

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

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36 **ABSTRACT**

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38 Infectious SARS-CoV-2 can be recovered from the oral cavities and saliva of
39 COVID-19 patients with potential implications for disease transmission.
40 Reducing viral load in patient saliva using antiviral mouthwashes may
41 therefore have a role as a control measure in limiting virus spread, particularly
42 in dental settings. Here, the efficacy of SARS-CoV-2 inactivation by seven
43 commercially available mouthwashes with a range of active ingredients were
44 evaluated *in vitro*. We demonstrate ≥ 4.1 to ≥ 5.5 \log_{10} reduction in SARS-CoV-
45 2 titre following a one minute treatment with commercially available
46 mouthwashes containing 0.01-0.02% stabilised hypochlorous acid or 0.58%
47 povidone iodine, and non-specialist mouthwashes with both alcohol-based
48 and alcohol-free formulations designed for home use. In contrast, products
49 containing 1.5% hydrogen peroxide or 0.2% chlorhexidine gluconate were
50 ineffective against SARS-CoV-2 in these tests. This study contributes to the
51 growing body of evidence surrounding virucidal efficacy of mouthwashes/oral
52 rinses against SARS-CoV-2, and has important applications in reducing risk
53 associated with aerosol generating procedures in dentistry and potentially for
54 infection control more widely.

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70 **MAIN TEXT**

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

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84 The commercial mouthwashes tested in this study are listed in Table 1. All
85 products were stored in their original packaging according to manufacturer's
86 instructions and were unopened prior to testing. *In vitro* SARS-CoV-2
87 inactivation assessments were performed in a containment level 3 facility, and
88 all virus manipulations were performed within a class III microbiological safety
89 cabinet. Briefly, one volume of virus preparation (SARS-CoV-2 England 2
90 strain, in tissue culture fluid [TCF] comprising Minimum Essential Media
91 [MEM] and 5% foetal calf serum) was mixed with ten volumes of product and
92 mixed well by inversion. Products were incubated at 20°C (+/- 2°C) for one
93 minute, then immediately titrated in phosphate-buffered saline (PBS) to
94 generate a ten-fold dilution series. Dilution series were directly applied to 96-
95 well plates of Vero E6 cells to determine the 50% tissue culture infectious
96 dose (TCID₅₀) as previously described (6). All products were tested in
97 triplicate, and a triplicate set of samples treated with an equivalent volume of
98 PBS was included in each experiment as a control for virus recovery. Mean
99 titre reductions were calculated by subtracting the mean log₁₀ titre of treated
100 samples from the mean log₁₀ titre of PBS-treated samples. The cytotoxicity of
101 treated samples varied between products, and a cytotoxic control sample

102 comprising one volume of PBS to ten volumes of product was evaluated in
103 parallel and used to calculate the limit of detection for each product.

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105 Two Listerine compositions were evaluated in this study: Listerine Advanced
106 Defence Sensitive and alcohol-free Listerine Total Care. Both formulations
107 reduced SARS-CoV-2 titre to below the limit of detection for the tests after a
108 one minute treatment: $\geq 3.5 \log_{10}$ reduction for Listerine Advanced Defence
109 Sensitive and $\geq 4.1 \log_{10}$ reduction for Listerine Total Care, respectively (Table
110 1). The high level of cytotoxicity associated with Listerine Advanced Defence
111 Sensitive meant that the reduction we could demonstrate for this product in
112 this test was below the $>4 \log_{10}$ reduction given in the standard for virucidal
113 quantitative suspension tests, BS EN 14476 (7). Previously, we have
114 conducted a wide range of chemical inactivation testing to inform risk
115 assessments around sample processing for the COVID-19 response (6, 8);
116 we have used purification methods extensively for these assessments to
117 remove components that are cytotoxic in cell culture and would otherwise
118 increase the limit of detection for treated samples. However, we have found
119 these methods unsuitable for evaluation of short (e.g. two minutes or less)
120 treatment times due to the additional time required for sample processing. To
121 see if we could increase the detectable titre reduction without performing a
122 post-treatment purification step, we tested these products using a
123 concentrated virus preparation, generated by concentrating TCF containing
124 virus through 100-kDa-cutoff Amicon Ultra-15 centrifugal filters. When tested
125 against this concentrated virus, we could demonstrate $\geq 4.2 \log_{10}$ titre
126 reduction for Listerine Advanced Defence Sensitive and $\geq 5.2 \log_{10}$ for
127 Listerine Total Care. Both of these products were therefore clearly effective at
128 inactivating SARS-CoV-2 in a TCF matrix, despite both products differing in
129 their active ingredients. The manufacturer lists 1.4% dipotassium oxalate as
130 the active ingredient in Listerine Advanced Defence Sensitive, while
131 eucalyptol, thymol, menthol, sodium fluoride and zinc fluoride are given as
132 active ingredients for Listerine Total Care, although the contribution of these
133 particular ingredients to the antiviral activity of these mouthwashes is unclear.
134 Alternative Listerine compositions have been evaluated for SARS-CoV-2
135 antiviral activity by others, including Listerine Cool Mint (9, 10), Listerine

136 Antiseptic (11) and Listerine Advanced Gum Treatment (10). This study
137 provides evidence that Listerine Advanced Defence Sensitive and Total Care
138 formulations are similarly effective against SARS-CoV-2.

