Antiviral Efficacy and Acceptability of Therapeutic Antiseptic Mouth Rinses for Inactivation of

COVID SARS-2 Virus

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Antiviral Efficacy and Acceptability of Therapeutic Antiseptic Mouth Rinses for Inactivation of COVID SARS-2 Virus

A randomized, placebo-controlled, double-masked clinical trial of antiseptic mouthwashes in the inactivation of COVID-19 SARS-2 virus in saliva

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PRÉCIS

Study Title

Antiviral Efficacy and Acceptability of Therapeutic Antiseptic Colgate Mouth Rinses for Inactivation of COVID SARS-2 Virus

Objectives

The primary objective is to quantify the impact of Colgate antiseptic mouthwashes on salivary infectivity in SARS-CoV-2+ patients. The secondary objectives are to quantitatively determine inflammatory cytokine and chemokine levels (pg/mL) in SARS-CoV-2+ patient blood and saliva and to evaluate the incidence of periodontal disease among study participants.

Design and Outcomes

Randomized, double-blind prospective trial to test the efficacy and acceptability of therapeutic, antiseptic mouth rinses to inactivate SARS-CoV-2 virus in saliva of SARS-CoV-2+ patients 18-65 years of age. All mouthrinses are commercially available Colgate products and will be used according to on-label instructions. Patients will be randomized to a mouthrinse and will be asked to give a saliva sample immediately before and after a 30-60 second mouthwash. Saliva samples will be collected from patients at 15-minute intervals thereafter up to one hour (15, 30, 45 and 60 min). The samples will be stored and used for RT-PCR detection of SARS-CoV-2 virus and viral infectivity assays, along with quantitative cytokine and chemokine concentration (pg/mL, Luminex). Patients will also complete a short survey on the taste and experience of using the mouthwash. Peripheral blood will be collected at the end of salivary collection. Participants, except controls, will be provided materials and oral hygiene instruction related to daily use of oral hygiene products provided by the study. In the seven-day period between study visits, participants will be directed to brush with Colgate toothpaste (at least twice per day) and rinse with the Colgate mouthrinse (according to on-label procedures). Controls are not given Colgate products and will be asked to carry out their typical oral hygiene regimen with the products they typically use. All participants will be asked to keep a daily diary of oral hygiene performance, product usage, COVID-19 symptoms and exposures. Daily diary data confirms compliance with oral hygiene protocol, and is needed for data inclusion and analysis, as it relates to secondary and tertiary outcomes for visit 2. Participants will be scheduled to return to the GoHealth Clinical Research Unit at one week after the baseline assessment, during which additional salivary (1 time point, 2 mL of saliva over 5 min, no rinse will occur) and blood samples will be collected. At the conclusion of sample collection, the participant will undergo a periodontal exam. Study participation concludes following the periodontal exam at the end of the second on site study visit. Patients with any periodontitis or gingivitis diagnosis will be informed of their diagnosis and will be referred to an oral healthcare provider for further treatment. Patients' final periodontal status will be a tertiary outcome of the periodontal exam and will be reported as the percentage of participants categorized into each diagnostic group; patients with a periodontal diagnosis will be informed of the findings and will be referred to an oral healthcare provider for further treatment. Participants who provide salivary and blood samples and follow study protocols will receive a $30 gift card as compensation at the end of visit 1 and $50 at the end of visit 2. This study involves two 90-minute visits.

Interventions and Duration

Subjects will have a brief oral exam and will be asked to rinse with an unlabeled/blinded antiviral mouth rinse for 30-60 sec and provide 2 mL of saliva prior to the rinse and 2 mL of saliva

immediately post-rinse, 15 min post-rinse, 30 min post-rinse, 45 min post-rinse, and 60 min post-rinse. Subjects will also be asked to complete a short survey about the rinsing experience. A blood sample will be taken. Subjects will be onsite for a 90 min initial visit appointment. Patients will be sent home with Colgate toothpaste and mouthrinse for a daily oral health regimen, where participants will brush and rinse twice daily and maintain a take-home diary on their oral care regimen and COVID-19 symptoms. Patients will return for a second visit one week later for a salivary sample, blood draw and periodontal exam.

Sample Size and Population

We will enroll 30 outpatient SARS-CoV-2+ subjects per mouth rinse, (to achieve a total of 25 subjects with full data sets per mouth rinse,) with 5 mouth rinses, requiring us to enroll 125 SARS-CoV-2+ patients. Patients will be randomized to their mouthrinse. These patients will have already had a confirmed SARS-CoV-2+ test prior to enrollment. There will be no stratification to our randomization. In addition to having a SARS-CoV-2+ test, patients will need to be at least 18 y.o.a. and at most 65 y.o.a. and in fair oral health without any known allergies or reactions to commercial dental products or mouthrinses. Patients who are pregnant, nursing/lactating, have a cognitive or developmental disability, have kidney dysfunction, have an active oral herpes flare up, have any oral viral infection and/or flare up, have significant mucosal tears, growths or damage to their mouth, have uncontrolled severe periodontal disease with bleeding gums, or those with severe xerostomia will be excluded from the study. There are no exclusion criteria based on gender or race.

Table of Abbreviations and Definition of Terms

Abbreviation / Term	Definition	Additional details or range of value
baseline	Refers to the blood and saliva sample collection time point prior to mouth rinse use at study visit 1.	
BOP	Bleeding on probing- sites where the gingiva/gum bleeds after probing	0 not bleeding; 1 bleeding
CDART	Carolina Data Acquisition and Reporting Tool	0mm to 20mm, recession plus probing depth
CAL	Clinical attachment level	Probing depth plus recession (mm)
Co-I	Co-investigator	
CT	Cycle threshold	refers to the number of cycles needed to amplify viral RNA to reach a detectable level
Cytokine / chemokines assessed in this study	Interleukin-6 (IL-6), Interleukin-10 (IL-10), Interleukin-8 (IL-8), C-X-C Motif Chemokine Ligand 10 (CXCL-10)	
EDTA	Ethylenediaminetetraacetic acid	

FBS	Fetal bovine serum	
GI	Modified Gingival index	0-3
hr	hours	
infectivity	Reduction factor in infectivity	
MEM	Minimum essential media	
mL	milliliters	
mRNA	Messenger ribonucleic acid	
OD	Optical density	Measured in nm on a spectrophotometer retrofitted with a plate reader
PBS	Phosphate buffered saline	
PFU	Plaque forming units	
pg	picogram	
PHI	Protected health information	
PI	Principle investigator or modified Plaque Index (depends on context)	0-3
PD, PPD	Periodontal Probing depth (PPD, PD); pocket depth- Refers to pocket depth (measured in mm) upon periodontal probing	0 to 15mm; measured with a periodontal probe
RDC	Respiratory Diagnostic Center	
RF	Reduction factor RF values [= log10 (loadpre / loadpost)]	RF will be calculated as the difference in the quotient of the infection titre before (‘control titration’) and after the mouthrinse (‘remaining virus’). The log10 titre and its (double) standard deviation (SD) will be calculated as well as the variance of the RF.
RNA	Ribonucleic acid	
RQ	Relative quantification for RQ the $-\Delta\Delta Ct$ values [$=\log2\ (2-\Delta\Delta Ct)$]	RQ will be calculated to determine viral transcript changes across the time course for each individual participant. RQ is calculated using the 2(-**delta delta Ct**) method.
RT-PCR	Reverse transcription - polymerase chain reaction	
SD	Standard deviation	

sec	seconds	
y.o.a.	Years of age	
°C	Degrees celsius	
µL	microliters	

1.1 Primary Objective:

The primary objective of this study is to determine the efficacy of antiseptic mouthwashes on reducing SARS-CoV-2 cellular infectivity in COVID+ patient saliva. *We hypothesize that SARS-CoV-2+ patients will have a >20% reduction in SARS-CoV-2 viral infectivity following a 60 second oral rinse with an antiseptic mouthrinse and that this reduction will persist for at least 30 minutes.* This primary objective will focus on quantifying SARS-CoV-2 viral infectivity (Reduction Factor (RF) calculated from plaque forming units [PFU] / mL) and viral titer (Relative Quantification (RQ) ratio) using an *in vitro* infectivity assay and RT-PCR. This objective provides urgently needed data to guide healthcare workers on the proper use of specific commercially available mouthwashes capable of reducing SARS-CoV-2 viral infectivity and potentially limiting SARS-CoV-2+ transmission.

1.2 Secondary Objective:

The secondary objectives are to measure cytokine and chemokine levels (pg/mL) in samples taken from SARS-CoV-2+ patients and also to determine the frequency of periodontal disease among SARS-CoV-2+ patients. *We hypothesize that SARS-CoV-2+ patients using Colgate oral health products will have lower inflammatory marker levels than controls. Furthermore, we hypothesize that SARS-Cov-2+ patients will have a greater incidence of periodontal disease than the general population.*

1.3 Tertiary Objective *(Exploratory):*

The tertiary objective of this study is to use post-rinse surveys to evaluate patient perceptions of the mouthrinse and feedback on their willingness to use the product (units: percentage of respondents). Additionally, a periodontal exam will be performed to assess periodontal health. Patients' final periodontal status will be the tertiary outcome of the periodontal exam and will be reported as the percentage of participants categorized into each diagnosis. This tertiary outcome is exploratory.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

The COVID-19 global pandemic, caused by SARS-CoV-2 virus, represents a public health emergency with severe societal and economic impacts. Evaluating the potential for antiviral mouthrinses to inactivate SARS-CoV2 holds great potential for quelling the virus' spread among healthcare workers, who comprise up to 10% of COVID-19 cases.[1,2] Healthcare providers like dentists, who deliver care in and near the oropharynx, suffer the highest risk of occupational

exposure.[3] This is because SARS-CoV-2 is transmitted primarily through aerosol and respiratory droplets and salivary glands are a site of early SARS-CoV-2 viral replication and transmission.[4,5] Dentists work in close proximity to the mouth and nasal passages with many procedures producing salivary airborne particles, particularly those involving ultrasonic and rotary instruments (hand pieces, "drills") and 3-way syringes.[5–8] High rates of SARS-CoV-2 transmission suggest that the minimum infectious dose is low compared to other viral diseases, and recent data suggest even one viral particle may be sufficient for infection.[9] Working in close proximity to the oropharynx and generating salivary aerosols increases viral load exposure and therefore the risk of infection among dentists, oral surgeons, and other interventional doctors of the face.[9] Furthermore, the proclivity of aerosols to remain airborne for up to 3 hours puts other patients, providers and staff at risk of exposure during aerosol-generating procedures, particularly in open bay clinics common to dental schools and large practices.[4–6,10]

One promising strategy to reduce clinical spread is use of antiviral mouthrinses to inactivate SARS-CoV-2 infectability in saliva. Several commercially available mouthrinses have promising *in vitro* data with SARS-CoV-2 and *in vivo* data with other enveloped viruses, with a proposed mechanism of membrane disruption.[3,11] Ethanol and Povidone-Iodine (PVP) solutions have been shown to reduce SARS-CoV-2 viral infectivity levels and titers *in vitro*.[12-15] Cetylpyridinium chloride has promising *in vitro* data for inactivating SARS-CoV-2 infectivity.[16] Chlorhexidine Gluconate (0.12%) has been widely adopted by practices with variable antiviral *in vitro* data.[3,11] Finally, hydrogen peroxide mouthrinse (e.g. 1.5% w/v H_2O_2) is a widely available oral antiseptic capable of hydroxylating membranes, and is the a preprocedural mouthrinse recommended by the ADA to prevent transmission.[17] No *in vivo* clinical data regarding SARS-COV-2 exists to-date for most oral rinses including H_2O_2 mouthrinses.

