Effective in vitro inactivation of SARS-CoV-2 by commercially available mouthwashes

Katherine Davies1, Hubert Buczkowski1, Stephen R. Welch1, Nicole Green1, Damian Mawer2, Neil Woodford3, Allen D. G. Roberts1, Peter J. Nixon2, David W. Seymour2 and Marian J. Killip1,*

Abstract

Infectious SARS-CoV-2 can be recovered from the oral cavities and saliva of COVID-19 patients with potential implications for disease transmission. Reducing viral load in patient saliva using antiviral mouthwashes may therefore have a role as a control measure in limiting virus spread, particularly in dental settings. Here, the efficacy of SARS-CoV-2 inactivation by seven commercially available mouthwashes with a range of active ingredients were evaluated in vitro. We demonstrate ≥4.1 to ≥5.5 log10 reduction in SARS-CoV-2 titre following a 1 min treatment with commercially available mouthwashes containing 0.01–0.02% stabilised hypochlorous acid or 0.58% povidone iodine, and non-specialist mouthwashes with both alcohol-based and alcohol-free formulations designed for home use. In contrast, products containing 1.5% hydrogen peroxide or 0.2% chlorhexidine gluconate were ineffective against SARS-CoV-2 in these tests. This study contributes to the growing body of evidence surrounding virucidal efficacy of mouthwashes/oral rinses against SARS-CoV-2, and has important applications in reducing risk associated with aerosol generating procedures in dentistry and potentially for infection control more widely.

SARS-CoV-2 is the virus responsible for causing COVID-19 [1], and infectious SARS-CoV-2 is detectable in the oral cavities and the saliva of COVID-19 patients [2, 3] with potential implications for disease transmission. Aerosol-generating procedures, particularly in the dental setting, therefore pose a potential infectious risk to health care teams working in close proximity to patients while these procedures are being carried out [4]. The World Health Organization recommends the use of pre-procedural mouth rinses for the reduction of SARS-CoV-2 viral load in patient saliva as a control measure for reduction of this infectious risk [5]. Here, we have assessed seven different commercially available mouthwashes with a range of active ingredients for the efficacy against SARS-CoV-2 in vitro.

The commercial mouthwashes tested in this study are listed in Table 1. All products were stored in their original packaging according to manufacturer’s instructions and were unopened prior to testing. In vitro SARS-CoV-2 inactivation assessments were performed in a containment level three facility, and all virus manipulations were performed within a Class III microbiological safety cabinet (MSC). Briefly, one volume of virus preparation (SARS-CoV-2 England 2 strain, in tissue culture fluid [TCF] comprising Minimum Essential Media [MEM] and 5% foetal calf serum, with a titre of 1.7×10^7 TCID50 ml^-1) was mixed with ten volumes of product and mixed well by inversion. Products were incubated at ambient temperature (20 ±2 °C) for 1 min, then immediately titrated in phosphate-buffered saline (PBS) to generate a ten-fold dilution series. Dilution series were directly applied to 96-well plates of Vero E6 cells to determine the 50% tissue culture infectious dose (TCID50) as previously described [6]. All products were tested in triplicate, and a triplicate set of samples treated with an equivalent volume of PBS was included in each experiment as a control for virus recovery. The cytotoxicity of treated samples varied between products, and a cytotoxic control sample comprising one volume of PBS to ten volumes of product was evaluated in parallel and used to calculate the limit of detection for each product (the lowest dilution at which no cytotoxic effect was observed). Mean titre reductions were calculated by subtracting the mean

Received 23 November 2020; Accepted 03 March 2021; Published 29 April 2021

Author affiliations: 1High Containment Microbiology, NIS Laboratories, National Infection Service, Public Health England, 61 Colindale Avenue, Colindale, London, NW9 5EQ, UK; 2York Hospitals NHS Foundation Trust, Wigginton Road, York, YO31 8HE, UK; 3National Laboratories, National Infection Service, Public Health England, 61 Colindale Avenue, Colindale, NW9 5EQ, London, UK.

*Correspondence: Marian J. Killip, marian.killip@phe.gov.uk

Keywords: COVID-19; coronavirus; inactivation; mouthwash; oral rinse; SARS-CoV-2

Abbreviations: MEM, minimum essential media; MSC, microbiological safety cabinet; PBS, phosphate-buffered saline; TCF, tissue culture fluid; TCID50, 50% tissue culture infectious dose; WHO, World Health Organization.

