Virucidal potential of oral rinses and nasal sprays against SARS-CoV-2 and their mode of action

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INTRODUCTION:

The ongoing SARS-CoV-2 pandemic creates a significant threat to global health. Recent studies suggested the significance of throat and salivary glands as major sites of virus replication and transmission during early COVID-19, thus advocating application of oral antiseptics. Here, we evaluated the virucidal activity of different available nasal sprays, oral rinses as well as individual compounds found in oral rinses against SARS-CoV-2.

METHODS:

According to European guidelines, virucidal activity of 8 oral rinses, 6 nasal sprays, 2 oral sprays and 10 antiseptic agents was determined in a quantitative suspension test with 30 s exposure time on VeroE6 cells. The experiments were performed under conditions mimicking nasopharyngeal secretions. To elucidate the mode of action of antiseptic agents density gradient centrifugation and a capsid protection assay were carried out.

EXPERIMENTAL SETUP:

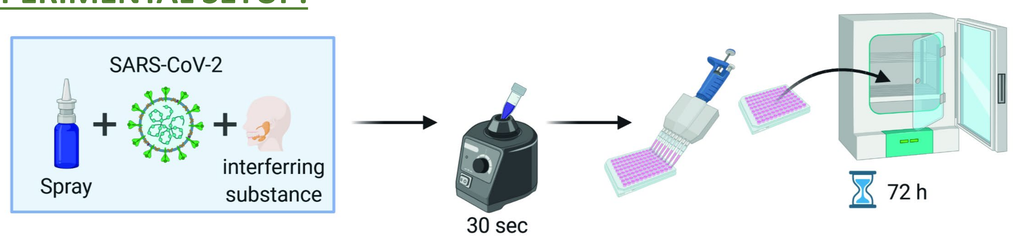

Figure 1: Experimental Setup of the quantitative suspension test. 8 parts test suspension was mixed with 1 part virus and 1 part interfering substance, incubated for 30 s and used to inoculate VeroE6 cells. After 72h cells were stained by crystal violet. Residual viral titres were determined by end-point dilution ($TCID_{50}$/mL) and compared to a medium control (grey). LLOQ: Lower limit of quantification (dotted line)

SELECTED NASAL SPRAYS, ORAL SPRAYS AND ORAL RINSES SIGNIFICANTLY REDUCED VIRAL INFECTIVITY TO UP TO THREE ORDERS OF MAGNITUDE TO BACKGROUND LEVELS IN VITRO

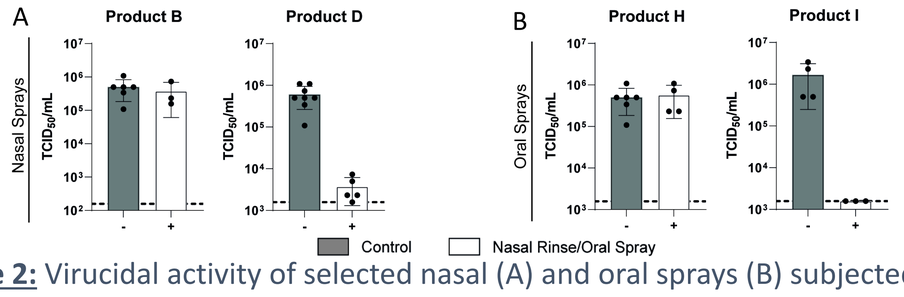

Figure 2: Virucidal activity of selected nasal (A) and oral sprays (B) subjected to a quantitative suspension test. Only product D and I based on sodium hypochlorite and essential oils, reduced infectious viral titres by 2.21 and ≥3.03 log_{10} $TCID_{50}$/mL, respectively.

