Mouth Rinses for Inactivation of COVID-19

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Clinical Intervention Study Protocol

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

Study Chairman or Principal Investigator:

Laura Jacox, DMD, PhD, MS, Assistant Professor of Orthodontics, UNC Adams School of Dentistry

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STUDY TEAM ROSTER

Laura Jacox, Principal Investigator, 2090 First Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (847) 702-0988, ljacox@live.unc.edu

Christina Graves, Co-Investigator, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3725, cxg@email.unc.edu

Shannon Wallet, Co-Investigator, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3359, shannon_wallet@unc.edu

Ralph Baric, Co-investigator, 3304 Michael Hooker Res Building CB #7435, Chapel Hill, NC 27599, (919) 966-3895, rbaric@email.unc.edu

Natalie Bowman, Co-investigator, CB 7030 Bioinformatics Building, 130 Mason Farm Road, Chapel Hill, NC 27514, natalie_bowman@med.unc.edu

S.T. Phillips, Study Coordinator, GO Health Clinic, 2040 Old Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3424, sherrill_phillips@unc.edu

Wendy Lamm, Research Assistant, GO Health Clinic, 2040 Old Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3418, wendy_lamm@unc.edu

Nare Ghaltakhchyan, Research Assistant, 2090 First Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 904-8493, nare@live.unc.edu

Eleanor Nanney, Research Assistant, 2090 First Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (704) 651-4579, ellien@ad.unc.edu

Mandy Bush, Research Assistant, DELTA Core Lab Manager, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3968, mbush43@email.unc.edu

PARTICIPATING STUDY SITES

Study Site: UNC Adams School of Dentistry

GO Health Clinic, Old Dental Building #2040

CB# 7450

385 S. Columbia St, Chapel Hill, NC 27599

(919) 537-3424 (P), (919) 843-1798 (F)

Laura Jacox, Principal Investigator, 2090 First Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (847) 702-0988, ljacox@live.unc.edu

Christina Graves, Co-Investigator, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3725, cxg@email.unc.edu

Shannon Wallet, Co-Investigator, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3359, shannon_wallet@unc.edu

Ralph Baric, Co-investigator, 3304 Michael Hooker Res Building CB #7435, Chapel Hill, NC 27599, (919) 966-3895, rbaric@email.unc.edu

Natalie Bowman, Co-investigator, CB 7030 Bioinformatics Building, 130 Mason Farm Road, Chapel Hill, NC 27514, natalie_bowman@med.unc.edu

S.T. Phillips, Study Coordinator, GO Health Clinic, 2040 Old Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3424, sherrill_phillips@unc.edu

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Nare Ghaltakhchyan, Research Assistant, 2090 First Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 904-8493, nare@live.unc.edu

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Mandy Bush, Research Assistant, DELTA Core Lab Manager, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3968, mbush43@email.unc.edu

PRÉCIS

Study Title

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

Objectives

The primary objectives are to quantify the impact of antiseptic mouthwashes on salivary protein antigen levels and viral RNA of the SARS-CoV-2 virus in COVID-19+ patients. The tertiary objectives are to quantify the impact of antiseptic mouthwashes on salivary viral infectivity in COVID+ patients, and to determine patients' acceptance of using antiseptic mouthwashes in healthcare settings.

Design and Outcomes

Randomized, double-blind prospective trial to test the efficacy and acceptability of therapeutic, antiseptic mouth rinses to inactivate the SARS-CoV-2 virus, and to reduce protein antigen levels and viral RNA in saliva of COVID-19 positive patients aged 18-65 years old. All mouthrinses are commercially available 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 one minute mouthwash. Saliva samples will be collected from patients at 15 minute intervals thereafter up to an hour (15, 30, 45 and 60 minutes). The samples will be stored and used for qRT-PCR detection of viral SARS-CoV-2 RNA, sandwich ELISA or rapid antigen tests for detection of SARS-CoV-2 protein antigens, and viral infectivity assays. Patients will also complete a short-survey on the taste and experience of using the mouthwash. This study involves one, 75-90 minute visit.

Interventions and Duration

Subjects will be asked to rinse with an unlabeled/blinded antiviral mouth rinse for 60 seconds and provide 5 mL of saliva prior to the rinse and 2 mL of saliva immediately post-rinse, 15 minutes post-rinse, 30 minutes post-rinse, 45 minutes post-rinse, and 60 minutes post-rinse. Subjects will also be asked to complete a short survey about the rinsing experience. Subjects will be onsite for a 70-90 minute, single visit appointment.

