

Comparative Analysis of Antiviral Efficacy of Four Different Mouthwashes against Severe Acute Respiratory Syndrome Coronavirus 2: An *In Vitro* Study

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ABSTRACT

Aim: The aim of the present study was to evaluate and compare the efficacy and cytotoxicity of four different mouthwashes containing 1.5% hydrogen peroxide, 0.2% povidone, 0.12% chlorhexidine and 100 ppm molecular iodine for their ability to inactivate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Materials and methods: The SARS-CoV-2 virus stocks were grown in minimum essential medium (MEM) test media, supplemented with 2.0% fetal bovine serum (FBS) and 50 µg/mL gentamicin. Test solutions and virus were incubated at room temperature for three contact times of 15, 30, and 60 seconds. The solutions were then neutralized by a 1/10 dilution in test media containing 10% FBS. Neutralized samples were then incubated for 5 days at 37 ± 2°C at 5% CO₂. The test plates were then scored for their cytopathic effect and the log-reduction value (LRV) of the test solution was compared to the negative control (water).

Results: The results showed that the LRV for a 100 ppm molecular iodine oral rinse was significantly greater, at each exposure time, compared with the other three oral rinses in the study with no associated cytotoxicity. The 100 ppm molecular iodine rinse exhibited an LRV of 2.6 at 15 seconds and complete inactivation of SARS-CoV-2 at both 30 seconds and also at 60 seconds with LRV greater than 3.6 for each of those contact times.

Conclusion: The spread of infection through aerosol and splatter has long been considered one of the main concerns in the dental community. A preprocedural rinse with 100 ppm molecular iodine will play a vital role in combating COVID-19 pandemic by preventing the spread of infection.

Keywords: COVID, Molecular iodine, Pandemic, Preprocedural.

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INTRODUCTION

The 2019 novel coronavirus (SARS-CoV-2) epidemic, which was first reported in December 2019 in Wuhan, China, and has been declared a public health emergency of international concern by the World Health Organization, has progressed to a pandemic associated with substantial morbidity and mortality.¹ Coronavirus is one of the major pathogens that primarily targets the human respiratory system. Previous outbreaks of coronaviruses (CoVs) include the severe acute respiratory syndrome (SARS)-CoV and the Middle East respiratory syndrome (MERS)-CoV, which have been previously characterized as agents that are a great public health threat.² Viruses do not replicate outside living cells but infectious virus may persist on contaminated environmental surfaces and the duration of persistence of viable virus is affected markedly by temperature and humidity. Contaminated surfaces are known to be significant vectors in the transmission of infections in the hospital setting as well as the community. The main route of transmission of SARS-CoV infection is presumed to be respiratory droplets.³ In response to the COVID-19 pandemic, dental offices have instituted unprecedented safeguards to protect patients and staff from viral transmission. A primary concern is the aerosolizing of the SARS-CoV-2 virus associated with dental procedures.⁴ The use of high-speed handpieces, three-way syringes, and ultrasonic scalers all generate dental aerosols.⁵ In order to mitigate the viral load in these aerosols, antimicrobial, preprocedural rinses are recommended.⁶

Therefore, the present *in vitro* study was designed to evaluate and compare the efficacy of four different mouthwashes containing

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1.5% hydrogen peroxide, 0.2% povidone, 0.12% chlorhexidine, and 100 ppm molecular iodine against SARS-CoV-2 and their relative cytotoxicity.