Meister et al; under revision

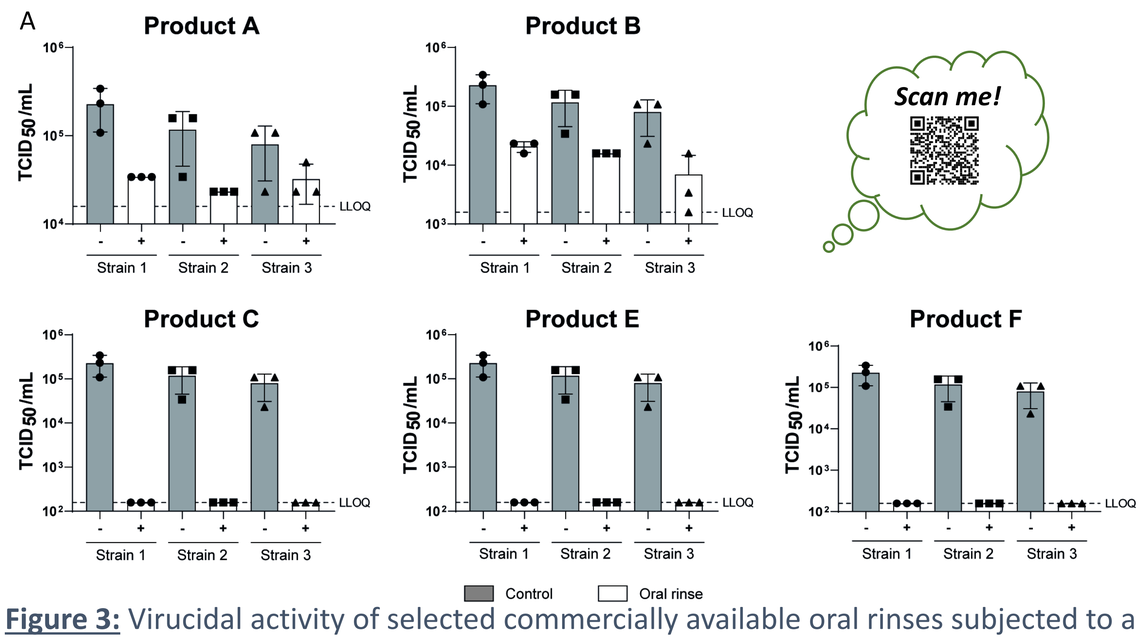

Figure 3: Virucidal activity of selected commercially available oral rinses subjected to a quantitative suspension test (A). Product C, E and F reduced infectious viral titres to background levels. These oral rinses contained a mixture of Dequaliniumchloride (DQ) and Benzalconiumchloride (BAC), Polyvidone-iodine (PVP-I) or ethanol and essential oils.

Meister et al 2020; JID

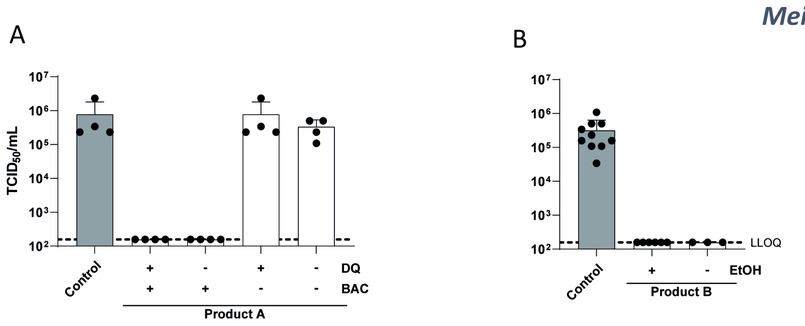

Figure 4: Depletion of ingredients of commercially available oral rinses can alter the inactivation capacity when subjected to a quantitative suspension assay. BAC and essential oils could possibly be two of many agents that successfully inactivate SARS-CoV-2

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CONCLUSION:

- **SARS-COV-2 CAN BE EFFICIENTLY INACTIVATED BY COMMERCIALLY AVAILABLE ORAL RINSES AND NASAL SPRAYS WITH RESPECT TO THEIR COMPOUND COMPOSITION WITHIN SHORT EXPOSURE TIMES**
- **AGENTS SUCH AS BAC, CPC, OCT-DIHCL, PVP-I AND SURFACTANTS DISRUPT THE VIRAL ENVELOPE**

TREATMENT WITH BENZALKONIUMCHLORIDE AND OTHER ANTISEPTIC AGENTS USED IN ORAL RINSES INACTIVATE SARS-COV-2 IN A DOSE-DEPENDENT MANNER