Sample Size and Population

We will enroll 80 outpatient COVID+ subjects per mouth rinse, with 6 mouth rinses, requiring us to enroll 480 COVID+ patients. Patients will be randomized to their mouthrinse. These patients will have already had a confirmed COVID+ test prior to enrollment. There will be no stratification to our randomization. In addition to having a COVID+ test, patients will need to be at least 18 years of age and at most 65 years of age and in good oral health without any known allergies or reactions to commercial

dental products, mouthrinses or iodine. Patients who are pregnant, nursing/lactating, who have a cognitive or developmental disability, who are taking lithium, who have or have ever had a thyroid problem, who have kidney dysfunction, who have an active oral herpes flare up, who have any oral viral infection and/or flare up, who have significant mucosal tears, growths or damage to their mouth, who have uncontrolled severe periodontal disease with bleeding gums, and those who have xerostomia will be excluded from the study. There is no exclusion criteria based on gender or race.

1. STUDY OBJECTIVES

1.1 Primary Objectives

The primary objectives of this study are to determine the efficacy of antiseptic mouthwashes on reducing SARS-CoV-2 viral RNA and protein antigen levels in COVID+ patient saliva. *We hypothesize that COVID-19+ patients will have a >95% reduction in SARS-CoV-2 viral RNA and protein antigen levels following a 60 second oral rinse with an antiseptic mouthrinse and that this reduction will persist for at least 30 minutes.* These primary objectives will assess SARS-CoV-2 viral load based on viral RNA measured by quantitative real time PCR and viral protein antigen levels measured by rapid antigen or sandwich ELISA assays. These objectives provides urgently needed data to guide healthcare workers on the proper use of specific commercially available mouthwashes capable of reducing SARS-CoV-2 in saliva and potentially limiting COVID-19+ transmission.

1.2 Tertiary Objectives

The first tertiary objective will focus on quantifying SARS-CoV-2 viral infectivity using an *in vitro* infectivity assay. *We hypothesize that COVID-19+ patients will have a >95% 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 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. Our second tertiary objective is to determine the **feasibility** and **acceptability** of using antiseptic mouthwashes by patients in healthcare settings. *We hypothesize that patients will welcome the use of commercially available antiseptic mouthrinses into clinical practice.* Patients enrolled in the randomized mouthrinse study will be surveyed on the taste, appearance, sensation, and acceptability of using the mouthrinse in healthcare settings. Understanding acceptability of a mouthrinse protocol is critical to implementation and therefore the ability to reduce COVID-19 transmission risk in clinical settings.

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 solutions (21% EtOH) have been shown to reduce SARS-CoV-2 viral infectivity levels *in vitro*, and in patients suffering Herpes Simplex Virus-1 flare-ups, a 30 second oral rinse significantly reduced salivary infectivity for over 30 minutes.[12] Povidone-iodine (PVP), is a fast-acting, broad-spectrum antiseptic with antiviral activity in vitro and is commonly encountered in dental settings outside the US.[13] PVP is effective in reducing SARS-CoV-2 viral titers by 99.99%, lasting up to 20 minutes *in vitro*, suggesting it could be effective in reducing transmission during dental procedures.[14,15] Chlorhexidine Gluconate (0.12%) has been widely adopted by practices despite discouraging 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 only preprocedural mouthrinse currently recommended by the ADA to prevent transmission, despite no studies evaluating its

efficacy against SARS-CoV-2.[16] No *in vivo* clinical data exists to-date for PVP or 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]

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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] Ethanol solutions (21% EtOH) have been shown to reduce SARS-CoV-2 viral infectivity levels *in vitro*, and in patients suffering Herpes Simplex Virus-1 flare-ups, a 30 second oral rinse significantly reduced salivary infectivity for over 30 minutes.[12] Povidone-iodine (PVP), is a fast-acting, broad-spectrum antiseptic with antiviral activity in vitro and is commonly encountered in dental settings outside the US.[13] PVP is effective in reducing SARS-CoV-2 viral titers by 99.99%, lasting up to 20 minutes *in vitro*, suggesting it could be effective

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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]

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: Based on similar viral infectivity studies evaluating mouth rinse efficacy against enveloped viruses, we used power calculations to estimate effect size and determine sample size as a function of length of mouthrinse time course. With ten simultaneous comparisons (T1-T0, T2-T1, T2-T0, T3-T0, T3-T2, T3-T1, T4-T3, T4-T2, T4-T1, T4-T0) = 60 minute time course, the sample size n=78 can detect the effect size in Cohen's d =0.7, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction.[12]

Subjects will be required to rinse their mouths with an unlabeled/blinded mouthrinse for 60 seconds. Per the instructions on the product label, it is recommended to rinse for 30-60 seconds. The longer rinse-time was chosen for this study in order to maximize the antiviral activity of the proposed mouthrinses. 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 minutes, 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.