MATERIALS AND METHODS

The present study was conducted at the Institute for Antiviral Research (IAR), Utah State University, in a level 3 BioContainment laboratory. The SARS-CoV-2 virus stocks were prepared in the laboratory by growing virus in Vero 76 cells. Test media used was minimum essential media (MEM) supplemented with 2% fetal bovine serum (FBS) and 50 µg/mL gentamicin. The four mouth rinses used in the study were the following:

I: 3% hydrogen peroxide (Walgreen Co., Deerfield, Illinois)

II: 10% povidone iodine (Walgreen Co., Deerfield, Illinois)

III: 0.12% chlorhexidine gluconate (Henry Schein Co., Melville, New York)

IV: Formula 100-S molecular iodine (Iotech International, Boca Raton, Florida)

Hydrogen peroxide was tested at 1.5%, povidone iodine at 0.2%, chlorhexidine gluconate at 0.12%, and Formula 100-S at 100 ppm molecular iodine. The SARS-CoV-2 virus stock was added to triplicate tubes of each prepared concentration (10% virus solution plus 90% test sample). Media only was added to one tube of each prepared concentration to serve as toxicity controls. Ethanol was tested in parallel as a positive control and water only to serve as the virus control. Test solutions and virus were incubated at room temperature for three contact times of 15, 30, and 60 seconds. Following the contact period, the solutions were neutralized by a 1/10 dilution in test media containing 10% FBS. Neutralized samples were combined for quantification for the average of triplicate tests. Samples were serially diluted using eight half-log dilutions in the test medium. Each dilution was added to four wells of a 96-well plate with 80–100% confluent Vero 76 cells. The toxicity controls were added to an additional four wells and two of these wells were infected with virus to serve as neutralization controls, ensuring that residual sample in the titer assay plated did not inhibit growth and detection of surviving virus. All plates were incubated at $37 \pm 2^\circ\text{C}$, 5% CO_2 . On day 5 after addition of samples, plates were scored for presence or absence of viral cytopathic effect (CPE). The Reed-Muench method was used to determine end-point titers (50% cell culture infectious dose, CCID_{50}) of the samples, and the log-reduction value (LRV) of the compound compared to the negative (water) control was calculated. Virus controls were tested in water and the reduction of virus in test wells compared to virus controls was calculated as the LRV. Toxicity controls were tested with media not containing virus to see if the samples were toxic to cells. Neutralization controls were tested to ensure that virus inactivation did not continue after the specified contact time, and that residual sample in the titer assay plates did not inhibit growth and detection of surviving virus. This was done by adding toxicity samples to titer test plates, then spiking each well with a low amount of virus that would produce an observable amount of CPE during the incubation period.

RESULTS

Virus titers and LRV for hydrogen peroxide, povidone iodine, chlorhexidine gluconate oral rinse, and Formula 100-S molecular iodine oral rinse are shown in Table 1. Full toxicity was observed in the top three dilutions of hydrogen peroxide (1/10, 1/100, and 1/1,000) and the top two dilutions of chlorhexidine gluconate oral rinse. Because of this toxicity, presence of virus could not be ruled out in those wells; therefore, the limit of detection was 3.7 and 2.7 \log_{10} CCID_{50} of virus per 0.1 mL, respectively. The average of virus

control samples was used for comparison of test samples and contained 4.3 \log_{10} CCID_{50} per 0.1 mL. Samples with <1 log reduction of virus compared to the virus control were not considered active for virucidal activity. Due to cytotoxicity, the virucidal activity of hydrogen peroxide could not be determined as ≥ 1 log reduction of virus. However, some virus was detected in samples from all time points, indicating no virucidal activity. Povidone iodine exhibited virucidal activity when tested at 0.2%, reducing virus by 2.0 (90%) when combined with virus for a 60-seconds contact time, but did not reduce by ≥ 1 log in shorter contact times. Iotech International's Formula 100-S molecular iodine oral rinse reduced virus below the limit of detection ($\text{LRV} > 3.6$, $>99.9\%$) when tested for a contact time of 30 seconds or longer. Further, it reduced virus by 2.6 logs ($>99\%$) when tested for a 15-seconds contact time. Neutralization controls demonstrated that residual sample did not inhibit virus growth and detection in the endpoint titer assays in wells that did not have cytotoxicity. Virus controls and positive controls performed as expected.