Despite promising *in vitro* data, there is a dearth of *in vivo* clinical trials interrogating the efficacy of mouthrinses on reducing salivary SARS-CoV-2 viral infectivity. Given the rapid transmission and widespread distribution of the COVID-19 pandemic, the development of protocols and treatments mitigating transmission represent a major and urgent unmet public health need; this study will rigorously address the *in vivo* utility of widely available oral rinses in limiting SARS-CoV-2 viral infectivity and their acceptability in the dental healthcare setting. [5–8]

2.2 Study Rationale

One promising strategy to reduce clinical spread is use of antiviral mouthrinses to inactivate SARS-CoV-2 infectability in saliva. Several commercially available mouthrinses have promising *in vitro* data with SARS-CoV-2 and *in vivo* data with other enveloped viruses, with a proposed mechanism of membrane disruption.[3,11-17] No *in vivo* clinical data regarding SARS-COV-2 exists to-date for most oral rinses including H_2O_2 mouthrinses.

Despite promising *in vitro* data, there is a dearth of *in vivo* clinical trials interrogating the efficacy of mouthrinses on reducing salivary SARS-CoV-2 viral infectivity. Given the rapid transmission and widespread distribution of the COVID-19 pandemic, the development of protocols and treatments mitigating transmission represent a major and urgent unmet public health need; this study will rigorously address the *in vivo* utility of widely available oral rinses in limiting SARS-CoV-2 viral infectivity. [5–8]

Like other enveloped viruses, coronaviruses are surrounded by a lipid bilayer which allows for spike glycoproteins required for infection to be inserted.[3] The mouth rinses proposed in this study have shown success in previous studies in disrupting this lipid envelope in other viruses, and there is

promise that they will do the same to the SARS-CoV-2 virus. Effect size: Using data from Meiller *et al.* we calculated power at four time points.[18] Time points included: Post-0 (immediately after rinse), Post-30 (30 min after rinse), and Post-60 (60 min after rinse). In addition, we calculated a Post-45, which was the midpoint between 30 and 60 min in the Meiller *et al.* data. Based on this viral study evaluating mouth rinse efficacy against infectivity of Herpes Simplex Virus, an enveloped virus, power calculations were determined to estimate effect size and determine sample size as a function of length of mouth rinse time course.[18] The sample size was justified by a paired T-test on outcomes between time points. With three simultaneous comparisons (t_1-t_0, t_2-t_1, t_2-t_0) over 30 min, an n=16 sample size can detect the effect size in Cohen's d=0.7, which is between medium and large, with 80% power and 0.016 type-I error rate by Bonferroni correction. Therefore, with four simultaneous comparisons (t_0-t_1, t_0-t_2, t_0-t_3, t_0-t_4) the sample size required for each arm is ~25 participants per group to discern oral rinse efficacy at any given time point.

Subjects will be required to rinse their mouths with an unlabeled/blinded mouthrinse for 30-60 sec. Per the instructions on the product label, it is recommended to rinse for 30-60 sec, depending on the rinse (on-label directions will be followed). The mouthrinses chosen for this study are all commercially available products with known antiviral effects.

The intervention will be administered orally because an oral mouth rinse will be utilized, and the dosage will range from 10 mL to 20 mL, dependent on the dosage instructions on the product label. The duration of the intervention will be 60 min, with collection of pooled saliva samples pre-rinse and every 15 minutes (0 min, 15 min, 30 min, 45 min, and 60 min) post-rinse. This time period was chosen to be similar to that of a typical dental appointment. Multiple saliva samples will be collected to show how long the proposed mouth rinses are successful in deactivating the virus.

Peripheral blood will be collected at the end of salivary collection.Participants will be provided materials and oral hygiene instruction related to daily use of oral hygiene products provided by the study. Participants will be asked to keep a daily diary of oral hygiene performance, product usage, COVID-19 symptoms and exposures. During the week between study visits, participants will be directed to brush with Colgate toothpaste (at least twice per day) and rinse with the Colgate mouthrinse (according to on label instructions). Participants will be scheduled to return to the GoHealth Clinical Research Unit at one week after the first visit, during which additional salivary (1 time point, 2 mL of saliva over 5 min, no antiseptic mouth rinse will occur) and blood samples will be collected (2 - 5 mL in EDTA-containing blood collection tubes). At the conclusion of sample collection, the participant will undergo a periodontal exam. Study participation concludes following the periodontal exam.

Samples will be evaluated at both timepoints for inflammation-related cytokines and chemokines using pre validated quantitative multiplex assays (Luminex technologies), diaries will be reviewed and periodontal charting will be evaluated for differences between patient groups. A periodontal exam will consist of periodontal probing depths (PD), clinical attachment level (CAL) and bleeding on probing (BOP) at six sites for each tooth excluding third molars. Once collected, the periodontal exam data will be evaluated and the periodontal status for each participant will be assigned via the case definitions for periodontitis, gingivitis, and clinical periodontal health as described in the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions and the 2012 Centers for Disease Control and Prevention and American Academy of Periodontology case definitions.[19, 20] Patients will be informed of their periodontal status. Patients with any periodontitis or gingivitis diagnosis will be informed of their diagnosis and will be

referred to an oral healthcare provider for further treatment. Patients' final periodontal status will be the tertiary outcome of the periodontal exam and will be reported as the percentage of participants categorized into each diagnostic group. This study involves two on-site 90-minute visits.

Known and potential risks include breach of confidentiality, slight discomfort, and a low chance of a previously undiagnosed allergy. To minimize risk of breach of confidentiality, all patients will be assigned a unique study ID. There will be no other subject identifiers on samples or data. The linkage file that relates patient's names to study ID numbers will be stored on a UNC secure server. All desktops computers and servers are kept in locked facilities and in accordance with UNC IT/security safeguards and policies.

All providers and study researchers have been trained in and abide by HIPAA procedures, and confidential patient information will be stored on secured, encrypted UNC servers. Patients will already have been provided with their SARS-CoV-2 status through their healthcare providers, and this confidential medical information will not be released by any study personnel. Some patients may perceive the mouth rinse as causing some discomfort if they dislike the flavor or the bubbling sensation. A small fraction of patients (rare <1%) may be allergic to the mouth rinse. We will ask detailed questions in our screening protocol if patients are allergic to any component of the mouthrinses, but if a patient answers no to all screening questions regarding allergy and then they are allergic, it may lead to an adverse allergic reaction.

The following table lists adverse effects associated with each mouthrinse proposed in this study:

mouthrinse*	***Adverse effects***
1.5% w/v hydrogen peroxide rinse (Peroxyl)	• If irritation persists for 7 days, is severe, is due to orthodontic appliances and/or dentures, or swelling or fever develops, the patient's condition needs to be re-evaluated by a doctor or dentist. • Overdose can injure the gums and continued use of hydrogen peroxide may cause reversible hypertrophy of the papillae of the tongue known as 'black hairy tongue' therefore using this product at high doses or for long periods of time is not recommended. • Some cases of mucosal irritation and swelling of the oral tissues have been reported specially with high doses or in continued use.
0.12% Chlorhexidine Gluconate (Periogard)	• The most common (1) an increase in staining of teeth and other oral surfaces, (2) an increase in calculus formation, and (3) an alteration in taste perception; see WARNINGS and PRECAUTIONS. Oral irritation and local allergy-type symptoms have been spontaneously reported as side effects associated with use of chlorhexidine gluconate rinse. The following oral mucosal side effects were reported during placebo-controlled adult clinical trials: aphthous ulcer, grossly obvious gingivitis, trauma, ulceration, erythema, desquamation, coated tongue, keratinization, geographic tongue, mucocele, and short frenum. Each occurred at a frequency of less than 1.0%. Among postmarketing reports, the most frequently reported oral mucosal symptoms associated with chlorhexidine gluconate oral rinse are stomatitis, gingivitis, glossitis, ulcer, dry mouth, hypesthesia, glossal edema, and paresthesia. Minor irritation and superficial desquamation of the oral mucosa have been noted in patients using chlorhexidine gluconate oral rinses. There have been cases of parotid gland swelling and inflammation of the salivary glands (sialadentitis) reported in patients using chlorhexidine gluconate oral rinse.

1:1.5% w/v hydrogen peroxide rinse (Peroxyl) 2nd: 0.12% Chlorhexidine Gluconate (Periogard)	• No additional adverse effects documented from sequential use. • Please see above for adverse effects of each rinse individually. • For the sequential Peroxyl (1st) and Periogard (2nd) rinses, both oral rinses will be conducted in an on label fashion. The patient will begin by rinsing with 10 mL of Peroxyl for 60 seconds and then expectorate the mouthrinse into an empty tube. Then the patient will rinse with 15 mL of Periogard for 30 seconds, and spit this mouthrinse into a separate tube.
0.075% Cetylpyridinium Chloride (Colgate Total Zero)	• If more than used for rinsing is accidentally swallowed, get medical help or contact a Poison Control Center right away
Sterile Water	**No contraindications**

**All mouthrinses are commercially available in the USA, routinely used in clinical settings, and will be used in an on-label manner.*

3. STUDY DESIGN

One of the aims of this placebo-controlled, randomized, double-blinded study is to measure the efficacy of antiseptic mouthwashes on reducing SARS-CoV-2 cellular infectivity in COVID+ patient saliva (described above). Asymptomatic and symptomatic SARS-CoV-2+ subjects will be enrolled in the study (according to Inclusion and Exclusion Criteria - Section A.3). Symptomatic SARS-CoV-2+ subjects will be enrolled within 7 days of symptom onset. Subjects' samples are collected in the GoHealth Clinic (Adams School of Dentistry, UNC Chapel Hill, North Carolina, USA). Asymptomatic SARS-CoV-2+ subjects will be enrolled within one week of likely exposure. Study participants who meet all inclusion criteria without any exclusion criteria will be randomized to either an experimental or control group using block randomization.

Participants give a baseline salivary sample (2 mL over 5 min) and then asked to rinse with an mouth rinse for 30-60 sec (on-label instructions) according to the table below:

mouthrinse*	***Number of final participants (sample size)***	***Group***
1.5% w/v hydrogen peroxide rinse (Peroxyl)	**25****	**Experimental**
0.12% Chlorhexidine Gluconate (Periogard)	**25**	**Experimental**

1st Peroxyl; 2nd Periogard^	**25**	**Experimental**
0.075% Cetylpyridinium Chloride (Colgate Total Zero)	**25**	**Experimental**
Sterile Water	**25**	**Control**

**All mouthrinses are commercially available in the USA, routinely used in clinical settings, and will be used in an on-label manner.*
***30 participants will be enrolled per regimen to ensure that complete data will be obtained from at least 25 participants per regimen. We assume that ≤ 5 enrollees will drop out or have incomplete data.30 participants will be enrolled per arm to collect full data sets from 25 participants per arm, assuming some patients will need to be replaced due to inability to complete a visit (missing data).*
^For the sequential Peroxyl (1st) and Periogard (2nd) rinses, both oral rinses will be conducted in an on-label fashion. The patient will begin by rinsing with 10 mL of Peroxyl for 60 sec and then expectorate the mouthrinse into an empty tube. Then the patient will rinse with 15 mL of Periogard for 30 seconds and spit this mouthrinse into another empty tube.

After the participant expectorates the mouth rinse, research staff will collect another salivary sample (T_0, 2 mL over 5 min). During a 10 min recuperation period, the study participants will answer demographics survey questions and questions regarding the taste, color, flavor and general acceptability of using the mouthrinse as part of routine dental and medical visits. At 15 minutes post-rinse, the participant will be asked to provide an additional saliva sample (2 mL over 5 min), followed by another 10-minute recuperation period. This sequence will be repeated for the 30, 45- and 60-minute time points post-rinse. At 60 minutes, the study participant will give their final salivary sample (2 mL over 5 min).

The samples will be stored and used for determining SARS-CoV-2 viral load by RT-PCR and inflammation-associated cytokine and chemokine concentration (pg/mL). The impact of mouthrinses on viral load in samples will be using a Relative quantification (RQ) ratio. A cycle threshold cut off (see sections 9.4-9.5) of 22 will be used for determining which samples will be used in downstream viral infectivity assays. Viral infectivity will be calculated (see sections 9.4-9.5) and yield a Reduction Factor as quantified output. Peripheral blood will be collected at the end of salivary collection (2 - 5 mL in EDTA-containing blood collection tubes).

Participants will be provided materials and oral hygiene instruction related to daily use of oral hygiene products provided by the study. During the week between study visits, participants will be directed to brush with Colgate toothpaste (at least twice per day) and rinse with the Colgate mouthrinse according to on-label instructions (10-20 mL, 30-60 seconds, 2-3 times per day depending on mouthrinse).