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 COVID 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***
26.9% ethanol plus essential oils	• If more than used for rinsing is accidentally swallowed, get medical help or contact a Poison Control Center right away
0.5% w/v povidone-iodide (PVI)	• Local irritation, skin burns and sensitivity reactions have been reported rarely. • Anaphylactic reactions, anaphylactoid reactions and anaphylactic shock have been reported uncommonly with products containing povidone-iodine or povidone. • Excess iodine can produce goitre and hypothyroidism or hyperthyroidism. Such effects have occasionally been seen with extensive or prolonged use of povidone iodine. • Other effects that have been reported are metabolic acidosis and acute renal failure. • Possible unwanted effects: Like all medicines, Betadine Gargle and Mouthwash can cause unwanted effects, although these are uncommon. Allergic-type reactions, including a rash or difficulty in breathing, have been reported uncommonly with products containing povidone iodine (the active ingredient in Betadine Gargle and Mouthwash). Although rare, Betadine Gargle and Mouthwash could also cause itching and burns on your skin. Applying very large

	amounts of medicines containing povidone iodine, or using such products over a long period of time, can occasionally cause thyroid disorders (symptoms of which include weight loss, increased appetite and sweating or lacking energy and weight gain). Other effects that have been reported are increased amounts of acid in your blood (the main symptoms are shortness of breath, confusion and lethargy) and kidney failure (symptoms include difficulty in urinating, thirst and a dry mouth and pain in one side of your back). Should you suffer from any of these unwanted effects, or if you notice any unwanted effects not listed in this leaflet, stop using Betadine Gargle and Mouthwash straight away and tell your doctor or pharmacist.
1.5% w/v hydrogen peroxide rinse	• 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	• 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.
0.1% Cetylpyridinium Chloride	• If more than used for rinsing is accidentally swallowed, get medical help or contact a Poison Control Center right away

0.9% NaCl	**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

Aims of this placebo-controlled, randomized, double-blinded study are to determine the efficacy of antiseptic mouthwashes on reducing SARS-CoV-2 viral RNA, protein antigen levels, and 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 10 days of symptom onset. Subjects will be seen and samples 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 will be asked to give a baseline salivary sample (5ml over 10 minutes) and then asked to rinse with an unlabeled (blinded) antiseptic mouth rinse for 60 seconds according to the table below:

mouthrinse*	***Number of participants (sample size)***	***Group***
26.9% ethanol plus essential oils	**80**	**Experimental**
0.5% w/v povidone-iodide (PVI)	**80**	**Experimental**
1.5% w/v hydrogen peroxide rinse	**80**	**Experimental**
0.12% Chlorhexidine Gluconate	**80**	**Experimental**
0.1% Cetylpyridinium Chloride	**80**	**Experimental**

0.9% NaCl	**80**	**Control**

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

After the participant expectorates the mouth rinse, research staff will collect another salivary sample (T_0, 2 mL over 5 minutes). During a 10 minute 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. Patients will be asked to smell an ammonia ampule ("smelling salts") to evaluate sense of smell, as loss of olfaction is indicative of the acute infection phase. At 15 minutes post-rinse, the participant will be asked to provide an additional saliva sample (2 mL over 5 minutes), followed by another 10 minute recuperation period. This sequence will be repeated for at 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), and study participation will conclude.

All salivary samples will be collected on ice in sterile 15 mL conical vials labelled "COVID Core" and using pre-printed labels including patient's random alphanumeric study ID. Saliva samples will be cataloged and stored at the UNC Delta COVID Translational Initiative (also known as the Covid-19 Core) for future qRT-PCR, viral infectivity, and rapid antigen or sandwich ELISA assays. Samples will be transported on ice 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).

Following study participation completion, participants will receive $50 compensation in the form of a Visa gift card.

During the period that the COVID+ study participants are present in the clinic and giving saliva samples, we will monitor oxygen saturation and inquire about their comfort. If participants exhibit signs of distress or if oxygen saturation drops, research study staff will contact emergency medical responders (present in the building) 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 determine if any of the antiseptic mouthwashes are effective at reducing SARS-CoV-2 in salivary secretions of COVID-19+ 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 hr in length). Further, the design will allow for a kinetic analysis of viral shedding in salivary secretions and to determine 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: Wallet and Bush) by pre-authorized personnel. Samples will be assessed for **1**) molecular presence of SARS-CoV-2, **2**) salivary antibody and cytokine responses to SARS-CoV-2 and **3**) in vitro infectibility.

1. Molecular analysis: For molecular analysis, including viral RNA and protein antigen analysis, samples will be inactivated by adding 2 mL of extraction buffer to each 500 uL saliva aliquot (4:1 ratio) and mixed by inversion. These samples will be further aliquoted at 250uL in 1.5 mL Eppendorf tubes and stored in an "Inactivated Samples -80 °C Freezer" until further analysis.