DISCUSSION

The oral cavity is a reservoir for a large number of microorganisms including bacteria and viruses. This ecological niche can be a pool for opportunistic and pathogenic microorganisms that can pose a risk for cross-contamination and infection and may even cause systemic infections. This is of particular importance in the case of routine dental practice, as the risk of exposure to microorganisms in the oral cavity is increased due to the open and invasive nature of the procedures.⁷ To better protect patients and staff, dental offices are seeking guidance in augmenting their infection control protocols against SARS-CoV-2.⁸ To a large extent, infection control products have not been directly tested for their efficacy against SARS-CoV-2 because it is extremely contagious. In the United States, *in vitro* testing on SARS-CoV-2 has only been authorized by the Center for Disease Control and Prevention (CDC) at a limited number of laboratories rated BioContainment Level 3 or higher.⁹ As a result, clinicians are left with well-meaning, but confusing, guidance on the antiviral efficacy of oral rinses against SARS-CoV-2, because no direct testing against the virus had been available.¹⁰ The American Dental Association has been recommending 1.5% hydrogen peroxide as a preprocedural rinse because of its antiviral efficacy. At the same time, CDC has published test data showing 18–20 minutes are required for 1.5% hydrogen peroxide to completely inactivate rhinovirus.^{8,11}

The present study directly compares, for the first time, the biocidal efficacy of three widely used oral rinses and a promising new rinse against SARS-CoV-2. These results validate the superior biocidal efficacy of iodine as an antiviral agent against SARS-CoV-2. Both povidone iodine and molecular iodine formula 100-S displayed robust antiviral activity in this study compared to the other rinses.

Table 1: Antiviral efficacy against SARS-CoV-2

Oral rinse	Log reduction value			Observed cytotoxicity
	15 seconds	30 seconds	60 seconds	
1.5% hydrogen peroxide	<1.0	<1.0	<1.0	Present at 1:1,000 dilution
0.2% povidone iodine	2.0	2.0	3.0	None
0.12% chlorhexidine gluconate	<1.0	<1.0	1.0	Present at 1:100 dilution
Iotech international formula 100-S (100 ppm molecular iodine)	2.6	>3.6 complete inactivation	>3.6 complete inactivation	None

Table 2: Antiviral efficacy of povidone iodine at varying molecular iodine concentrations

Measured molecular iodine concentration	Log reduction value	
	Adenovirus (1 minute)	Poliovirus (15 minutes)
0.17 ppm	0.6	0.6
1.58 ppm	2.4	2.5
4.88 ppm	4.3	4.2

Iotech International's formula 100-S displayed the greatest antiviral activity of all the tested rinses, completely inactivating SARS-CoV-2 within 30 seconds. The antiviral efficacy of povidone iodine is directly dependent on the concentration of molecular iodine (free iodine) present. A previous study reported that 10% povidone iodine contains just a few ppm of molecular iodine and that its biocidal efficacy increases, in a dose-dependent fashion, as the concentration of molecular iodine is increased¹² as summarized in Table 2. The present study results were somewhat compromised by the observed cytotoxicity of both the hydrogen peroxide and chlorhexidine gluconate rinses.

The high rates of transmission, morbidity, and mortality of SARS-CoV-2¹³ suggest that dental offices utilize the most effective measures, possible, to counteract this deadly virus. A preprocedural rinse that can rapidly, safely and effectively knock down viral load, is an important link in this chain of defense.¹⁴ However, the clear differential in efficacy among the tested rinses highlights the need for an evidence-based approach in the selection of oral rinses. The 100 ppm molecular iodine rinse was recently developed and is based on a unique iodine chemistry.¹⁵ It was found to be the most effective rinse in reducing viral load in the *in vitro* lab study.

CONCLUSION

The routine use of evidence-based, antiviral, preprocedural rinses should provide both dental patients and dental staff with an additional measure of protection against a uniquely communicable and deadly virus. This *in vitro* laboratory study demonstrated that iodine-based oral rinses have superior antiviral efficacy with no associated cytotoxicity. Their use preprocedurally will significantly aid in controlling cross-infection in closed dental settings. However, further investigation of their antiviral applications and more studies in clinical settings are needed.

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