Participants will be asked to keep a daily diary of oral hygiene performance, product usage, COVID-19 symptoms and exposures. Participants will be given a $30 gift card for their participation in visit 1. Participants will be scheduled to return to the GoHealth Clinical Research Unit at one week after the baseline assessment, during which additional salivary (1 time point, 2 mL

of saliva over 5 min, no rinse will occur) and blood samples (2 - 5 mL in EDTA-containing blood collection tubes) will be collected.

At the conclusion of sample collection, the participant will undergo a periodontal exam and will receive a $50 gift card for their participation in visit 2. Study participation concludes following the periodontal exam after visit 2.

Missing data may occur due to participants being unable to complete a study visit due to health concerns or insufficient salivary production. Any missing data will be excluded from the study analysis. We do not anticipate that this will bias the data in any way. To accommodate this eventuality, we plan to enroll 30 participants per arm, to be able to have 25 participants per arm with full datasets.

All salivary samples will be collected on ice in sterile 50 mL conical vials and blood will be collected in EDTA-containing blood collection tubes. All collection tubes will be labelled "COVID Core" using pre-printed labels including the participants random alphanumeric study ID. Saliva samples will be cataloged and stored at the UNC Delta COVID Translational Initiative (also referred to as the COVID Core) for future RT-PCR, cytokine and chemokine concentration (Luminex) and viral infectivity assays. Samples will be transported on ice in a secondary container from the collection site to 4403A Koury Hall in leak proof biohazard bags. Samples will be processed according to the **sample processing and outcomes** procedure (below).

Participants who successfully provide salivary and blood samples and follow study protocols will receive a $30 gift card as compensation at the end of visit 1 and $50 at the end of visit 2. This study involves two, 90 minute on site visits.

During the period that the study participants are present in the clinic and giving saliva samples, we will monitor patient wellbeing and inquire about their comfort. If participants exhibit signs of distress or if oxygen saturation drops, research study staff will contact emergency medical responders to visit the patient and if deemed appropriate, the participants will be escorted to UNC hospital (adjacent building).

This time course analysis design will allow us to investigate if any of the antiseptic mouthwashes are effective at reducing SARS-CoV-2 in salivary secretions of study participants as well as to determine the therapeutic window if effective. We have designed this study to recapitulate a standard oral exam and procedure time (~1.5 hrs in length). Further, the design will allow for a kinetic analysis of viral shedding in salivary secretions and to identify the most effective mouth rinse.

Sample processing and outcomes

Sample processing: Following collection, saliva samples will be processed according to existing BSL2+ SOP protocols (COVID Core, UNC (PI: Webster, Wallet and Maile) by pre-authorized personnel. Samples will be assessed for **1**) molecular presence of SARS-CoV-2, **2**) salivary antibody responses to SARS-CoV-2, **3**) in vitro infectibility and **4)** inflammatory biomarkers.

> **1. Molecular analysis:** For molecular analysis, samples will be inactivated by adding 2 mL of TriZol LS reagent to each 500 µL saliva aliquot (4:1 ratio) and mixed by inversion. These samples will be further aliquoted at 250 µL in 1.5 mL Eppendorf tubes and stored in an "Inactivated Samples -80 °C Freezer" until further analysis.

2. Antibody responses: an aliquot of saliva will be inactivated in a 56 °C water bath for 30 min. 250 µL aliquots will be transferred into 1.5 mL cryovials and stored in the "Inactivated Samples -80 °C Freezer" in 4403A Koury Hall until further analysis.

3. In vitro infectivity: Samples will be stored in the "Activated Samples -80 °C Freezer" in 4403A Koury Hall until further analysis.

At analysis, all samples will be serially diluted in sterile PBS (10^{-10}-fold dilutions). Plaque assays will be performed using Vero E6 cells at confluency in 96-well cell culture plates. Briefly, cell cultures will be washed with sterile PBS and samples containing virus will then be plated in duplicate (100 µL per well). Plates will be incubated at 37 °C for 45 min with occasional rocking. Then 2 mL of 0.5% agarose in minimal essential media (MEM) containing 2% FBS and antibiotics will be added per well. Plates will be incubated at 37 °C for 72 hours, fixed with 10% buffered formalin, followed by the removal of the overlay, and then stained with 0.2% crystal violet to visualize plaque forming units (PFU). Average PFU will be evaluated by OD. All assays will be performed in BSL-3 laboratory setting. (PMID: 32475066)

Unprocessed saliva exceeding immediate experimental capacity will be stored in aliquots of 250 µL volume in 1.5 mL Eppendorf tubes and stored in "Activated Samples -80C Freezer."

Survey data: The survey data will be analyzed and used to identify clinical and other factors impacting applicability of each of the mouth rinses.

4. Detection of Inflammatory biomarkers: Inflammation-associated cytokine and chemokine concentrations (IL-6, CXCL-10, IL-8, and IL-10 [pg/mL]) will be quantified from saliva and blood samples using multiplex analyte analysis (Luminex technology) and using internal, manufacturer supplied standards of known concentration (pg/mL). These analyses will be performed by the DELTA Translational Core according to COVID Core SOP (PI: Wallet and Maile). The COVID Core staff routinely performs these analyses on samples from SARS-CoV-2+ research study participant samples.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment in the study:

- Diagnosed SARS-CoV-2+ status. Either became symptomatic in the prior 7 days, or if not symptomatic, likely infected/exposed within the prior 7 days. All patients listed from the UNC Respiratory Diagnostic Center (RDC) have a confirmed SARS-CoV-2 infection and have consented to be contacted for research purposes. For patients contacting study coordinators for enrollment, who were not tested in the RDC, they must provide written proof of positive SARS-CoV-2 status in the prior 7 days.
- Individuals (all sex, all gender) at least 18 y.o.a. and at most 65 y.o.a. and in good oral health without any known allergies to commercial dental products or cosmetics. ASA class I or II prior to SARS-CoV-2 infection.
- Evidence of a personally signed and dated informed consent document indicating the subject (or legally acceptable representative) has been informed of all pertinent aspects of the trial and all of their questions have been answered.
- Able to comprehend and follow the requirements of the study (including availability on scheduled visit dates) based on research site personnel's assessment.

- Females of childbearing potential will have a negative urine pregnancy test (on site) or be physically incapable of pregnancy (implants or injections, Intrauterine device, Bilateral tubal ligation, Hysterectomy, Ovariectomy, Women post-menopausal)

4.2 Exclusion Criteria

Subjects presenting with any of the following will not be included in the study:

- Patients who have been eating or drinking within an hour of the study
- Patients under 18 y.o.a. and older than 65 y.o.a.
- Subjects presenting with and/or self-reporting any of the following will not be included in the study:
  - history of significant adverse effects following use of oral hygiene products such as toothpastes and mouthrinses. (self-reported)
  - Self-reported allergy to hydrogen peroxide, peroxyl, chlorhexidine gluconate, periogard, peridex, colgate total zero, colgate total, cetylpyridinium chloride, essential oils (Eucalyptol, Menthol, Methyl salicylate, Thymol), and other components in the mouth rinses (methyl salicylate, ethanol, saccharin sodium, glycerin, propylene glycol, sorbitol, *FD&C blue no. 1,* Poloxamer 407, Benzoic acid, Zinc chloride, Sodium benzoate, Sucralose, PEG-40 sorbitan diisostearate, potassium sorbate, citric acid*)*.
  - History of serious medical conditions that, at the discretion of the Investigator, will disqualify the subject. (Self-reported)
  - A history of severe dry mouth (xerostomia), severe drug-induced xerostomia (antidepressants, anticonvulsants, antihypertensives), or Sjogren's syndrome
  - A history of recent (within the last 30 days) or current **recent oral herpes flare up**, candida (thrush) infection, apthous ulcer flare up, current/active severe periodontal disease, or other recent oral viral infection or flare up within the past 30 days (self-reported)
  - Current history of alcohol or drug abuse (self-reported).
  - History of drinking water or eating food within an hour of the study visit.
  - History of drinking alcohol within 12 hours of the study visit.
  - History of using a commercial mouthrinse within 24 hours of the study visit.
  - Participation in any study involving oral care products, concurrently or within the previous 30 days. (self-reported)
  - Positive pregnancy test reported pregnancy or lactation (this criterion is due to oral tissue changes related to pregnancy and nursing which can affect interpretation of study results. Additionally, women are advised to check with their physician before using Chlorhexidine Gluconate during pregnancy and lactation, which cannot occur in a blinded, randomized trial.)
  - Other severe acute or chronic medical or psychiatric conditions or laboratory abnormality that may increase the risk associated with trial participation or investigational product administration or may interfere with the interpretation of trial results and, in the judgment of the Investigator, would make the subject inappropriate for entry into this trial.
  - Patient with developmental/cognitive disability that cannot self-consent, comprehend and follow the requirements of the study based on research site personnel's assessment.
  - Patients with sizable mucosal tears, abrasions, growths or burns in the mouth
  - Patients with kidney dysfunction

The following table lists exclusion criteria specific to each mouth rinse proposed in the study:

Mouthrinse*	***Exclusions***

1.5% w/v hydrogen peroxide rinse (Peroxyl)	• Pregnancy and lactation
	  o Although there is not sufficient specific clinical data on the use of 1.5
	  o % w/v hydrogen peroxide rinse in this patient group, self-administration without medical advice is not recommended.
	• Do not use in children under 12

0.12% Chlorhexidine Gluconate (Periogard)	• Carcinogenesis, Mutagenesis, Impairment of Fertility
	  o In a drinking water study in rats, carcinogenic effects were not observed at doses up to 38 mg/kg/day. Mutagenic effects were not observed in two mammalian in vivo mutagensis studies with chlorhexidine gluconate. The highest doses of chlorhexidine used in a mouse dominant-lethal assay and a hamster cytogenetics test were 1000 mg/kg/day and 250 mg/kg/day, respectively. No evidence of impaired fertility was observed in rats at doses up to 100 mg/kg/day.
	• Pregnancy: Teratogenic Effects
	  o Reproduction studies have been performed in rats and rabbits at chlorhexidine gluconate doses up to 300 mg/kg/day and 40 mg/kg/day, respectively, and have not revealed evidence of harm to the fetus. However, adequate and well-controlled studies in pregnant women have not been done. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.
	• Nursing Mothers
	  o It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when 0.12% Chlorhexidine Gluconate is administered to a nursing woman. In parturition and lactation studies with rats, no evidence of impaired parturition or of toxic effects to suckling pups was observed when chlorhexidine gluconate was administered to dams at doses up to 100 mg/kg/day.
	• Pediatric Use
	  o Clinical effectiveness and safety of 0.12% Chlorhexidine Gluconate have not been established in children under the age of 18.

1st: Peroxyl 2nd: Periogard	• No additional exclusions from sequential use are documented. • Please see above for adverse effects of each rinse individually.
0.075% Cetylpyridinium Chloride (Colgate Total Zero)	• Do not use in children under 6
Sterile Water	**No contraindications**

**All mouthrinses are commercially available in the USA, routinely used in clinical settings, and will be used in an on-label manner.*

Summary of Inclusion and Exclusion Criteria for Mouthrinse RCT

Inclusion Criteria	Exclusion Criteria
Individuals (all sex, all gender) at least 18 years of age and at most 65 years of age in good oral health and with stable physical health (ASA Class I or II prior to SARS-CoV-2 infection), decided at hte discretion of the study coordinator.	Known allergies or significant adverse reactions following the use of oral hygiene products (toothpastes, mouthrinses), commercial cosmetics, and any ingredient in the mouthrinses (list will be provided).
Diagnosed SARS-CoV-2+ status (within the past 48 hours) who became symptomatic in the prior 7 days, or if not symptomatic, was likely infected/exposed within the prior 7 days.	Established history of severe Xerostomia (drug-induced or autoimmune dry mouth), renal disease, Hepatitis C Virus (HCV), severe periodontal disease with actively bleeding gums, significant oral abrasions/ulcers or growths, current alcohol abuse, and/or current recreational drug abuse. Other severe acute or chronic medical or psychiatric conditions or laboratory abnormality that may increase the risk associated with trial participation or investigational product administration or may interfere with the interpretation of trial results and, in the judgment of the Investigator, would make the subject inappropriate for entry into this trial.
Evidence of a personally signed and dated informed consent document indicating the subject (or legally acceptable representative) has been informed of all pertinent aspects of the trial and all of their questions have been answered.	Drinking or eating within an hour of the study visit. Consuming alcohol within 12 hours of the study visit. Rinsing with a commercial mouthrinse within 24 hours of the study visit.
Able to comprehend and follow the requirements of the study (including availability on scheduled visit dates) based on research site personnel's assessment.	Developmental/cognitive disability such that the participant cannot self-consent, comprehend and follow the requirements of the study based on research site personnel's assessment.
Females of childbearing potential will have a negative urine pregnancy test (on site) or be physically incapable of pregnancy (implants or injections, Intrauterine device, Bilateral tubal ligation, Hysterectomy, Ovariectomy, Women post-menopausal)	Positive pregnancy test, reported pregnancy or lactation.
	A history of recent (within the last 30 days) or current recent oral herpes flare up, candida (thrush) infection, apthous ulcer flare up, current/active severe periodontal disease with bleeding gums, or other recent oral viral infection or flare up within the past 30 days.