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140 Povidone contains 0.58% povidone iodine, and reduced SARS-CoV-2 titre by
141 $\geq 4.1 \log_{10}$ in our tests using unconcentrated TCF and $\geq 5.2 \log_{10}$ using
142 concentrated TCF (Table 1). This is consistent with previous studies of
143 povidone iodine-based products, where efficacy in vitro against coronaviruses
144 has been demonstrated, including against SARS-CoV-1 and Middle East
145 respiratory syndrome-associated coronavirus MERS-CoV (12, 13). More
146 recently, oral rinse products containing between 0.5% and 1.0% povidone
147 iodine have been demonstrated to be effective against SARS-CoV-2 in vitro
148 (9, 10, 14, 15) and in reducing viral load in the saliva of human COVID-19
149 patients (16).

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151 OraWize+, a product containing 0.01-0.02% hypochlorous acid (HOCl) as its
152 active ingredient, reduced virus titre in unconcentrated TCF by $\geq 5.5 \log_{10}$
153 TCID₅₀/ml, to below the limit of detection for the assay (Table 1). A potential
154 role for hypochlorous acid-based products as oral rinses to combat SARS-
155 CoV-2 has been proposed (17, 18), but to our knowledge this is the first *in*
156 *vitro* evidence for efficacy of a hypochlorous acid-based mouthwash against
157 SARS-CoV-2. It is important to note however that OraWize+ was not effective
158 when tested against concentrated TCF (Table 1), potentially due to high levels
159 of protein in this sample matrix, suggesting that the chemistry of this product
160 may be affected by complex samples types. This is an observation we have
161 also made for other hypochlorous acid-based inactivants (unpublished data)
162 and further testing is required to determine the significance of this observation
163 for product use.

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165 Two chlorhexidine gluconate-based products were evaluated in this study:
166 Corsodyl (alcohol-free) and Ecolabs Chlorhexidine Gluconate Antiseptic Wash
167 (containing ethanol). Neither were effective at inactivating SARS-CoV-2
168 (Table 1), consistent with previous studies demonstrating only a very small
169 effect on SARS-CoV-2 (9, 10). Peroxyl (containing 1.5% hydrogen peroxide)

170 was similarly ineffective. This last observation was initially surprising
171 considering that one minute treatment with 0.5% hydrogen peroxide has been
172 reported to be effective against human coronavirus 229E in virus suspension
173 tests (19) and that 1% hydrogen peroxide pre-procedural mouth rinse is
174 recommended by the World Health Organisation (WHO) and others for
175 reduction of infectious risks in the context of COVID-19 (4, 5). However, ours
176 is not the only study to demonstrate minimal *in vitro* effectiveness of hydrogen
177 peroxide-based mouth rinses against SARS-CoV-2 and the superior
178 effectiveness of other types of oral rinses (9, 15).

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

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190 In conclusion, we have demonstrated effective inactivation of SARS-CoV-2 by
191 by Listerine Advanced Defence Sensitive and Total Care formulations, and by
192 commercial mouthwashes containing 0.01-0.02% hypochlorous acid or 0.58%
193 povidone iodine in *in vitro* tests using TCF. Our data support the use of these
194 products, but not the use of hydrogen peroxide or chlorhexidine gluconate
195 mouthwashes, for reduction of SARS-CoV-2 viral load, and thus indicate a
196 potential use for these products in the reduction of infectious risk associated
197 with aerosol generating dental procedures and for SARS-CoV-2 infection
198 control more generally. Our evidence supports inclusion of several of these
199 mouthwashes into a randomised controlled trial to evaluate their efficacy and
200 substantivity against SARS-CoV-2 *in-vivo*.

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202 **Conflicts of interest**

203 The authors declare that there are no conflicts of interest.

204

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

Product	Manufacturer	Active ingredient/s [‡]	Mean titre reduction; log ₁₀ TCID ₅₀ /ml (95% CI)	
			TCF unconcentrated	TCF concentrated
Chlorhexidine Gluconate Antiseptic Mouthwash (Peppermint Flavour)	Ecolabs	0.2% chlorhexidine gluconate (formulation contains ethanol)	0.5 (0.1-0.9)	Not tested
Corsodyl (Alcohol Free Mint Flavour)	GlaxoSmithKline	0.2% chlorhexidine gluconate (alcohol-free formulation)	0.4 (-0.2-0.7)	Not tested
Listerine Advanced Defence Sensitive	Johnson & Johnson	1.4% dipotassium oxalate (alcohol-free formulation)	≥ 3.5** (3.2-3.8)	≥ 4.2** (3.9-4.4)
Listerine Total Care	Johnson & Johnson	Eucalyptol, thymol, menthol, sodium fluoride, zinc fluoride	≥ 4.1* (3.8-4.4)	≥ 5.2* (4.9-5.4)
OraWize+	Aqualution Systems	0.01-0.02% stabilised hypochlorous acid	≥ 5.5 [†] (5.2-5.8)	0.4 (0.0-0.8)
Peroxyl	Colgate	1.5% hydrogen peroxide	0.2 (-0.1-0.5)	Not tested
Povident	Huddersfield Pharmacy Specials	0.58% povidone iodine	≥ 4.1* (3.8-4.4)	≥ 5.2* (4.9-5.4)

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[‡]Principal active ingredient/s listed by the manufacturer only are given; refer to manufacturer documents for full ingredients
[†]Limit of detection was 0.7 log₁₀ TCID₅₀/ml
^{*}Limit of detection was 1.7 log₁₀ TCID₅₀/ml due to product cytotoxicity
^{**}Limit of detection was 2.7 log₁₀ TCID₅₀/ml due to product cytotoxicity