001578 © 2021 Crown Copyright

This is an open-access article distributed under the terms of the Creative Commons Attribution License. This article was made open access via a Publish and Read agreement between the Microbiology Society and the corresponding author’s institution.
log_{10} titre of treated samples from the mean log_{10} titre of PBS-treated samples, or for products with no detectable virus remaining following treatment, the mean log_{10} titre of PBS-treated samples minus the limit of detection.

Two Listerine compositions were evaluated in this study: Listerine Advanced Defence Sensitive and alcohol-free Listerine Total Care. Both formulations reduced SARS-CoV-2 titre to below the limit of detection for the tests after a 1 min treatment: ≥3.5 log_{10} reduction for Listerine Advanced Defence Sensitive and ≥4.1 log_{10} reduction for Listerine Total Care, respectively (Fig. 1, Table 1). The high level of cytotoxicity associated with Listerine Advanced Defence Sensitive meant that the reduction we could demonstrate for this product in this test was below the >4 log_{10} reduction given in the standard for virucidal quantitative suspension tests, BS EN 14476 [7]. Previously, we have conducted a wide range of chemical inactivation testing to inform risk assessments around sample processing for the COVID-19 response [6, 8]; we have used purification methods extensively for these assessments to remove components that are cytotoxic in cell culture and would otherwise increase the limit of detection for treated samples. However, we have found these methods unsuitable for evaluation of short (e.g. 2 min or less) treatment times due to the additional time required for sample processing. To see if we could increase the detectable titre reduction without performing a post-treatment purification step, we tested these products using a concentrated virus preparation, generated by concentrating TCF containing virus through 100-kDa-cutoff Amicon Ultra-15 centrifugal filters. When tested against this concentrated virus, we could demonstrate ≥4.2 log_{10} titre reduction for Listerine Advanced Defence Sensitive and ≥5.2 log_{10} for Listerine Total Care. Both of these products were therefore clearly effective at inactivating SARS-CoV-2 in a TCF matrix, despite both products differing in their active ingredients. The manufacturer lists 1.4 % dipotassium oxalate as the active ingredient in Listerine Advanced Defence Sensitive, while eucalyptol, thymol, menthol, sodium fluoride and zinc fluoride are given as active ingredients for Listerine Total Care, although the contribution of these particular ingredients to the antiviral activity of these mouthwashes is unclear. Alternative Listerine compositions have been evaluated for SARS-CoV-2 antiviral activity by others and found to be effective, including Listerine Cool Mint [9, 10], Listerine Antiseptic [11] and Listerine Advanced Gum Treatment [10]. This study provides evidence that Listerine Advanced Defence Sensitive and Total Care formulations are similarly effective against SARS-CoV-2.

Povident contains 0.58 % povidone iodine, and reduced SARS-CoV-2 titre by ≥4.1 log_{10} in our tests using unconcentrated TCF and ≥5.2 log_{10} using concentrated TCF (Fig. 1, Table 1). This is consistent with previous studies of povidone iodine-based products, where efficacy in vitro against coronaviruses has been demonstrated, including against SARS-CoV-1 and Middle East respiratory syndrome-associated coronavirus MERS-CoV [12, 13]. More recently, oral rinse products containing between 0.5 and 1.0 % povidone iodine have been demonstrated to be effective against SARS-CoV-2.

Table 1. SARS-CoV-2 inactivation by commercial mouthwashes

| Product                                      | Manufacturer                                   | Active ingredient/s*                          | Mean titre reduction; log_{10} TCID50 ml⁻¹ (95% CI) |
|----------------------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------------|
| Chlorhexidine Gluconate                      | Ecolabs                                       | 0.2% chlorhexidine gluconate (formulation contains ethanol) | 0.5 (0.1–0.9)                                     |
| Antiseptic Mouthwash (Peppermint Flavour)    |                                               |                                               | Not tested                                        |
| Corsodyl                                     | GlaxoSmithKline                               | 0.2% chlorhexidine gluconate (alcohol-free formulation) | 0.2 (-0.2–0.7)                                    |
| (Alcohol Free Mint Flavour)                  |                                               |                                               | Not tested                                        |
| Listerine Advanced Defence Sensitive         | Johnson and Johnson                           | 1.4% dipotassium oxalate (alcohol-free formulation) | ≥3.5† (3.2–3.8)                                   |
| Listerine Total Care                         | Johnson and Johnson                           | Eucalyptol, thymol, menthol, sodium fluoride, zinc fluoride | ≥4.1‡ (3.8–4.4)                                   |
| OraWize+                                     | Aqualution Systems                            | 0.01–0.02% stabilised hypochlorous acid       | ≥5.5§ (5.2–5.8)                                   |
| Peroxyl                                      | Colgate                                       | 1.5% hydrogen peroxide                        | 0.2 (-0.1–0.5)                                    |
| Povident                                     | Huddersfield Pharmacy Specials                | 0.58% povidone iodine (surfactant-free)        | ≥4.1‡ (3.8–4.4)                                   |