4.3 Study Enrollment Procedures

Outpatient subjects will be recruited from patients seen in the UNC Respiratory Diagnostic Center (RDC) who have tested positive for SARS-CoV-2+ and consented to be contacted for participation in COVID-related research studies. The potential subjects will have signed a prior facility consent at the RDC confirming their willingness to share their name and contact information to be contacted for study participation in COVID-19 related research. We will not receive information on subjects that decline this internal consent. A brief review of the patient's Epic chart will take place for medical screening and to identify any missing contact information, as needed. Subjects will be contacted for recruitment by phone and (if unreachable by phone) by secure email by study personnel; patients will have the study rationale and risks explained and will be provided time to ask questions and consider participation. If patients are interested in participating, both appointments will be scheduled for them at the Adams School of Dentistry Go Health Clinical research core. Consent documentation will be signed in person and in private in the Go Health Clinical Research Core.

For Spanish speaking patients and/or parents, Spanish forms will be provided and communication will occur through a Spanish translator. All patients are adults and over normal cognitive capacity, and therefore will be able to consent for themselves. A list of SARS-CoV-2+ subjects that have consented to be contacted for research, will be provided by the RDC clinic, sent via secure UNC servers or secure UNC email to our research team's clinical coordinator, and this list will include names and contact details (PHI). This list is updated daily and provided to approved research sites, including ours.

At the visit, a consented patient will answer screening questions regarding inclusion and exclusion criteria. Answers will be entered into the CDART research database managed by UNC. Prior to finalizing these screening questions, women participants of childbearing potential will be asked to provide a urine sample in the restroom, to undergo a rapid pregnancy test by study personnel. Any pregnant or lactating patients will be excluded.

All of the consenting eligible patients will be enrolled in the study and will then be allocated to the treatment regimens by randomization A statistician will create a block randomization schedule and perform these computations. All patients will be consented and then fill out our screening questions for inclusion and exclusion to confirm eligibility prior to being assigned to the randomization schedule.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Patients who meet all inclusion criteria without any exclusions will be asked to give a baseline salivary sample of 2 mL over 5 min. The patient will then be asked to rinse with an unlabeled/blinded antiseptic mouth rinse for 60 sec. Once the participant expectorates the mouth rinse, a time 0, 2 mL salivary sample will be collected over 5 min. During the 10 min recuperation period post rinse, the participant will answer survey questions about their demographics and regarding the taste, color, flavor and acceptability of using the mouth rinse as part of routine dental and medical visits. At 15 min post-rinse, the patient will be asked to provide an additional 2 mL saliva sample over the course of 5 min, followed by another 10 min break. This sequence repeats for

the 30, 45 and 60-min time points. At 60 min, the patient will give a final 2 mL salivary sample. Peripheral blood will be collected at the end of salivary collection (2 - 5 mL in EDTA-containing blood collection tubes).

Participants will be provided materials and oral hygiene instruction related to daily use of oral hygiene products provided by study sponsors. In the one week between study visits, participants will be directed to brush with Colgate toothpaste (at least twice per day) and rinse with the Colgate mouthrinse (2-3 times per day based on-label directions). Participants will be asked to keep a daily diary of oral hygiene performance, product usage, COVID symptoms and exposures. Study personnel will monitor daily diary adherence and will reach out to participants between visits 1 and 2 to ensure home oral hygiene protocol adherence by phone or email. Participants will be given a $30 gift card for their participation in visit 1.

Participants will be scheduled to return to the GoHealth Clinical Research Unit at one week after the baseline assessment, during which additional salivary (1 time point, 2 mL of saliva over 5 min, no rinse will occur) and blood samples will be collected. At the conclusion of sample collection, the participant will undergo a periodontal exam and will receive a $50 gift card for their participation in visit 2. Once collected, the periodontal exam data will be evaluated and the periodontal status for each participant will be assigned via the case definitions for periodontitis, gingivitis, and clinical periodontal health as described in the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions and the 2012 Centers for Disease Control and Prevention and American Academy of Periodontology case definitions.[19, 20] Patients will be informed of their periodontal status. Patients with any periodontitis or gingivitis diagnosis will be informed of their diagnosis at the time of the visit and will be referred to an oral healthcare provider for further treatment. This study involves two on-site 90 minute visits. Study participation concludes at the end of the second on site visit with no additional follow-up, as results are not relevant to the patient's clinical course and treatment management.

5.2 Handling of Study Interventions

Mouthrinses will be acquired from the following sources:

mouthrinse*	***Source: Colgate company providing their commercially available, commercially packaged and ready-for-sale products***
1.5% w/v Hydrogen Peroxide rinse	Peroxyl- Colgate
0.12% Chlorhexidine Gluconate	Periogard- Colgate
0.075% Cetylpyridinium Chloride	Colgate Total Zero- Colgate

Sterile water	CVS store

**All mouthrinses are commercially available in the USA, routinely used in clinical settings, and will be used in an on-label manner.*

Mouthrinses will be prepared by research study staff in a 50 mL sterile conical tube. The conical tube will be unlabeled so both the participant and study personnel will be blinded to the solution. Any adverse events or protocol deviations will be reported to the UNC IRB. Weekly meetings of the internal quality control committee will allow us to closely monitor such events.

5.3 Concomitant Interventions

There are no concomitant interventions other than the mouthrinse and a short survey.

5.3.1 Allowed Interventions

There are no additional interventions, other than mouth rinsing with one of the mouthrinse options and using commercially available Colgate toothpaste and mouthrinse at home, as previously described. If patients have an allergic reaction, EMS will be contacted for management, and therefore no rescue medications are indicated.

5.3.2 Required Interventions

The patient will rinse with a blinded mouthrinse for 30-60 seconds (as specified on the label) and then will spit it out, followed by donating saliva and blood samples.

5.3.3 Prohibited Interventions

Patients who have brushed their teeth, drank or eaten within an hour of the study visit will be directed to wait until an hour has elapsed; if they cannot wait, the patient will be excluded. Patients who have used a mouthrinse within 48 hours of the study will be excluded.

Patients who have participated in a study of other oral products (toothpaste, mouthrinses) within the prior 30 days will be excluded.

Patients who are currently abusers of alcohol or recreational drugs, and have used such products within 48 hours will be excluded

5.4 Adherence Assessment

Adherence would be defined as rinsing with the mouthrinse for 30-60 sec (as directed on the label) before expectorating, and then providing saliva samples at 15 min time points and a blood sample.

6. STUDY PROCEDURES

6.1 Schedule of Evaluations

Assessment	Screening: Visit-1 (Day-14 to Day -1)	Baseline, Enrollment, Randomization, Intervention: Visit 1 (Day 0)	Visit 2
Informed Consent Form	X	X	
Demographics	X	X	
Medical History	X	X	X
Current Medications	X	X	X
Blood Chemistries		X	X
Urine Analysis- pregnancy		X	
Vital Signs		X	X
Brief Oral Exam	X	X	
Periodontal Exam			X
Inclusion/Exclusion Criteria	X	X	
Enrollment/Randomization	X	X	
Treatment Admin.Form		X	
Concomitant Medications	X	X	X
Adverse Events	X	X	X

6.2 Description of Evaluations

Subjects are required to be onsite for two 90-minute appointments. Subjects will be asked to rinse with an unlabeled/blinded antiviral mouth rinse for 30-60 sec and provide 2 mL of saliva prior to the rinse and 2 mL of saliva immediately post-rinse, 15 min post-rinse, 30 min post-rinse, 45 min post-rinse, and 60 min post-rinse. Subjects will also be asked to complete a short survey about the rinsing experience. A blood sample will also be collected, and a brief oral exam will occur. At the end visit, a single saliva sample and blood sample will be taken, along with a periodontal exam.

6.2.1 Screening Evaluation

Consenting Procedure: Patients will be contacted first by phone and, if not available, then by email. The consent process will be conducted by trained study coordinators. The study's purpose and requirements will be explained and the patient will have the opportunity to ask questions and will have time to consider participation. Interested patients will make an appointment to visit the Go Health Clinical Research Unit at Adams School of Dentistry for this study. Study participation. The consent forms will be reviewed and signed by interested patients in person in the Go Health Clinical Research Unit at Adams School of Dentistry prior to study participation. For Spanish speaking patients and/or parents, Spanish forms will be provided and communication will occur through a Spanish translator. All patients are adults and over normal cognitive capacity, and therefore will be able to consent for themselves.

Screening

- At the visit, a consented patient will answer screening questions regarding inclusion and exclusion criteria. Answers will be entered into the CDART research database managed by UNC. A brief oral exam will take place to evaluate for existing severe oral trauma, severe periodontal disease, tumors or infection.
- Prior to finalizing these screening questions, women participants of childbearing potential will be asked to provide a urine sample in the restroom, to undergo a rapid pregnancy test by study personnel. Any pregnant or lactating patients will be excluded.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

Outpatient subjects will be recruited from patients seen in the UNC RDC who have tested positive for SARS-CoV-2+ and consented to be contacted for participation in COVID-19-related research studies. The subjects being approached by phone or email have signed a prior facility consent at the RDC confirming their willingness to share their name and contact information to be contacted for study participation in COVID-19-related research. We will not receive information on subjects that decline this internal consent. A brief review of the patient's Epic chart will take place for medical screening and to identify any missing contact information, as needed. Subjects will be contacted for recruitment by phone and (if unreachable by phone) by secure email by study personnel; patients will have the study rationale and risks explained and will be provided time to ask questions and consider participation.

If patients are interested in participating, appointments will be scheduled for them at the Adams School of Dentistry Go Health Clinical research core. Subjects must be able to attend both visits, to be enrolled. Consent documentation will be signed in person and in private in the Go Health Clinical Research Core. A list of SARS-CoV-2+ subjects that have consented to be contacted for research, will be provided by the RDC clinic, sent via secure UNC servers or secure

UNC email to our research team's clinical coordinator, and this list will include names and contact details (PHI). This list is updated daily and provided to approved research sites, including ours.

Baseline Assessments

In this application, "baseline" refers to the initial saliva sample taken before the participant uses a mouthwash." For this baseline saliva sample, we will measure the salivary viral load and infectivity. The initial brief oral exam will also be a baseline visual inspection.

Randomization

A statistician will create a block randomization schedule and perform these computations; assignments will be placed in organized sequentially numbered opaque envelopes (SNOEs), which are opened as participants are enrolled. Block randomization will provide for group equality. Our sample is limited to adults aged 18-65years, to limit age variability and exclude people at highest risk. We will not preemptively stratify our sample based on our demographic variables (age, gender, race, BMI). However, after we have collected our data, we will check if there is an effect of these demographic features and then include an adjustment if appropriate.