2. Antibody and cytokine responses: an aliquot of saliva will be inactivated in a 56 °C water bath for 30 minutes. 250 uL 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 minutes 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 PFUs 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 uL 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 determine clinical and other factors impacting applicability of each of the mouth rinses.

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 COVID+ status by physician. Either became symptomatic in the prior 10 days, or if not symptomatic, likely infected/exposed within the prior 10 days.
- Individuals (all sex, all gender) at least 18 years of age and at most 65 years of age and in good oral health without any known allergies to commercial dental products or cosmetics.
- 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 years old and older than 65 years old
- 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 iodine, ethanol, essential oils (Eucalyptol, Menthol, Methyl salicylate, Thymol), hydrogen peroxide, chlorhexidine gluconate, peroxyl, listerine, betadine, peridex, cetylpyridinium chloride, 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), 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 Povidone-iodine during pregnancy and lactation, which cannot occur in a blinded, randomized trial.)
- Other severe acute or chronic medical or psychiatric condition 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.
- Patient who has or ever had a thyroid problem, including swelling (nodular colloid goitre, endemic goitre or Hashimoto's thyroiditis)
- Patients currently having lithium therapy for depression
- 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***
26.9% ethanol plus essential oils	• Do not use in children under 12

1% w/v povidone-iodide (PVI)	● Hypersensitivity to iodine, polyvinyl pyrrolidine or to any excipient. ● History of abnormal thyroid function or goitre (in particular nodular colloid goitre, endemic goitre and Hashimoto's thyroiditis). ○ Use of this preparation may interfere with tests of thyroid function. Iodine is absorbed through burns and broken skin and to a lesser extent through intact skin and may lead to toxic levels of iodine in the blood, particularly in patients with renal insufficiency. If symptoms occur suggesting changes in thyroid function, these should be investigated. In patients with impaired renal function, blood levels of iodine should be monitored ● Regular use should be avoided in patients on concurrent lithium therapy. ○ Use with concurrent lithium therapy has been shown to exhibit additive hypothyroidic effects. Absorption of iodine from povidone iodine through either intact skin or broken skin may interfere with thyroid function tests. Contamination with povidone iodine of several types of tests for the detection of occult blood in faeces or blood in urine may produce false-positive results. ● Do not use in children of 6 years and under. ● Pregnant or nursing mothers ○ Iodine freely crosses the placenta and is secreted in breast milk. Thyroid function disorders have been reported in the offspring of mothers exposed to pharmacological doses of iodine. Povidone iodine should not be used regularly during pregnancy unless there is no alternative treatment available. ● Do not use Betadine Gargle and Mouthwash 10mg/ml Oral Solution: - if you are allergic (hypersensitive) to povidone iodine or any of the other ingredients listed - if you currently have or have ever had a thyroid problem, including swelling (nodular colloid goitre, endemic goitre or Hashimoto's thyroiditis), as using Betadine Gargle and Mouthwash in these circumstances can further affect the function of your thyroid - if you are currently having lithium therapy for depression as this type of medicine can interact with Betadine Gargle and Mouthwash to affect the function of your thyroid - on children of 6 years and under. Talk to your doctor or pharmacist before using Betadine Gargle and Mouthwash 10mg/ml Oral Solution - if your skin is broken (for example, due to a burn) as using Betadine Gargle and Mouthwash in these circumstances may cause toxic levels of iodine to be absorbed into your blood - if you currently have or have ever had a kidney problem as using Betadine Gargle and Mouthwash in these circumstances may cause toxic levels of iodine to be absorbed into your blood - when you know you are due to have a thyroid, faeces (stool) or urine test as Betadine Gargle and Mouthwash may affect the results.

1.5% w/v hydrogen peroxide rinse	● Pregnancy and lactation ○ Although there is not sufficient specific clinical data on the use of 1.5 ○ % 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	● Carcinogenesis, Mutagenesis, Impairment of Fertility ○ 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 ○ 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 ○ 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 ○ Clinical effectiveness and safety of 0.12% Chlorhexidine Gluconate have not been established in children under the age of 18.