*Principal active ingredient/s listed by the manufacturer only are given; refer to manufacturer documents for full ingredients.
†Limit of detection was 2.7 log_{10} TCID50 ml⁻¹ due to product cytotoxicity.
‡Limit of detection was 1.7 log_{10} TCID50 ml⁻¹ due to product cytotoxicity.
§Limit of detection was 0.7 log_{10} TCID50 ml⁻¹.
OraWize+, a product containing 0.01–0.02% hypochlorous acid (HOCl) as its active ingredient, reduced virus titre in unconcentrated TCF by ≥5.5 log₁₀ TCID₅₀ ml⁻¹, to below the limit of detection for the assay (Fig. 1, Table 1). A potential role for hypochlorous acid-based products as oral rinses to combat SARS-CoV-2 has been proposed [17, 18], but to our knowledge this is the first in vitro evidence for efficacy of a hypochlorous acid-based mouthwash against SARS-CoV-2. It is important to note however that OraWize+ was not effective when tested against concentrated TCF (Fig. 1, Table 1), potentially due to high levels of protein in this sample matrix, suggesting that the chemistry of this product may be affected by complex samples types. This is an observation we have also made for other hypochlorous acid-based inactivants (unpublished data) and further testing is required to determine the significance of this observation for product use.

Two chlorhexidine gluconate-based products were evaluated in this study: Corsodyl (alcohol-free) and Ecolabs Chlorhexidine Gluconate Antiseptic Wash (containing ethanol). Neither were effective at inactivating SARS-CoV-2 (Fig. 1, Table 1), consistent with previous studies demonstrating only a very small effect on SARS-CoV-2 [9, 10]. Peroxyl (containing 1.5% hydrogen peroxide) was similarly ineffective. This last observation was initially surprising considering that 1 min treatment with 0.5% hydrogen peroxide has been reported to be effective against human coronavirus 229E in virus suspension tests [19] and that 1% hydrogen peroxide pre-procedural mouth rinse is recommended by the World Health Organisation (WHO).
and others for reduction of infectious risks in the context of COVID-19 [4, 5]. However, ours is not the only study to demonstrate minimal in vitro effectiveness of hydrogen peroxide-based mouth rinses against SARS-CoV-2 and the superior effectiveness of other types of oral rinses [9, 15].

The availability and stability of these products vary, and these factors may impact their utility in different settings. OraWize+ has a much shorter shelf life than other products tested (1 month after opening) and must be protected from light; we have found that it can lose effectiveness when stored incorrectly (unpublished data). Povienst has a relatively short shelf life, and is not widely available in the UK (indeed, currently there is no widely commercially available povidone iodine mouthwash in the UK). In contrast, the Listerine formulations tested have a considerably longer shelf life, are far more widely available and are designed for use by the general public.

The mean temperature of the oral cavity, where mouthwash products are designed to be used, is 36.6°C [20]. Temperature is known to affect virus stability and the half-life of SARS-CoV-2 infectivity in suspension has been shown to be longer at 22 °C than at 37 °C [21]. Our testing was carried out at ambient temperature, at which virus is likely to be more stable than at body temperature; any deviation between our results and effects at 36–37 °C therefore potentially errs on the conservative side, with observed effects of products on virus viability being less pronounced than may be seen at higher temperatures.