All patients will be consented and then fill out our screening questions for inclusion and exclusion to confirm eligibility prior to being assigned to the randomization schedule. A secondary study coordinator will use a block randomization schedule, organized into SNOEs, to determine the mouthrinse for the next patient, and load it into an unmarked tube. This secondary study coordinator will have no interaction with the patients and the primary study coordinators and lab technicians. The primary study coordinator in charge of collecting the salivary samples will be blinded to the mouthwash solution, as they will be given a pre-aliquoted mouthrinse in an unlabeled storage tube to provide to the patient for the rinse.

6.2.3 Blinding

At their onsite visit, subjects will be provided with an unlabeled mouthrinse. If a subject identifies the mouthrinse due to sensation or taste, this will not affect the primary and secondary outcomes as viral load and infectivity are quantitative laboratory assays. The primary study coordinator in charge of collecting the salivary samples will be blinded to the mouthwash solution as well, as they will be given a pre-aliquoted mouthrinse in an unlabeled storage conical to provide to the patient for the rinse. The laboratory technician who will conduct the RT-PCR, viral infectivity and cytokine/chemokine analyte analyses will be blinded to the mouthrinse used prior to the salivary sample collection.

Prior to enrollment of participants, the trial statistician, Kevin Moss, will prepare the randomization schedule which will be concealed from all other study personnel. To conceal the treatment assignments, Wendy Lamm will prepare a set of sequentially numbered, opaque envelopes (SNOEs). The SNOEs will be used during recruitment to allocate enrollees to treatment regimens; i.e., for the next patient enrolled, the next envelope will be opened by an approved research staff who has been delegated responsibility for preparing the mouthrinse tubes for the enrollees.

Kevin Moss will set up a block randomization schedule that the team will abide by for assignment of enrolled subjects using sequentially numbered, opaque envelopes (SNOEs).

6.2.4 Follow-up Visits

This study requires two 90 minute visits. No diagnostic tests will be run as part of this clinical trial. All participants will already have known COVID+ status, and therefore no follow-up reporting is needed.

6.2.5 Completion/Final Evaluation

This study requires two 90 minute visits. Participation is completed after the 2nd visit. For the second visit, participants will be scheduled to return to the GoHealth Clinical Research Unit at one week after the baseline assessment, during which additional salivary (1 time point, 2 mL of saliva over 5 min, no rinse will occur) and blood samples will be collected. At the conclusion of sample collection, the participant will undergo a periodontal exam and will receive a $50 gift card for their participation in visit 2. Study participation concludes after visit 2 with no additional follow-up, as results are not relevant to the patient's clinical course and treatment management.

Subjects may withdraw from the trial at any time at their own request, or they may be withdrawn at any time at the discretion of the Investigator or Sponsor for safety, behavioral or administrative reasons.

The Investigator may discontinue a subject if, in the opinion of the Investigator, the subject is no longer a suitable candidate for the study. Possible reasons for the discontinuation of a subject are including but not limited to: adverse event, protocol deviation, insufficient saliva production, missed appointment, or subject no longer meets the eligibility criteria. The reason for discontinuation will be documented in the CDART record.

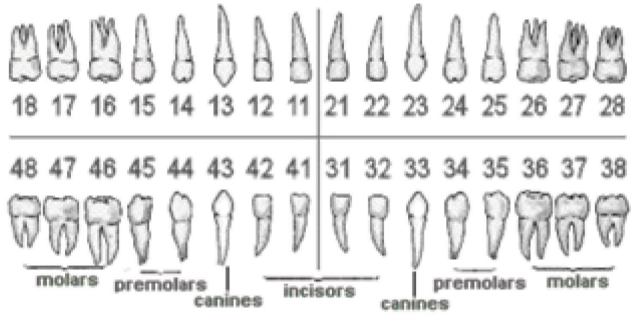
Patients may be removed from the study due to an allergic reaction to a mouth rinse or due to failure or inability to comply with study procedures.

If a patient suffers from xerostomia/dry mouth, such that s/he cannot produce the baseline salivary sample volume or the immediate, 15 minute and 30 minute post-rinse saliva volumes, s/he will be removed from the study with a parking voucher, but without a visit 1 $30 gift card and will be replaced. This would lead to missing data at the uncompleted time points. If a patient suffers from dry mouth, such that s/he cannot produce salivary sample volumes needed for later time points (45 min or 60 min post-rinse) but s/he produced sufficient baseline, immediate, 15 min and 30 min post-rinse samples, the participant will still be compensated with the visit 1 $30 gift card and parking vouchers, and will remain in the study for visit 2 participation, if willing. At the conclusion of the visit 2 on site appointment (after saliva and blood samples have been collected and the periodontal exam is completed), participants will receive a $50 gift card and parking vouchers. If a participant fails to carry out the at-home oral hygiene regimen with diary, cannot provide salivary or blood samples at visit 2, or cannot undergo the oral exam, they will not be provided the $50 gift card for visit 2. Missing or deficient samples will be noted in CDART.

7.0 Clinical Dental Measures

Clinical dental measures include a comprehensive periodontal examination including the following measures and indices: UNC Modified Plaque Index (Greene & Vermillion); Modified Gingival Index, Löe and Silness ; Periodontal Probing Depth (PPD), Clinical attachment level (CAL) and Bleeding on Probing (BOP).

The examination for all measurements will follow the sequence below (FDI numbering system):
Quadrant 1 Buccal: teeth 8 - 1
Quadrant 2 Buccal: teeth 1 – 8
Quadrant 2 Lingual: teeth 8 – 1
Quadrant 1 Lingual: teeth 1 - 8
Quadrant 3 Buccal: teeth 8 – 1
Quadrant 4 Buccal: teeth 1 – 8
Quadrant 4 Lingual: teeth 8 – 1
Quadrant 3 Lingual: teeth 1 – 8



The comprehensive periodontal exam will yield a diagnosis of periodontal disease or health. Once collected, the periodontal exam data will be evaluated and the periodontal status for each participant will be assigned via the case definitions for periodontitis, gingivitis, and clinical periodontal health as described in the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions and the 2012 Centers for Disease Control and Prevention and American Academy of Periodontology case definitions.[19, 20] Patients will be informed of their periodontal status. Patients with any periodontitis or gingivitis diagnosis will be informed of their diagnosis and will be referred to an oral healthcare provider for further treatment. Patients' final periodontal status will be the tertiary outcome of the periodontal exam and will be reported as the percentage of participants categorized into each diagnostic group.

<u>Study procedures and Periodontal evaluations</u>

Prior to the first subject study visit, study examiner(s) will be trained and calibrated for accuracy and repeatability in using the UNC Modified Gingival Index (GI), Loe and Silness, UNC Modified Plaque Index (Green & Vermillion), periodontal pocket depths, and clinical attachment levels using a UNC 15 periodontal probe. All examiners have a kappa > 0.90 for all calibration sessions. Calibration training will be conducted on an annual basis according to the guidelines set forth in this document including: Periodontal determination of plaque (PI), gingival inflammation (GI), gingival bleeding (BOP), pocket probing depths (PD) and clinical attachment levels (CAL). These parameters are customarily used in clinical dental studies to measure the disease status of

gingivitis and periodontitis. Probe penetration and depth may vary with the degree of inflammation, probing force, angulation, position, and instrument tip diameter.

Other confounding factors include patient discomfort, accuracy of probe markings, anatomical differences in tooth crown and roots, and technique variability within and between examiners. Thus, all of these measurements are important to consider in periodontal clinical studies and this training session is designed to minimize the inter-examiner variance.

7.1 UNC Modified Gingival Index (GI), Loe and Silness

Full mouth gingival scores shall be visually assessed by segmenting marginal and papillary units, 6 sites per tooth: distobuccal, buccal, mesiobuccal and distolingual, lingual, mesiolingual surfaces**.**
0 = Normal gingiva
1 = Mild inflammation; slight change in color, slight edema. No bleeding on probing.
2 = Moderate inflammation: redness, edema, and glazing. Bleeding on probing
3= Severe inflammation; marked redness and edema. Ulceration. Tendency for spontaneous bleeding.

7.2 UNC Modified Plaque Index (Greene & Vermillion)

Full mouth plaque assessment shall be assessed using the UNC Modified Plaque Index (Greene and Vermillion). Plaque scores shall be visually assessed at 6 sites per tooth (distobuccal, buccal, mesiobuccal and distolingual, lingual, mesiolingual surfaces) on a scale of 0-3.

0 =No debris or stain present on the clinical crown.
1 =Soft debris covering not more than 1/3 of the clinical crown (cervical 3rd), or presence of extrinsic stains without other debris regardless of surface area covered.
2 =Soft debris covering more than 1/3, but not more than 2/3 (middle 3rd) of the clinical crown.
3 =Soft debris covering more than 2/3 of the clinical crown

7.3 Pocket depth (PD): Linear distance from the gingival margin (GM) to base of the pocket. If a PD reading falls between two millimeter readings, the rule shall be to round down and the lower of the two readings will be recorded.

7.4 Bleeding on Probing (BOP): Presence or absence of bleeding to manual probing recorded as a dichotomous variable.

0 =No bleeding within 10 seconds after probing.
1 =Bleeding within 10 seconds after probing.

7.5 Clinical Attachment Level (CAL): Linear distance from the cemento-enamel junction (CEJ) to base of the pocket. If a CAL reading falls between two millimeter readings, the rule shall be to round down and the lower of the two readings will be recorded.

8. SAFETY ASSESSMENTS

The following table lists possible adverse effects associated with each mouthrinse proposed in this study:

mouthrinse*	***Adverse effects***
1.5% w/v Hydrogen Peroxide rinse (Peroxyl)	• If irritation persists for 7 days, is severe, is due to orthodontic appliances and/or dentures, or swelling or fever develops, the patient's condition needs to be re-evaluated by a doctor or dentist. • Overdose can injure the gums and continued use of hydrogen peroxide may cause reversible hypertrophy of the papillae of the tongue known as 'black hairy tongue' therefore using this product at high doses or for long periods of time is not recommended. • Some cases of mucosal irritation and swelling of the oral tissues have been reported specially with high doses or in continued use.
0.12% Chlorhexidine Gluconate (Periogard)	• The most common side effects associated with chlorhexidine gluconate oral rinses are: (1) an increase in staining of teeth and other oral surfaces, (2) an increase in calculus formation, and (3) an alteration in taste perception; see WARNINGS and PRECAUTIONS. Oral irritation and local allergy-type symptoms have been spontaneously reported as side effects associated with use of chlorhexidine gluconate rinse. The following oral mucosal side effects were reported during placebo-controlled adult clinical trials: aphthous ulcer, grossly obvious gingivitis, trauma, ulceration, erythema, desquamation, coated tongue, keratinization, geographic tongue, mucocele, and short frenum. Each occurred at a frequency of less than 1.0%. Among postmarketing reports, the most frequently reported oral mucosal symptoms associated with chlorhexidine gluconate oral rinse are stomatitis, gingivitis, glossitis, ulcer, dry mouth, hypesthesia, glossal edema, and paresthesia. Minor irritation and superficial desquamation of the oral mucosa have been noted in patients using chlorhexidine gluconate oral rinses. There have been cases of parotid gland swelling and inflammation of the salivary glands (sialadentitis) reported in patients using chlorhexidine gluconate oral rinse.
1st: 1.5% w/v Hydrogen Peroxide rinse (Peroxyl) 2nd: 0.12% Chlorhexidine Gluconate (Periogard)	• No additional adverse effects documented from sequential use. • Please see above for adverse effects of each rinse individually.
0.075% Cetylpyridinium Chloride (Colgate Total Zero)	• If more than used for rinsing is accidentally swallowed, get medical help or contact a Poison Control Center right away
Sterile Water	**No contraindications**

**All mouthrinses are commercially available in the USA, routinely used in clinical settings, and will be used in an on-label manner.*

The use of on-label, widely used mouthrinses and collection of saliva carry minimal risk for participants, apart from the very rare, undiagnosed allergy, and the unlikely event that the sample is

swallowed. If the case of allergy, UNC Hospital emergency services will be contacted. In the case of swallowing, a Poison Control Center will be contacted.