0.1% Cetylpyridinium Chloride	• Do not use in children under 6
0.9% NaCl	**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, decided at the discretion of the study coordinator.	Known allergies or significant adverse reactions following the use of oral hygiene products (toothpastes, mouthrinses), commercial cosmetics, iodine, and any ingredient in the mouthrinses (list will be provided).
Diagnosed COVID+ status (within the past 48 hours) who became symptomatic in the prior 10 days, or if not symptomatic, was likely infected/exposed within the prior 10 days.	Established history of Xerostomia (drug-induced or autoimmune dry mouth), renal disease, thyroid problems (including swelling, Hoshimoto's thyroiditis, Goiter), Hepatitis C Virus (HCV), severe periodontal disease with bleeding gums, significant oral abrasions/ulcers or growths, alcohol abuse, recreational drug abuse, and/or current treatment with lithium. Other severe acute or chronic medical or psychiatric condition 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 patient 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 Distress Clinic (RDC) and other UNC testing facilities who have tested positive for COVID+ and consented to be contacted for participation in COVID-related research studies. The subjects are being approached by phone or email have signed a prior facility consent at the Respiratory Distress Clinic confirming their willingness to share their name and contact information to be contacted for study participation in COVID related research. We will not receive information on subjects that decline this internal consent. 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, a single appointment 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 COVID+ 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.

Our study statistician, Dr. Kevin Moss, 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 5ml over 10 minutes. The patient will then be asked to rinse with an unlabeled/blinded antiseptic mouth rinse for 60 seconds. Once s/he expectorates the mouth rinse, a time 0, 2ml salivary sample will be collected over 5 minutes. Then the patient will have 10 minutes to recuperate, while answering 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. Patients will be asked to smell an ammonia ampule ('smelling salt') to evaluate sense of smell, as loss of olfaction is indicative of the acute infection phase. At 15 minutes post-rinse, the patient will be asked to provide an additional 2ml saliva samples over 5 minutes, followed by another 10 minute break. This sequence repeats for the 30, 45 and 60 minute time points. At 60 minutes, the patient will give their last 2ml salivary sample, and study participation will conclude. Participants will be provided with a gift card for their participation.

5.2 Handling of Study Interventions

Mouthrinses will be acquired from the following sources:

mouthrinse*	***Source:*** Online pharmacies and reputable marketplaces with over the counter mouthrinses
26.9% ethanol plus essential oils	Listerine's Original Mouthrinse- Amazon or CVS online store
1% w/v Povidone-iodide (PVI)	Betadine Mouthrinse- Amazon or CVS online store
1.5% w/v Hydrogen Peroxide rinse	Oral B Mouth Sore Mouthrinse- Amazon or CVS online store
0.12% Chlorhexidine Gluconate	Peridex- CVS Pharmacy and Adams School of Dentistry Dispensary
0.1% Cetylpyridinium Chloride	Crest Pro-Health Mouthrinse- Amazon or CVS online store
0.9% NaCl	Amazon or CVS online 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 under sterile conditions in a 10mL sterile syringe. The syringe will be unlabeled and opaque so both the participant and study personnel will be blinded to the color of 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 6 options 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 60 seconds and then will spit it out, followed by donating saliva samples.

5.3.3 Prohibited Interventions

Patients who have drank or eaten within an hour of the study visit 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 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 a full 60 seconds before expectorating, and then providing saliva samples at 15 minute time points.

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)
Informed Consent Form	X	X
Demographics	X	X
DXA	X	X
Medical History	X	X
General Physical Examination		
Current Medications	X	X
Blood Chemistries		
Hematology		
Urine Analysis-pregnancy		X
Vital Signs		X
Inclusion/Exclusion Criteria	X	X
Enrollment/Randomization	X	X

Treatment Administration Form		X
Concomitant Medications	X	X
Adverse Events	X	X

6.2 Description of Evaluations

Subjects are required to be onsite for a 70-90 minute, single visit appointment. Subjects will be asked to rinse with an unlabeled/blinded antiviral mouth rinse for 60 seconds and provide 5 mL of saliva prior to the rinse and 2 mL of saliva immediately post-rinse, 15 minutes post-rinse, 30 minutes post-rinse, 45 minutes post-rinse, and 60 minutes post-rinse. Subjects will also be asked to complete a short survey about the rinsing experience.

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 Samantha Jhingree. 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 only one visit. 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.
- 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 Respiratory Distress Clinic (RDC) who have tested positive for COVID+ and consented to be contacted for participation in COVID-related research studies. The subjects being approached by phone or email have signed a prior facility consent at the Respiratory Distress Clinic confirming their willingness to share their name and contact information to be contacted for study participation in COVID related research. We will not receive information on subjects that decline this internal consent. 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, a single appointment 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. A list of COVID+ 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

There are no baseline assessments required as this study only requires a 70-90 minute single visit appointment.

Randomization

Our statistician Dr. Kevin Moss 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.

The researcher 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/blinded mouthrinse. The researcher 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 syringe to provide to the patient for the rinse. Our statistical consultant, Dr. Kevin Moss,

will set up a block randomization schedule that the team will abide by for assignment of enrolled subjects.