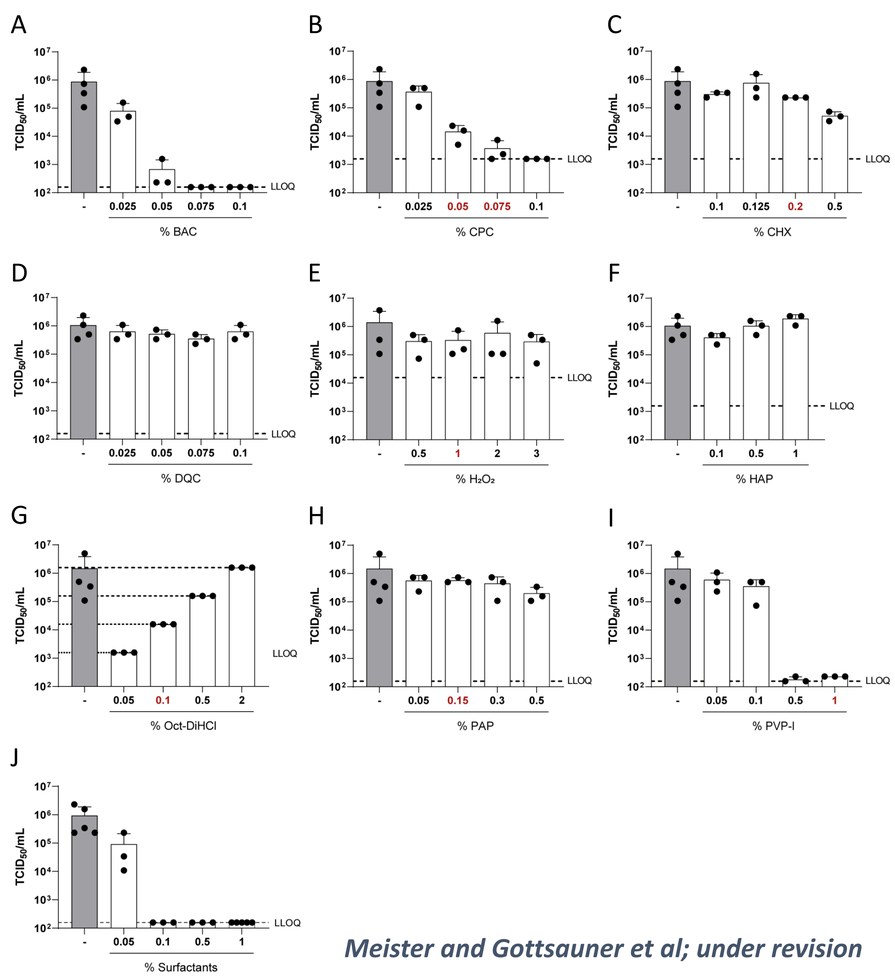

Figure 5: Virucidal activity of Benzalkoniumchloride (BAC), Cetylpyridiniumchloride (CPC), Chlorhexidine digluconate (CHX), Dequaliniumchloride (DQC), Hydrogen peroxide (H2O2), Hydroxyapatite (HAP), Octenidine-Dihydrochloride (Oct-DiHCl), Polyaminopropyl-Biguanide (PAP), Polyvenylpyrrolidone iodine (PVP-I), and Surfactants (Sodium Lauryl Sulfate, Sodium Methyl Cocoyl Taurate, Sodium Myristoyl Sarcosinate) subjected to a quantitative suspension test. Each agent was tested in up to 4 different concentrations, that may occur in commercially available oral rinses (red number). Residual viral titres were determined by end-point dilution ($TCID_{50}$/mL) and compared to a medium control (grey). LLOQ: Lower limit of quantification (dotted line)

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TREATMENT WITH BENZALCONIUMCHLORIDE AND OTHER ANTISEPTIC AGENTS DISRUPTED THE VIRAL ENVELOPE, WITHOUT AFFECTING VIRAL RNA INTEGRITY