8.1 Specification of Safety Parameters

Mouthrinse and saliva collection carries low risk and minimal to no safety concerns to the participants. Blood draws and oral exams are routine healthcare screening procedures that carry minimal safety concern to participants. Results of analysis from known COVID+ patients will not require any medical follow-up or safety concerns. As a result, the investigator will monitor subject data, without a safety monitoring board. The investigator and team will meet weekly to review safety concerns, unanticipated problems or adverse events.

8.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The investigators and study coordinators will meet weekly to review unanticipated problems with recruitment or adverse events. There is only one site for this study. The use of on-label, widely used mouthrinses, tooth pastes, and collection of saliva and blood carry minimal risk for participants, apart from the very rare, undiagnosed allergy, which would be managed through proper referral to the neighboring UNC hospital.

8.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is generally defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recorded regardless of their relationship to the study intervention.

A **serious adverse event (SAE)** is generally defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly.

The use of on-label, widely used mouthrinses and toothpastes and collection of saliva and blood carry minimal risk for participants, apart from the very rare, undiagnosed allergy, or the unlikely event that a participant slips or falls during the study, which would be managed through proper referral to the neighboring UNC hospital.

The investigator and team will meet weekly to review safety concerns, unanticipated problems or adverse events. All adverse events will be reported to the IRB and the committee overseeing the study within 30 days.

8.4 Reporting Procedures

The PI will be responsible to monitor the overall study, including both research data and clinical procedures. The PI will be responsible for reporting all adverse events to the necessary parties. The investigator and team will meet weekly to review safety concerns, unanticipated problems or adverse events. All adverse events will be reported to the IRB and the committee overseeing the study within 30 days.

8.5 Follow-up for Adverse Events

In the case of an adverse event, participants will be referred to the neighboring UNC hospital. All follow-up visits and procedures regarding adverse events will be handled through the UNC Hospital system. A representative from our team will call the participant one week after the adverse event occurs to check-in.

8.6 Safety Monitoring

The investigator will monitor subject data, without a safety monitoring board. The investigator and team will meet weekly to review safety concerns, unanticipated problems or adverse events.

9. INTERVENTION DISCONTINUATION

Subjects may withdraw from the trial at any time at their own request, or they may be withdrawn at any time at the discretion of the Investigator or Sponsor for safety, behavioral or administrative reasons.

The Investigator may discontinue a subject if, in the opinion of the Investigator, the subject is no longer a suitable candidate for the study. Possible reasons for the discontinuation of a subject are including but not limited to: adverse event, protocol deviation, insufficient saliva production, missed appointment, failure to comply with oral hygiene regimen, subject no longer meets the eligibility criteria. The reason for discontinuation will be documented in the patient's CDART record.

Patients may be removed from the study due to an allergic reaction to a mouth rinse or due to failure or inability to comply with study procedures. If a patient suffers from xerostomia/dry mouth, such that s/he cannot produce the baseline salivary sample volume or the immediate, 15 minute and 30 minute post-rinse saliva volumes, s/he will be removed from the study with a parking voucher, but without a visit 1 $30 gift card and will be replaced. If a participant suffers from dry mouth, such that they cannot produce salivary sample volumes needed for later time points (45 min or 60 min post-rinse) but produced sufficient baseline, immediate, 15 min and 30 min post-rinse samples, s/he will still be compensated with the visit 1 $30 gift card and parking vouchers, and will remain in the study for visit 2 participation, if willing.

At the conclusion of the visit 2 appointment (after saliva and blood samples have been collected and the periodontal exam is completed), participants will receive a $50 gift card and parking vouchers. If a participant fails to carry out the at-home oral hygiene regimen with diary, cannot provide salivary or blood samples at visit 2, or cannot undergo the oral exam, s/he will not be provided the $50 gift card for visit 2. Missing or deficient samples will be noted in CDART.

10. STATISTICAL CONSIDERATIONS

General Design Issues

A double-blinded, randomized controlled trial was chosen as the study design. It allows us to collect human based data on the effects of commercially available mouthrinses on salivary infectivity and viral load. A crossover study was not pursued because the repeated use of different mouthrinses is likely to confound the results. A 1-hour time-course was chosen to correspond with the average length of most dental procedures. Fifteen-minute intervals were chosen to evaluate viral kinetics over time, and to see when infectivity rebounds post-mouthrinse.

10.2 Research Hypotheses

The central hypothesis of this study is that COVID-19+ patients will have a >20% reduction in SARS-CoV-2 viral infectivity following oral rinse with an antiseptic mouthrinse and that this reduction will persist for at least 30 minutes.

The secondary hypothesis is that COVID-19+ patients using Colgate oral health products will have lower inflammatory marker levels than controls. Furthermore, we hypothesize that COVID-19+ patients will have a greater incidence of periodontal disease than the general population.

Sample Size Considerations

Using data from Meiller *et al.* we calculated power at four time points.[18] Time points included: Post-0 immediately after rinse, Post-30 (30 min post rinse), and Post-60 (60 min post rinse). In addition, we calculated a Post-45, the midpoint between 30 and 60 min, in the Meiller *et al.* data. Based on this viral study evaluating mouth rinse efficacy against viral infectivity of Herpes Simplex Virus, an enveloped virus, power calculations were determined to estimate effect size and determine sample size as a function of length of mouth rinse time course.[18] The sample size was justified by a paired T-test on outcomes between time points. With three simultaneous comparisons (t_1-t_0, t_2-t_1, t_2-t_0) over 30 min, an n=16 sample size can detect the effect size in Cohen's d=0.7, which is between medium and large, with 80% power and 0.016 type-I error rate by Bonferroni correction. Therefore, with four simultaneous comparisons (t_0-t_1, t_0-t_2, t_0-t_3, t_0-t_4) the sample size required for each arm is ~25 participants per group to discern oral rinse efficacy at any given time point (minutes post rinse).
Statistical analysis of our data will be performed by Kevin Moss, the statistician on this project.Statistical analysis of our data will be performed by Kevin Moss, the statistician on this project. Kevin Moss will be involved in all stages of this project to help ensure statistical rigor.

Primary Outcomes

The analyses will compare viral titre (RT-PCR) and viral infectivity levels between mouth rinse types and across the time course.

Table: Variable Types for Primary Outcomes

	Viral Infectivity (Continuous)	Viral Load (Continuous)
Age, Years (Continuous)		
Race (Categorical)		
Sex (Categorical)		
BMI (Continuous)		
Time (Categorical)		
StudyGroup (Categorical)		

Data generated from the RT-PCR assays (relative quantification (RQ) ratio, numerical value) will be presented as mean (standard deviation) for normally distributed data and as median [Interquartile range] for non-normally distributed data. P values will be reported as continuous numerical scales, with multiple testing adjustments. After performing the main analysis using a linear model specified a priori, various sensitivity analyses will be performed. These will include an analysis of residuals which will rely on graphical methods, examination of skewness, kurtosis, and other descriptive statistics. The sensitivity analyses may also include a Shapiro-Wilk test of the null hypothesis Ho 'the underlying distribution of the residuals is Gaussian'. The sensitivity analyses may also include assessment of the impact of using a modified version of the linear model analysis in which the scale of the dependent variable is transformed (e.g., log10 or some other Box-Cox transformation). The sensitivity analyses will be used only to guide our level of trust in the main results.

For the primary analysis, viral infectivity and viral load, we will first determine if these variables are normally distributed. However, we anticipate these variables to be right skewed. They will be log transformed if they are not normally distributed. We will then compare the demographic variables (age, race, sex, age and BMI) and groups (study arms). We will use Chi-square and ANOVA statistics to determine if the demographic variables are evenly distributed amongst the groups. If not we will adjust for these variables in subsequent analyses. In the rare event there are multiple demographic variables that are found to be significantly different by group we will create a propensity score to adjust for these variables in subsequent analyses.

The primary analysis will be ANCOVA models with viral infectivity at time Post 0 minute, Post 1 minute, post 15 minute, post 30 minute, post 45 minute and post 60 minute adjusting for time Pre 0 minute. We will also perform a repeated measures analysis looking at viral infectivity over time, with the advantage of having one model with more power, while the ANCOVA model may give us a clearer answer if the rebound effect of viral infectivity is exponential. We will adjust for any demographic variables that are found to be significantly different between groups. We will adjust for any demographic variables that are found to be significantly different between groups.

The viral infectivity analysis will compare infectivity levels between mouth rinse types and across the time course for calculation of the reduction factor (RF calculated from PFU/mL, numerical value). The RF will be calculated as the difference in the quotient of the infection titre before ('control titration') and after the mouthrinse ('remaining virus'). Therefore, the log10 titre and its (double) standard deviation (SD) were calculated as well as the variance of the RF.

Secondary Outcomes

Inflammation-associated cytokine and chemokine concentration (pg/mL) will be quantified using a Luminex bioanalyzer containing pre-validated internal standards of known concentration. Saliva and serum analyte concentrations will be compared between groups using descriptive statistics. Periodontal exam results will be reviewed to categorize patients' periodontal status which will be reported as frequencies.

Tertiary Outcomes

The tertiary outcomes of this study are a periodontal exam and post-rinse surveys to evaluate patient perceptions of the mouthrinse, their symptoms and feedback on their willingness to use the product. Percentage of respondents will be gathered and reported for surveys. Periodontal exam data will be evaluated and the periodontal status for each participant will be assigned via the case definitions for periodontitis, gingivitis, and clinical periodontal health as described in the 2017 World Workshop

on the Classification of Periodontal and Peri-Implant Diseases and Conditions and the 2012 Centers for Disease Control and Prevention and American Academy of Periodontology case definitions.[19, 20] Patients' final periodontal status will be the tertiary outcome of the periodontal exam and will be reported as the percentage of participants categorized into each diagnostic group.

10.2 Sample Size and Randomization

Using data from Meiller *et al.* we calculated power at four time points.[18] Time points included: Post-0 immediately after rinse, Post-30 (30 min post rinse), and Post-60 (60 min post rinse). In addition, we calculated a Post-45, which was the mid-point between 30 and 60 min in the Meiller *et al.* data. Based on this viral study evaluating mouth rinse efficacy against infectivity of Herpes Simplex Virus, an enveloped virus, power calculations were determined to estimate effect size and determine sample size as a function of length of mouth rinse time course.[18] The sample size was justified by a paired t-test on outcomes between time points. With three simultaneous comparisons (t_1-t_0, t_2-t_1, t_2-t_0) over 30 min, an n=16 sample size can detect the effect size in Cohen's d=0.7, which is between medium and large, with 80% power and 0.016 type-I error rate by Bonferroni correction. Therefore, with four simultaneous comparisons (t_0-t_1, t_0-t_2, t_0-t_3, t_0-t_4) the sample size required for each arm is ~25 participants per group to discern oral rinse efficacy at any given time point (minutes post rinse).

Kevin Moss, who is the statistical consultant on this project, will create a block randomization schedule and perform these computations. SAS Institute (SAS Cary, NC) Proc PLAN will be used to create the randomization schedule. Block randomization will be used to ensure equal and random assignment into each study arm. All patients will be consented and then fill out our screening questions for inclusion and exclusion to confirm eligibility prior to being assigned to the randomization schedule. We will closely collaborate on Kevin Moss throughout all stages of this study to ensure statistical rigor.

The researcher responsible for collecting the salivary samples will be blinded to the mouthwash solution, as they will be given a pre-aliquoted mouthrinse in an unlabeled conical tube to provide to the patient for the rinse.

The PI, Co-I, study coordinators and study personnel who work for GoHealth are authorized to break the blinding if a patient suffers an allergic or adverse reaction to one of the mouthrinses. Though highly unlikely, as all proposed mouth rinses are commercially available over the counter, adverse or allergic reactions are possible. Using the numerical code associated with the participant and mouthrinse syringe, the study personnel can reference the block randomization schedule to determine the mouthrinse type, to inform the emergency medical workers, poison control or the emergency room what type of mouthrinse product led to the reaction.

10.3 Definition of Populations

Our population includes patients 18-65 y.o.a. who have tested positive with COVID-19 and are either asymptomatic but exposed in the prior 7 days or are symptomatic with symptom development in the prior 7 days. Inclusion and exclusion criteria (described elsewhere) will be met. We are not treating patients for SARS-CoV-2.