6.2.4 Follow-up Visits

This study requires only one 70-90 minute, single visit appointment. 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 only one 70-90 minute, single visit appointment. Subjects will be asked to rinse with an unlabeled/blinded antiviral mouth rinse for 60 seconds and provide 5 mL of saliva prior to the rinse and 2 mL of saliva immediately post-rinse, 15 minutes post-rinse, 30 minutes post-rinse, 45 minutes post-rinse, and 60 minutes post-rinse. Subjects will also be asked to complete a short survey about the rinsing experience.

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, missed appointment, subject no longer meets the eligibility criteria.

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 gift card and will be replaced. If a patient suffers from dry mouth, such that s/he cannot produce salivary sample volumes needed for later time points (45 minutes or 60 minutes post-rinse) but s/he produced sufficient baseline, immediate, 15 minute and 30 minute post-rinse samples, s/he will still be compensated with a gift card and parking vouchers, and will remain in the study. Missing or deficient salivary samples will be noted in CDART.

7. SAFETY ASSESSMENTS

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

mouthrinse*	***Adverse effects***
26.9% ethanol plus essential oils	• If more than used for rinsing is accidentally swallowed, get medical help or contact a Poison Control Center right away
1% w/v povidone-iodide (PVI)	• Local irritation, skin burns and sensitivity reactions have been reported rarely. • Anaphylactic reactions, anaphylactoid reactions and anaphylactic shock have been reported uncommonly with products containing povidone-iodine or povidone. • Excess iodine can produce goitre and hypothyroidism or hyperthyroidism. Such effects have occasionally been seen with extensive or prolonged use of povidone iodine. • Other effects that have been reported are metabolic acidosis and acute renal failure. • Possible unwanted effects: Like all medicines, Betadine Gargle and Mouthwash can cause unwanted effects, although these are uncommon. Allergic-type reactions, including a rash or difficulty in breathing, have been reported uncommonly with products containing povidone iodine (the active ingredient in Betadine Gargle and Mouthwash). Although rare, Betadine Gargle and Mouthwash could also cause itching and burns on your skin. Applying very large amounts of medicines containing povidone iodine, or using such products over a long period of time, can occasionally cause thyroid disorders (symptoms of which include weight loss, increased appetite and sweating or lacking energy and weight gain). Other effects that have been reported are increased amounts of acid in your blood (the main symptoms are shortness of breath, confusion and lethargy) and kidney failure (symptoms include difficulty in urinating, thirst and a dry mouth and pain in one side of your back). Should you suffer from any of these unwanted effects, or if you notice any unwanted effects not listed in this leaflet, stop using Betadine Gargle and Mouthwash straight away and tell your doctor or pharmacist.
1.5% w/v hydrogen peroxide rinse	• 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	● 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.
0.1% Cetylpyridinium Chloride	● If more than used for rinsing is accidentally swallowed, get medical help or contact a Poison Control Center right away
0.9% NaCl	**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 (like Listerine) 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.

7.1 Specification of Safety Parameters

Mouthrinse and saliva collection carries low risk and minimal to no safety concerns to the 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.

7.2 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 (like Listerine) 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.

7.3 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 (like Listerine) and collection of saliva 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.

7.4 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.

7.5 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.

7.6 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.

8. 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, missed appointment, subject no longer meets the eligibility criteria.

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 gift card and will be replaced. If a patient suffers from dry mouth, such that s/he cannot produce salivary sample volumes needed for later time points (45 minutes or 60 minutes post-rinse) but s/he produced sufficient baseline, immediate, 15 minute and 30 minute post-rinse samples, s/he will still be compensated with a gift card and parking vouchers, and will remain in the study. Missing or deficient salivary samples will be noted in CDART.

9. STATISTICAL CONSIDERATIONS

9.1 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.

Statistical Hypothesis

The central hypotheses of this study are that a 60 second antiseptic mouth rinse will effectively reduce detectable viral RNA and protein antigen levels of SARS-CoV-2 for a minimum of 30 minutes duration.

The tertiary hypotheses are that a 60 second antiseptic mouth rinse will effectively reduce viral infectivity (>50%) of SARS-CoV-2 for a minimum of 30 minutes duration, and that mouth rinses will be well tolerated in a clinical care setting by a majority of patients and providers.