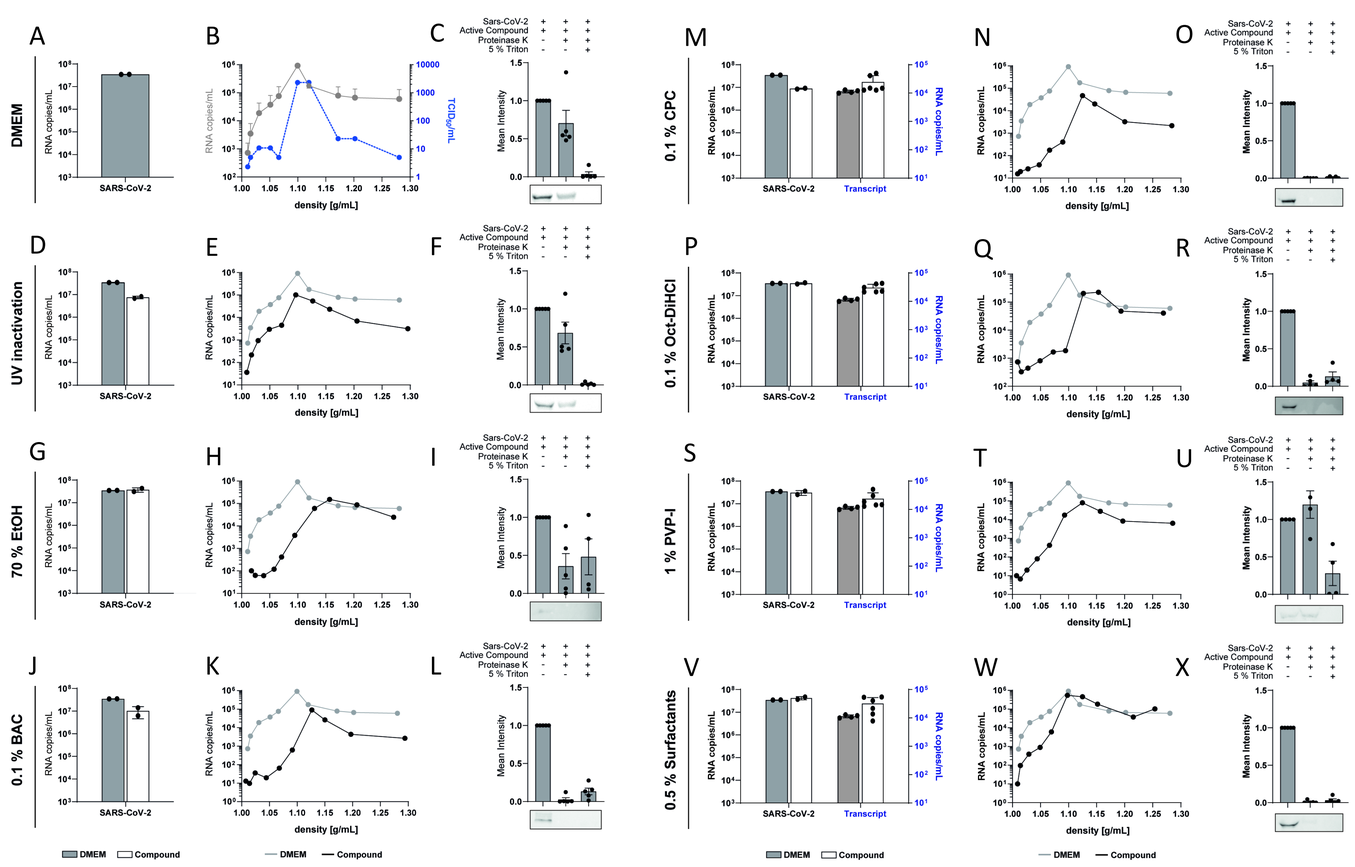

Figure 6: Mode of action. SARS-CoV-2 was either incubated with BAC, CPC, Oct-DiHCl, PVP-I, or Surfactants and an interfering substance for 30 s. Medium was used as a control (A-C). UV inactivation served as a control for RNA damage (D-F), while 70% EtOH served as a control for envelope disruption (G-I). RNA integrity (A, D, G, J, M, P, S and V) for each treatment (white bar) was investigated by RT-qPCR and compared to DMEM (grey bar). Additionally, M-gene transcripts were spiked into each agent and recovered by RNA isolation and RT-qPCR (blue; M, P, S, V). Sucrose step gradient ultracentrifugation was performed to evaluate viral envelope integrity after exposure to antiseptic agents (B, E, H, K, N, Q, T and W). RNA copy numbers in each fraction were determined by RT-qPCR (black line) and compared to DMEM (grey line). The viral envelope was further assessed by a capsid protection assay (C, F, I, L, O, R, U and X). Therefore, one replicate was left untreated, one part was treated with proteinase K for 1 h at 4 °C, and another part was lysed in 5% Triton X-100 prior to proteinase K treatment. The amount of protease-resistant nucleocapsid protein was quantified by Western blot. Data indicate averages.

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