10.4 Study Stopping Rules

Analysis will occur at several points, while enrolling and collecting samples from 125 patients, in batches of 25 randomized participants. Analysis will occur after enrollment of 25, 50, 100 and then 125 participants and will include viral load and replication (as measured by changes in genomic, strand, and subgenomic expression and will be evaluated and compared between treatment groups). No interim analyses of data will be performed. mRNA obtained from salivary samples will be analyzed and will be assessed by RT-PCR at time points 0 and 30 min. Infectivity assays will be subsequently performed on salivary samples collected at time points 0 min ('baseline') and 30 min post rinse with cycle threshold of 22 or lower (CT22) as determined by RT-PCR using primers specific for SARS-CoV-2 viral products. If the cycle threshold is above 22 (CT22), samples are considered to contain insufficient virus for use in viral infectivity assays. This cycle threshold has been empirically determined by the Baric laboratory (personal communication, Dr. Ralph Baric).

If saliva samples from patients 6-7 days post-diagnosis around found to routinely have insufficient viral load at interim analysis (at n=25 or 50), the study will be modified for more stringent enrollment criteria (with inclusion up to 5 days post-diagnosis rather than 7, as salivary viral load diminishes with time after infection). Because all oral rinse products are commercially available, low risk products that have previously undergone extensive safety testing and approval procedures, we do not anticipate a situation where safety concerns to participants necessitates halting the study. Adverse events will be reported and reviewed by our internal quality control committee. Medical problems that occur in our facility will be reported as needed to the IRB board and emergency medical personnel will be contacted to care for the patient and potentially bring them to the adjacent UNC medical hospital. If a significant number of adverse events or reactions are witnessed (defined as more than 1 per 20 patients), a safety review will be triggered and study personnel will look at the records in an unblinded fashion to see if one mouthrinse is causing reactions. If that is the case, the mouthrinse study arm associated with adverse events will be discontinued, but the mouthrinse arms associated with minimal adverse events will continue.

10.5 Outcomes

All oral rinse products are commercially available, low risk products. Therefore, adverse outcomes are anticipated to be extremely rare, and will be reviewed weekly by the study team/committee. Any adverse event will be documented in our CDART database system.

The primary and secondary outcomes of RT-PCR (RQ), viral infectivity (RF), and cytokine/chemokine analyte concentration (pg/mL) for each sample type and each participant will be stored as a comma-separated values file analyzed using Excel (or similar) software. This data will be available to research study personnel weeks to months after sample collection when the experiments are conducted. Samples will be run in batches resulting in large spreadsheets of data. Periodontal exam data will be entered into CDART and can be exported as a comma-separated values file.

The outcomes do not include patient treatment response, and therefore the outcomes will not be reviewed and adjudicated by an outside committee. The study personnel will constitute their own committee that meets weekly, and they will review and evaluate the data once these experiments are conducted. The committee will not be masked to the participant's group assignment; the statistician will conduct analyses and then the committee will evaluate the data to reach scientific conclusions on which mouthrinse is more effective.

10.5.1 Primary Outcome

The primary objective of this study is to determine the efficacy of antiseptic mouthwashes on reducing SARS-CoV-2 cellular infectivity in participant saliva. *We hypothesize that COVID-19+ patients will have a >20% reduction in SARS-CoV-2 viral infectivity following a 60 second oral rinse with an antiseptic mouthrinse and that this reduction will persist for at least 30 minutes.* This primary objective will focus on quantifying SARS-CoV-2 viral infectivity and mRNA level using an *in vitro* infectivity assay (Reduction factor (RF) calculated from PFU/mL) and RT-PCR (relative quantification [RQ] ratio). This objective provides urgently needed data to guide healthcare workers on the proper use of specific commercially available mouthwashes capable of reducing SARS-CoV-2 viral infectivity and potentially limiting COVID-19+ transmission.

Outcome assessments will be conducted weeks to months after the study visit, when RT-PCR and viral infectivity assays are routinely conducted in the COVID Core and the laboratory of Dr. Ralph Baric, respectively.

10.5.2 Secondary Outcomes

The secondary objectives are to measure inflammation-associated cytokine and chemokine concentration (pg/mL) in saliva and blood samples taken from SARS-CoV-2+ participants and also to determine the frequency of periodontal disease among participants. *We hypothesize that SARS-CoV-2+ participants using Colgate oral health products will have lower blood and salivary inflammation-associated cytokine and chemokine concentrations (pg/mL) than non-intervention controls. Furthermore, we hypothesize that SARS-CoV-2+ participants will have a greater incidence of periodontal disease than the general population.* Outcome assessments will be conducted weeks to months after the study visit, when statistical analyses are conducted.

10.5.3 Tertiary Outcomes

The tertiary outcomes of this study are a periodontal exam and post-rinse surveys to evaluate patient perceptions of the mouthrinse, their symptoms and feedback on their willingness to use the product. Percentage of respondents will be gathered and reported for surveys. Periodontal exam data will be evaluated and the periodontal status for each participant will be assigned via the case definitions for periodontitis, gingivitis, and clinical periodontal health as described in the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions and the 2012 Centers for Disease Control and Prevention and American Academy of Periodontology case definitions.[19, 20] Patients' final periodontal status will be the tertiary outcome of the periodontal exam and will be reported as the percentage of participants categorized into each diagnostic group. These tertiary outcomes are exploratory and therefore have no hypotheses assigned to them.

Table: Study Variables and Covariates

X Independent variables	Y Dependent variables for primary and secondary outcomes	Covariates
Mouthrinse Group	Viral infectivity (continuous positive variable)	Age (years)
Time (in minutes, 0, 15, 30, 45 and 60 minutes, visit 1 and 2)	Viral load (continuous positive variable)	Gender (m/f)
	Cytokine level (continuous positive variable)	Race (White, Native Hawaiian or other pacific islander, Asian, African American, Hispanic or Latino, American Indian or Alaska Native)
		BMI Body mass index (continuous range: 0-56)

10.6 Data Analyses

qRT-PCR: Data will be presented as mean (standard deviation) for normally distributed data and as median [Interquartile range] for non-normally distributed data. P values will be reported as two tailed. Between group comparisons will be performed using a Student's T test or Mann-Whitney test. Normality will be assessed using the Kolmorgorov-Smirnov test, and logistic regression will be performed with robust standard errors. Methods adapted from Bullard *et al* 2020.

Viral infectivity: The viral infectivity analysis will compare infectivity levels between mouth rinse types and across the time course for calculation of the reduction factor (RF). The RF will be calculated as the difference in the quotient of the infection titre before ('control titration') and after the mouthrinse ('remaining virus'). Therefore, the log10 titre and its (double) standard deviation (SD) were calculated as well as the variance of the RF.

Inflammatory Markers: Inflammation-associated cytokine and chemokine concentrations (IL-6, CXCL-10, IL-8, and IL-10 [pg/mL]) will be quantified from saliva and blood samples collected at each visit and across the salivary collection time course using multiplex analyte analysis (Luminex technology) and using internal, manufacturer supplied standards of known concentration (pg/mL).

The analysis plan will include use of sensitivity analyses to evaluate the robustness/fragility of the study's main results to reasonable perturbations of the statistical methods and assumptions used in the main analysis. We will first describe the demographics (age, race, sex and BMI) for each study arm. If significant differences are found between groups, we will adjust the remainder of the analysis. The main analysis will be a repeated measures linear regression model(s). If multiple demographics are found to be significantly different the propensity scores will be created and final models will be adjusted by propensity scores. In the primary analysis, viral load and replication as measured by changes in genomic, strand, and subgenomic expression, will be evaluated and compared between treatment groups. Repeated measures linear regression models will be utilized to look at changes in viral load logarithmically before and after treatment. Similarly,

in the secondary analyses, the repeated measures linear regression will be used to compare the changes in other continuous outcomes (such as immunological parameters) from baseline to specific time points outlined. Statistical significance will be tested using a two-sided 0.05 level with adjustments for multiple testing. Multiple testing correction will be made by Tukey adjustments (for multiple study arms) and False Discovery Rate (FDR) where appropriate (i.e. multiple inflammatory markers).

All statistical estimates of population parameters will be tabulated along with corresponding confidence intervals (CIs) and/or standard errors (SEs) to convey level of precision. All P- values will be reported as continuous numerical outputs (and not as dichotomous results). All hypothesis tests yielding large p-value (e.g., $p > \alpha$) will be reported as being inconclusive.

Power Calculation: Using data from Meiller *et al.* we calculated power at four time points.[18] Time points included: Post-0 (immediately post rinse), Post-30 (30 min post rinse), and Post-60, (60 min post rinse). In addition, we calculated a Post-45, which was the midpoint between 30 and 60 min in the Meiller *et al.* data. Based on this viral study evaluating mouth rinse efficacy against infectivity of Herpes Simplex Virus, an enveloped virus, power calculations were determined to estimate effect size and determine sample size as a function of length of mouth rinse time course.[18] The sample size was justified by a paired t-test on outcomes between time points. With three simultaneous comparisons (t_1-t_0, t_2-t_1, t_2-t_0) over 30 min, an n=16 sample size can detect the effect size in Cohen's d=0.7, which is between medium and large, with 80% power and 0.016 type-I error rate by Bonferroni correction. Therefore, with four simultaneous comparisons (t_0-t_1, t_0-t_2, t_0-t_3, t_0-t_4) the sample size required for each arm is ~25 participants per group to discern oral rinse efficacy at any given time point (minutes post rinse).

11. DATA COLLECTION AND QUALITY ASSURANCE

11.1 Data Collection Forms

Consented patients' data (including screening questions, demographics (age in years, race, sex (m/f) and BMI (continuous)), and the survey on mouth rinse acceptability) will be collected using the CDART dental toolkit program, which stores data on a secure UNC maintained server and meets federal guidelines for clinical study data acquisition. The CDART electronic forms and database will serve as our CRFs and source data. IRB approved study personnel will ask participants the questions and will enter responses into this encrypted, password protected database. Data will be collected in this program using participant's study ID and no other personal health identifiers (PHI). A separate linkage file, securely stored on UNC servers, will be utilized to associate the random alphanumeric study ID with patient identifier, so samples and data will not be associated with patient identifiers. Data will only be transmitted among the research team using the school of dentistry secure research servers. Demographic data will be transmitted among the research team through access to the secure CDART research database system, stored on secure UNC servers, which study personnel will access only through encrypted computers via secure user accounts.

11.2 Data Management

An Excel key will be created with study ID (random alphanumeric code) to identify patients, saved in a secure folder on a UNC SOD server. This linkage file will be stored separately from data and destroyed at the close of the study. All data collected from participants and results from sample

analysis will be coded with unique numerical identifiers and stored in the UNC CDART clinical research database and on a secure UNC-CH server. Only trained, IRB-approved study personnel will enter and access data in the secure CDART research database.

Consented patients' data (including screening questions, demographics, and the survey on mouth rinse acceptability) will be collected using the CDART dental toolkit program, which stores data on a secure UNC maintained server and meets federal guidelines for clinical study data acquisition. Only HIPAA and human subjects trained IRB-approved study personnel will collect, enter and manage patient data; study coordinators will serve in this capacity.

Data will be collected in the CDART research database using participant's study ID and no other PHI identifiers, on approved, carefully designed digital forms within CDART. CDART forms will include questions with drop downs, radio button multiple choice and text field entry, depending on the question type. There will be separate digital forms for inclusion/exclusion screening questions (before sample collection begins), medical history, substance use history, demographic questions, and questions regarding mouthrinse experience (taste, color, sensation) and willingness to use a mouthrinse in various clinical settings (*e.g.* dentist or doctor appointments). A separate linkage file, securely stored on UNC servers, will be utilized to associate the random alphanumeric study ID with patient identifier, so samples and CDART data will not be associated with patient identifiers. Home diary entries will be made on paper or using a secure UNC Qualtrix account link, depending on patients' technological capabilities.

Data will only be transmitted among the research team using the SOD secure research server. Demographic data will be transmitted among the research team through access to the secure CDART research database system, stored on secure UNC servers, which study personnel will access only through encrypted computers via secure user accounts.