Sample Size Considerations

Based on similar viral infectivity studies evaluating antiviral mouth rinse efficacy, we were able to run power calculations to estimate effect size and determine sample size as a function of length of mouthrinse time course. The sample size is justified by a paired t-test on outcomes between time points. With three simultaneous comparisons (T1-T0, T2-T1, and T2-T0 = 30 minute time course), the sample size n=6 can detect the effect size in Cohen's d =0.7, which is between medium and large, over 80% power and 0.016 type-I error rate by Bonferroni correction. With six simultaneous comparisons (T1-T0, T2-T1, T2-T0, T3-T0, T3-T2, T3-T1) = 45 minute time course, the sample size n=53 can detect the effect size in Cohen's d =0.7, which is between medium and large, over 80% power and 0.016 type-I error rate by Bonferroni correction. With ten simultaneous comparisons (T1-T0, T2-T1, T2-T0, T3-T0, T3-T2, T3-T1, T4-T3, T4-T2, T4-T1, T4-T0) = 60 minute time course, the sample size n=78 can detect the effect size in Cohen's d =0.7, which is between medium and large, over 80% power and 0.016 type-I error rate by Bonferroni correction.

Statistical analysis of our data will be performed by Dr. Kevin Moss, the statistician on this project.

Primary Outcomes

The analyses will compare viral RNA level (qRT-PCR) and protein antigen level (sandwich ELISA or rapid antigen) of SARS-CoV-2 between mouth rinse types and across the time course.

Data generated from the qRT-PCR and sandwich ELISA or rapid antigen assays 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 U test. Normality will be assessed using the Kolmorgorov-Smirnov test, and logistic regression will be performed with robust standard errors.

Tertiary Outcomes

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.

Survey- Statistical analysis will be conducted by Odum Institute's statistical consultant, Chris Wiesen, PhD. We will use descriptive statistics, frequency counts and means to evaluate feasibility and acceptability of using point-of-care mouthrinses in clinical workflow.

9.2 Sample Size and Randomization

Based on similar viral infectivity studies evaluating antiviral mouth rinse efficacy, we were able to run power calculations to estimate effect size and determine sample size as a function of length of mouthrinse time course. The sample size is justified by a paired t-test on outcomes between time points. With three simultaneous comparisons (T1-T0, T2-T1, and T2-T0 = 30 minute time course), the sample size n=6 can detect the effect size in Cohen's d =0.7, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction. With six simultaneous comparisons (T1-T0, T2-T1, T2-T0, T3-T0, T3-T2, T3-T1) = 45 minute time course, the sample size n=53 can detect the effect size in Cohen's d =0.7, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction. With ten simultaneous comparisons (T1-T0, T2-T1, T2-T0, T3-T0, T3-T2, T3-T1, T4-T3, T4-T2, T4-T1, T4-T0) = 60 minute time course, the sample size n=78 can detect the effect size in Cohen's d =0.7, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction.

Dr. Kevin Moss, who is the statistical consultant on this project, 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.

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 storage syringe to provide to the patient for the rinse. The syringe will be opaque to mask any coloring of the solution.

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 mouthrinses are sold commercially at Walgreens and CVS, 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.

9.3 Definition of Populations

Our population includes patients 18-65 years old who have tested positive with COVID-19 and are either asymptomatic but exposed in the prior 10 days, or are symptomatic with symptom development in the prior 10 days. Inclusion and exclusion criteria (described elsewhere) will be met. We are not treating patients for their COVID-19 infection.

9.4 Interim Analyses and Stopping Rules

An interim analysis will be conducted after 120 patients are enrolled and samples collected; block randomization will be planned to randomize in batches of 120 participants, with 20 randomized to each mouthrinse. Therefore, we can do an interim analysis with an n=120. This sample size is sufficient to determine antiviral efficacy at 30 minutes post-mouthrinse. If mouthrinses are ineffective at reducing viral infectivity at timepoints 15 and 30 minutes, then that mouth rinse arm will be discontinued for further enrollment and will be removed from the block randomization calendar, with initiation of a subsequent trial with fewer arms. This will allow us to enroll sufficient numbers of patients to statistically evaluate longer time point efficacy for the mouthrinses with antiviral efficacy (the longer time points require larger samples sizes for sufficient statistical power).

If no mouthrinse has antiviral effects at this interim analysis point, the study will be stopped due to lack of efficacy. 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 every 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.

9.5 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 qRT-PCR assay for our primary outcome will be documented in an excel spreadsheet generated by the qRT-PCR assay quantification device, available weeks to months after sample collection when the experiments are conducted. If qRT-PCR proves unsuccessful, antigen-based assays will be used instead to quantify viral protein antigen level (Spike or N protein) using a sandwich ELISA or rapid antigen assays. These outcomes will be documented in excel spreadsheets generated by study personnel, available weeks to months after sample collection when the experiments are conducted. Samples will be run in large batches to limit batch effects.

