mixed with one part interfering substance (bovine serum albumin, 0.3 g/L final concentration) were spiked on sterile stainless steel discs and incubated for indicated time-periods: 0, 15, 30, 60, 120, and 360 min, and 1, 2, 3, 4, 5, 6, and 7 days post contamination; remaining infectious viral titres were recovered from the specimens stored at room temperature. Simultaneously, environmental data regarding temperature (black) and humidity (grey) were measured every 24 h (A). Titres were determined by an endpoint dilution assay and calculated as 50% tissue culture infectious dose (TCID50)/mL (B). A non-linear model based on Weibull distribution was used to fit data. Dotted line indicates the lower limit of detection. Grey area represents 95% confidence interval.

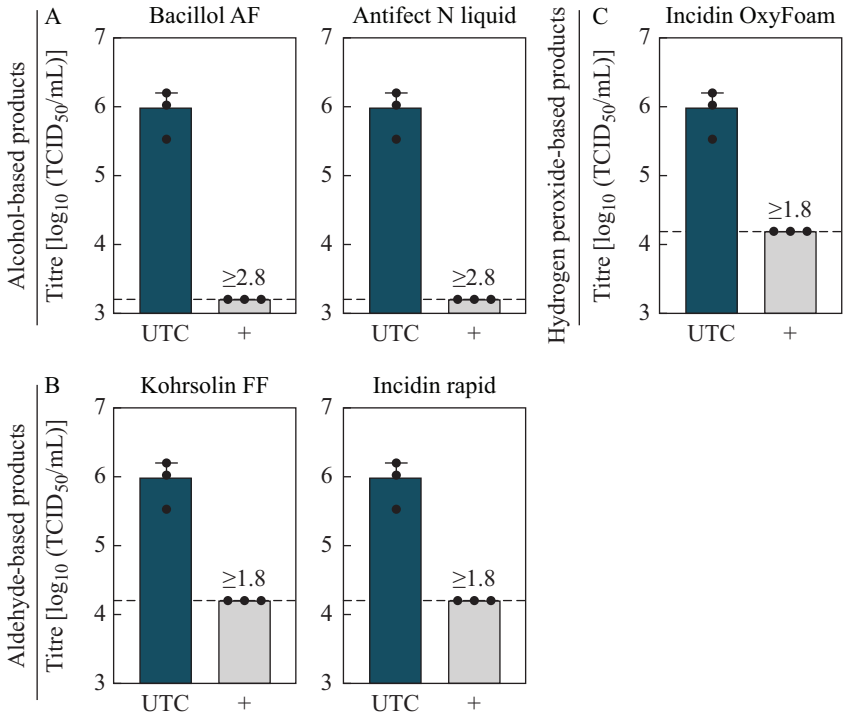

Figure 4. Inactivation of respiratory syncytial virus (RSV) by surface disinfectants. Five surface disinfectants based on alcohol (A), aldehyde (B), and hydrogen peroxide (C) were tested according to EN16777 to evaluate their virucidal activity. All products were tested as suggested by the manufacturers. Nine parts RSV mixed with one part interfering substance (bovine serum albumin, 0.3 g/L final concentration) were spiked on sterile stainless steel discs and inactivated with undiluted Bacillol AF, Antifect N liquid, and Incidin OxyFoam for 30 s. Kohrsolin FF and Incidin Rapid were diluted to 0.5% working solutions and incubated for 5 min on contaminated stainless steel discs. Remaining infectious viral titres were determined in an endpoint dilution assay and are displayed as 50% tissue culture infectious dose (TCID50)/mL. The untreated control (UTC) is displayed as the dark blue bar. The light grey bar shows viral titres recovered after exposure to the surface disinfectants. Reduction factors (RFs) are shown above the grey bars. Dotted line indicates the lower limit of detection.

was observed for hepatitis E virus, hepatitis C virus, hepatitis A virus, murine norovirus, bovine rotavirus, and MPXV [23,31,38–42]. Studies have shown that RSV is stable for hours on inanimate surfaces commonly found in healthcare settings, such as gloves, gowns, and stethoscopes, highlighting the risk of transmission from inanimate surfaces [43]. We found that infectious RSV could still be recovered seven days post contamination of stainless steel discs, which is of comparable stability to other respiratory viruses such as SARS-CoV-2 and influenza A virus [31,44]. Thereby, we showed that transmission by contaminated fomites cannot easily be ruled out and surface disinfection should be considered. Five different surface disinfectants based on alcohol, aldehyde and hydrogen peroxide were tested regarding their potential to inactivate RSV. All surface disinfectants completely abolished detection of infectious RSV. Similarly, others observed efficient inactivation of RSV by disinfectants based on sodium hypochlorite and ethanol, as well as isopropanol and aqueous chlorhexidine detergent solutions, or soap [45–48]. By contrast, MPXV was only inactivated by alcohol- and aldehyde-based surface disinfectants but not by hydrogen peroxide-based disinfectants [39]. Hydrogen peroxide-, ethanol-, sodium hypochlorite- and quaternary ammonium-based disinfectants efficiently reduced Ebola virus infectious viral titres [49,50]. Such data from other enveloped viruses suggest that RSV might also be susceptible to disinfectants based on quaternary ammonium, chlorine, and others [51]. For HEV elimination, alcohol-based disinfectants were insufficient, but disinfectants based on aldehyde, peracetic acid, oxygen, and/or quaternary ammonium inactivated HEV, highlighting that hygiene measures need to be considered carefully and cannot be generalized [38]. Furthermore, the process of translating in-vitro findings to real-life scenarios requires careful assessment of the limitations, complexities, and differences between these two contexts. Yet, proper surface disinfection in clinical settings has far-reaching implications for infection prevention, protection of vulnerable populations, reduced healthcare-associated infections, and overall public health. Implementing and maintaining effective disinfection protocols is essential to ensure the highest standards of care and safety within healthcare environments. Since all evaluated substances — encompassing oral rinses, alcohols, WHO formulations, and surface disinfectants — effectively inactivated RSV in laboratory settings, their application within healthcare systems and during viral outbreaks is endorsed for curtailing RSV transmission.

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Author contributions

T.L.M.: data curation, formal analysis, investigation, methodology, visualization, writing — original draft; M.F.: data curation, investigation, writing — review and editing; N.F.: data curation, investigation, writing — review and editing; M.W.: resources, writing — review and editing; S.H.: resources, writing — review and editing; J.S.: resources, writing — review and editing; D.T.: formal analysis, writing — review and editing; T.P.: resources, supervision, writing — review and editing; E.S.: conceptualization, resources, supervision, writing — original draft.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2023.08.009.

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