Linkage files containing identifiers will be deleted and permanently removed from all servers and study computers at the conclusion of the study with acceptance of associated manuscripts. Saliva specimens will be labeled with a study ID (random alphanumeric code) that is not identifiable without the linkage file, and therefore will not be identifiable after linkage file destruction.

10.3 Quality Assurance

11.3.1 Training

All study personnel have been trained in health privacy (HIPAA), human subjects research and good clinical practices through the CITI program and are certified to work with patient data on clinical trials. Study coordinators and healthcare providers are trained in and approved for the use of the CDART electronic research database and EPIC medical record system. Technical research personnel performing viral PCR and infectivity assays are approved to operate in their BL3 laboratory facilities and have specialized, lab-based training in these molecular techniques. They have also undergone training in the use of universal precautions and management of human biological samples along with bloodborne pathogen training. Finally, the GoHealth Clinical Research core has highly experienced research coordinators who will monitor all study staff to ensure protocol compliance and quality assurance.

11.3.2 Quality Control Committee

The investigators and study coordinators will meet weekly as an internal quality control committee to review consent documents, CDART data forms, and unanticipated problems with recruitment or adverse events. There is only one site for this study. The use of on-label, widely used mouthrinses and collection of saliva carry minimal risk for participants, apart from the very rare, undiagnosed allergy, which would be managed through proper referral to the neighboring UNC hospital. Therefore, there is no study quality control committee.

11.3.3 Metrics

Every salivary sample will be collected in a conical tube with millimeter markings to monitor salivary volume. If insufficient saliva is collected, the study personnel collecting the samples from participants will note insufficient volume in the CDART database for each applicable time point and will indicate in CDART if the subject must be withdrawn and replaced. If a patient is unable to generate sufficient saliva for the 0 min, 15 min or 30 min post-rinse time points, the subject will be removed from the study and this will be noted in CDART along with their insufficient samples. If a subject is unable to generate sufficient saliva for time points 45 or 60 min post-rinse, the participant will remain in the study but their insufficient samples will be noted in CDART. If a patient is unable to tolerate the blood draws or periodontal exam, this will be noted in CDART and the patient will be withdrawn from the study.

For RT-PCR, viral infectivity and multiplex Luminex assays, all salivary samples will be run in triplicate with standard experimental controls (negative and positive) and protocol quality checks performed by research technical personnel in the COVID Core, Baric, Webster and Wallet laboratories. SARS-CoV-2 viral infectivity and RNA level will be measured using an *in vitro* infectivity assay (Reduction factor [RF] calculated from PFU / mL) and RT-PCR (relative quantification [RQ] ratio). Cytokine and chemokine concentration (pg/mL) will be measured using a prevalidated multiplexed quantitative analyte assay on a bioanalyzer (Luminex technologies). This objective provides urgently needed data to guide healthcare workers on the proper use of specific commercially available mouthwashes capable of reducing SARS-CoV-2 viral infectivity and potentially limiting COVID-19+ transmission.

Outcome assessments will be conducted weeks to months after the study visit, when RT-PCR, viral infectivity and cytokine/chemokine assays are conducted in the COVID Core and the laboratory of Dr. Ralph Baric.

The secondary objectives are to measure inflammation-associated cytokine and chemokine concentration (pg/mL) in saliva and blood samples taken from SARS-CoV-2+ participants and also to quantify the frequency of periodontal disease among study participants. The tertiary objectives of this study are a periodontal exam and to use post-rinse surveys to evaluate patient perceptions of the mouthrinse and feedback on their willingness to use the product (units: percentage of respondents). These tertiary outcomes are exploratory. Periodontal exams will be scored and reported according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, co-presented by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP).[19]

11.3.4 Protocol Deviations

Protocol deviations will be recorded within source documentation (CDART) for each participant by study personnel during the study visits. The investigator and study coordinators will meet weekly to review unanticipated problems, adverse events and protocol deviations. All such events will be appropriately reported to the IRB board of UNC.

11.3.5 Monitoring

The quality control committee will meet weekly to review adverse events, protocol deviations, data quality in CDART, and collection of consent forms. The GoHealth Clinical Research core has highly experienced research coordinators who will monitor all study staff to ensure protocol compliance. A representative from the GoHealth core will participate in the weekly quality control committee meetings to review CDART records and consent forms.

12. PARTICIPANT RIGHTS AND CONFIDENTIALITY

12.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. Consent forms are separate from this protocol document and were submitted as part of the IRB application.

12.2 Informed Consent Forms

Outpatients, who have tested positive for SARS-CoV-2 and consented to be contacted for study enrollment in the UNC Respiratory Diagnostic Center (RDC), will be contacted to participate in this study by research personnel. The subjects being approached by phone or email have signed a prior facility consent at the RDC confirming their willingness to share their name and contact information to be contacted for study participation in COVID-19 related research. We will not receive information on subjects that decline this internal consent. Patients will be contacted first by phone and, if not available, then by email. The study's purpose and requirements will be explained and the patient will have the opportunity to ask questions and will have time to consider participation. Interested patients will make an appointment to visit the Go Health Clinical Research Unit at Adams School of Dentistry for this study. Study participation includes two visits, one week apart. The consent forms will be reviewed and signed by interested patients in person in the Go Health Clinical Research Unit at Adams School of Dentistry.

A signed consent form will be obtained from each participant. For participants who cannot consent for themselves, such as those with a legal guardian (e.g., person with power of attorney), this individual must sign the consent form, though minors and patients who are intellectually delayed and unable to consent for themselves will be excluded. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record. For Spanish speaking patients and/or parents, Spanish forms will be provided and communication will occur through a Spanish translator. All patients are adults and over normal cognitive capacity, and therefore will be able to consent for themselves.

At the visit, a consented patient will answer screening questions regarding inclusion and exclusion criteria. Answers will be entered into the CDART research database managed by UNC. Prior to finalizing these screening questions, women participants of childbearing potential will be asked to provide a urine sample in the restroom, to undergo a rapid pregnancy test by study personnel. Any pregnant or lactating patients will be excluded.

Our study statistician,will create a block randomization schedule and perform these computations. All patients will be consented and then fill out our screening questions for inclusion and exclusion to confirm eligibility prior to being assigned to the randomization schedule.

12.3 Participant Confidentiality

An Excel Linkage File key will be created with study ID (random alphanumeric code) to identify patients, saved in a secure folder in a UNC School of Dentistry (SOD) server. This linkage file will be stored separately from data and destroyed at the close of the study. All data collected from participants and results from sample analysis will be coded with unique numerical identifiers and stored in the UNC CDART clinical research database and on a secure UNC-CH server. Data will only be transmitted among the research team using the SOD secure research server. Demographic data will be transmitted among the research team through access to the secure CDART research database system, stored on secure UNC servers, which study personnel will access only through encrypted computers via secure user accounts. All research personnel will be trained in maintaining patient confidentiality and HIPAA.

Any data, specimens, forms, reports, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. We do not anticipate any data, specimens, forms reports and other records leaving the site as sample storage and analysis will occur on site. All paper records will be kept in a locked file cabinet, and all digital records will be stored in the encrypted, secure, password protected CDART research database. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NCCIH, and the OHRP.

There is little to no potential for deductive disclosures from the survey portion of this study. Patients will be asked about the experience rinsing with a commercially available mouth rinse, about their COVID-19 related symptoms and about their oral healthcare. General demographics will be asked such as gender, race, ethnicity and age bracket. Their identity cannot be deduced from this information.

12.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCIH, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

13. COMMITTEES

The IRB committee will review and approve all study documents, and adverse events and protocol deviations will be reported to them for review as well.

14. PUBLICATION OF RESEARCH FINDINGS

Any presentation, abstract or manuscript will be made available for review and approval by the sponsor and all authors prior to submission.

15. REFERENCES

1. State Officials Announce Latest COVID-19 Facts Including New Data on Racial Demographics and Expanded Health Care Worker Data. *California Department of Public Health*(2020).
2. Sorveglianza Integrata COVID-19 in Italia. *Instituto Superiore di Sanita*https://portale.fnomceo.it/wp-content/uploads/2020/04/Infografica_9aprile-ITA.pdf (2020).
3. O'Donnell, V. B. *et al.*Potential Role of Oral Rinses Targeting the Viral Lipid Envelope in SARS-CoV-2 Infection. *Function* **1**, (2020).
4. Harrel, S. K. & Molinari, J. Aerosols and splatter in dentistry: A brief review of the literature and infection control implications. *J. Am. Dent. Assoc.*(2004) doi:10.14219/jada.archive.2004.0207.
5. Izzetti, R., Nisi, M., Gabriele, M. & Graziani, F. COVID-19 Transmission in Dental Practice: Brief Review of Preventive Measures in Italy. *Journal of Dental Research*(2020) doi:10.1177/0022034520920580.
6. Ather, A., Patel, B. & Ruparel, N. Coronavirus Disease 19 (COVID-19): Implications for Clinical Dental Care. *J. Endod.*(2020).
7. Baghizadeh Fini, M. What dentists need to know about COVID-19. *Oral Oncology*(2020) doi:10.1016/j.oraloncology.2020.104741.
8. Cleveland, J. L. *et al.*Transmission of blood-borne pathogens in US dental health care settings: 2016 update. *J. Am. Dent. Assoc.*(2016) doi:10.1016/j.adaj.2016.03.020.
9. Pfefferle, S. *et al.*Low and high infection dose transmission of SARS-CoV-2 in the first COVID-19 clusters in Northern Germany. *medRxiv*(2020) doi:10.1101/2020.06.11.20127332.
10. Ge, Z. yu, Yang, L. ming, Xia, J. jia, Fu, X. hui & Zhang, Y. zhen. Possible aerosol transmission of COVID-19 and special precautions in dentistry. *Journal of Zhejiang University: Science B*(2020) doi:10.1631/jzus.B2010010.
11. Carrouel, F. *et al.*COVID-19: A Recommendation to Examine the Effect of Mouthrinses with ß-Cyclodextrin Combined with Citrox in Preventing Infection and Progression. *J. Clin. Med.*(2020) doi:10.3390/jcm9041126.
12. Dennison, D. K., Meredith, G. M., Shillitoe, E. J. & Caffesse, R. G. The antiviral spectrum of Listerine antiseptic. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol.*(1995) doi:10.1016/S1079-2104(05)80124-6.
13. Hasheminia, D. *et al.*The efficacy of 1% betadine mouthwash on the incidence of dry socket after mandibular third molar surgery. *J. Clin. Exp. Dent.*(2018) doi:10.4317/jced.54444.
14. Challacombe, S. J., Kirk-Bayley, J., Sunkaraneni, V. S. & Combes, J. Povidone iodine. *British Dental Journal*(2020) doi:10.1038/s41415-020-1589-4.
15. Kirk-Bayley, J., Challacombe, S., Sunkaraneni, V. & Combes, J. The Use of Povidone Iodine Nasal Spray and Mouthwash During the Current COVID-19 Pandemic

May Protect Healthcare Workers and Reduce Cross Infection. *SSRN Electron. J.*(2020) doi:10.2139/ssrn.3563092.
16. Statkute, E., Rubina, A., O'Donnell, V., Thomas, D., & Stanton, R. The Virucidal Efficacy of Oral Rinse Components Against SARS-CoV-2 In Vitro. *BiorXiv.* doi.org/10.1101/2020.11.13.381079
17. COVID-19 Frequently Asked Questions. *American Dental Association: Center for Professional Success* (2020).
18. Meiller TF, Silva A, Ferreira SM, Jabra-Rizk MA, Kelley JI, DePaola LG. Efficacy of Listerine Antiseptic in reducing viral contamination of saliva. *J Clin Periodontol*. 2005;32(4):341-346. doi:10.1111/j.1600-051X.2005.00673.x
19. Tonetti et al. J Periodontol 89 (Suppl 1): S159-S172 [2018]; Trombelli et al. J Periodontol 89 (Suppl 1): S46-S73 [2018]; Chapple et al. J Periodontol 89 (Suppl 1) S74-S84 [2018]
20. Eke et al. J Periodontol 83: 1449-1454 [2012]

16. SUPPLEMENTS/APPENDICES

No supplements or appendices. All forms are submitted as part of the IRB application.