The viral infectivity assay for our tertiary outcome will be documented in an excel spreadsheet generated by the viral infectivity assay quantification device, available weeks to months after sample collection when the experiments are conducted. Survey results will also yield excel spreadsheets of data, available shortly after data collection completion.

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.

9.5.1 Primary Outcomes

The primary objectives of this study are to determine the efficacy of antiseptic mouthwashes on reducing SARS-CoV-2 viral RNA and protein antigen levels in COVID+ patient saliva. *We hypothesize that COVID-19+ patients will have a >95% reduction in SARS-CoV-2 viral RNA and protein antigen levels following a 60 second oral rinse with an antiseptic mouthrinse and that this reduction will persist for at least 30 minutes.* These primary objectives will assess SARS-CoV-2 viral load based on viral RNA measured by quantitative real time PCR and viral protein antigen levels measured by rapid antigen or sandwich ELISA assays. These objectives provides urgently needed data to guide healthcare workers on the proper use of specific commercially available

mouthwashes capable of reducing SARS-CoV-2 in saliva and potentially limiting COVID-19+ transmission.

Outcome assessments will be conducted weeks to months after the study visit, when qRT-PCR and sandwich ELISA or rapid antigen assays are conducted in the COVID core and the Baric lab.

9.5.2 Tertiary Outcomes

The first tertiary objective will focus on quantifying SARS-CoV-2 viral infectivity using an *in vitro* infectivity assay. *We hypothesize that COVID-19+ patients will have a >95% 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 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. Our second tertiary objective is to determine the **feasibility** and **acceptability** of using antiseptic mouthwashes by patients in healthcare settings. *We hypothesize that patients will welcome the use of commercially available antiseptic mouthrinses into clinical practice.* Patients enrolled in the randomized mouthrinse study will be surveyed on the taste, appearance, sensation, and acceptability of using the mouthrinse in healthcare settings. Understanding acceptability of a mouthrinse protocol is critical to implementation and therefore the ability to reduce COVID-19 transmission risk in clinical settings.

Outcome assessments will be conducted weeks to months after the study visit, when viral infectivity assays are conducted in the COVID core and the Baric lab.

Survey results will be evaluated using bivariate, descriptive statistics and non-parametric methods as appropriate for the question type. Outcome assessments will be conducted weeks to months after the study visit, when statistical analyses are conducted.

9.6 Data Analyses

qRT-PCR and sandwich ELISA or rapid antigen: 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 Students 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.

Survey results will be evaluated using bivariate, descriptive statistics and non-parametric methods as appropriate for the question type.

Power Calculation: Based on similar viral infectivity studies evaluating mouth rinse efficacy against other enveloped viruses, we used power calculations to estimate effect size and determine sample size as a function of length of mouthrinse time course.[12] The sample size is justified by a paired t-test on outcomes between time points. With ten simultaneous comparisons (T1-T0, T2-T1, T2-T0, T3-T0, T3-T2, T3-T1, T4-T3, T4-T2, T4-T1, T4-T0) = 60 minute time course, the sample size n=78 can detect the effect size in Cohen's d =0.7, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction. Analyses will compare viral RNA level (qRT-PCR), protein level (sandwich ELISA and rapid antigen) or viral infectivity levels between mouth rinse types and across the time course. ANCOVA models will be utilized to look at changes in viral load and infectivity logarithmically before and after treatment.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

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. 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.

10.2 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), 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.

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 account.

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.

The quality control committee (described below) will be in charge of monitoring these forms and the CDART database.

10.3 Quality Assurance

10.3.1 Training

All study personnel have been trained in health privacy (HIPAA) and human subjects research 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.

10.3.2 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 (like Listerine or Crest) 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.

10.3.3 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 minutes or 30 minutes 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 minutes post-rinse, the patient will remain in the study but their insufficient samples will be noted in CDART.

For qRT-PCR, viral infectivity, and sandwich ELISA or rapid antigen 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, and Wallet laboratories.

10.3.4 Protocol Deviations

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

10.3.5 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.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 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.

11.2 Informed Consent Forms

Outpatients, who have tested positive for COVID-19 and consented to be contacted for study enrollment in the UNC Respiratory Distress Clinic (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 Respiratory Distress Clinic confirming their willingness to share their name and contact information to be contacted for study participation in COVID 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 only one visit. 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, Dr. Kevin Moss, 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.

11.3 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 account. 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 the experience rinsing with a commercially available mouth rinse (such as Listerine). They will asked to rate the taste, appearance/color, sensation, and their willingness to use the mouth rinse in the future. General demographics will be asked such as gender, race, ethnicity and age bracket. Their identity cannot be deduced from this information.

11.4 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.

12. 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.

13. 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.

14. REFERENCES

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15. SUPPLEMENTS/APPENDICES

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