In conclusion, we have demonstrated effective inactivation of SARS-CoV-2 by Listerine Advanced Defence Sensitive and Total Care formulations, and by commercial mouthwashes containing 0.01–0.02% hypochlorous acid or 0.58% povidone iodine in in vitro tests using TCF. Our data support the use of these products, but not the use of hydrogen peroxide or chlorhexidine gluconate mouthwashes, for reduction of SARS-CoV-2 viral load, and thus indicate a potential use for these products in the reduction of infectious risk associated with aerosol generating dental procedures and for SARS-CoV-2 infection control more generally. Their applicability for these purposes is highly dependent on being able to demonstrate reproducibility of our findings of in real-world settings, and determination of the length of time after product use that any antiviral effect persists. Our evidence supports inclusion of several of these mouthwashes into a randomised controlled trial to evaluate their efficacy and substantivity against SARS-CoV-2 in vivo.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med Overseas Ed 2020;382:727–733.
2. Jeong HW, Kim SM, Kim HS, Kim YI, Kim JH et al. Viable SARS-CoV-2 in various specimens from COVID-19 patients. Clin Microbiol Infect 2020;26:1520–1524.
3. To KK-W, Tsang OT-Y, Yip CC-Y, Chan K-H, Wu T-C et al. Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis 2020;71:841–843.
4. Peng X, Xu X, Li Y, Cheng L, Zhou X et al. Transmission routes of 2019-nCoV and controls in dental practice. Int J Oral Sci 2020;12:9.
5. World Health Organization. Considerations for the provision of essential oral health services in the context of COVID-19: Interim guidance 2020 https://www.who.int/publications/i/item/who-2019-nCoV-oral-health-2020.1.
6. Welch SR, Davies KA, Buczkowski H, Hettiarachchi N, Green N et al. Analysis of inactivation of SARS-CoV-2 by specimen transport media, nucleic acid extraction reagents, detergents, and fixatives. J Clin Microbiol 2020;58.
7. British Standards Institution. Chemical disinfectants and antiseptics- quantitative suspension test for the evaluation of virucidal activity in the medical area- test method and requirements (phase 2/Step 1). BSI Standards Limited 2019.
8. Public Health England. 2021. COVID-19: Phe laboratory assessments of inactivation method (phase 2019-0913). Public Health England 2021. COVID-19: Phe laboratory assessments of inactivation methods.
9. Meister TL, Brüggemann Y, Todt D, Conzelmann C, Müller JA et al. Virucidal efficacy of different oral rinses against severe acute respiratory syndrome coronavirus 2. J Infect Dis 2020;222:1289–1292.
10. Statkute E, Rubina A, O’Donnell VB, Thomas DW, Stanton RJ. Brief report: the virucidal efficacy of oral rinse components against SARS-CoV-2 in vitro. bioRxiv 2020.
11. Meyers C, Robison R, Milici J, Alam S, Quilien D. Lowering the transmission and spread of human coronavirus. J Med Virol 2021;93:1605–1612.
12. Eggers M, Koburger-Janssens T, Eickmann M, Zorn J. In vitro bactericidal and virucidal efficacy of povidone-iodine Gargle/Mouthwash against respiratory and oral tract pathogens. Infect Dis Ther 2018;7:249–259.
13. Kariwa H, Fujii N, Takashima I. Inactivation of SARS coronavirus by means of povidone-iodine, physical conditions and chemical reagents. Dermatology 2016;232:119–123.
14. Anderson DE, Sivalingam V, Kang AEZ, Ananthanarayanan A, Arumugam H et al. Povidone-Iodine demonstrates rapid in vitro virucidal activity against SARS-CoV-2, the virus causing COVID-19 disease. Infect Dis Ther 2020;9:669–675.
15. Bidra AS, Pelletier JS, Westover JB, Frank S, Brown SM et al. Comparison of in vitro inactivation of SARS-CoV-2 with hydrogen peroxide and povidone-iodine oral antiseptic rinses. J Prosthodont Res 2020;64:246–251.
16. Martínez Lamas L, Diz Dios P, Pérez Rodríguez MT, Pérez DC V, Cabrera Alvaranzoles JJ et al. Is povidone iodine mouthwash effective against SARS-CoV-2? first in vivo tests. Oral Dis 2020;26:1520–1524.
17. Block MS, Rowan BG. Hypochlorous acid: a review. J Oral Maxillofac Surg 2020;78:1461–1466.
18. Banakar M, Bagheri Lankarani K, Jafarpour D, Moayedi S, Banakar MH et al. COVID-19 transmission risk and protective protocols in dentistry: a systematic review. BMC Oral Health 2020;20:275.
19. Kämpf G, Todt D, Pfender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020;104:246–251.
20. Geneva II, Cuzzo B, Fazili T, Javadi W. Normal body temperature: a systematic review. Open Forum Infect Dis 2019;6:ofz325-0fz.
21. Chin AWH, Chu JTS, Perera MRA, Hui KPY, Yen H-L et al. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 2020;1:e10-e.

Funding information

This work was supported by Public Health England.

Acknowledgements

The views expressed in this article are those of the authors and are not necessarily those of Public Health England, the National Health Service or the Department of Health and Social Care.

Conflicts of interest

The authors declare that there are no conflicts of interest.