Efficacy of Oral Rinses for Inactivation of COVID-19 (MOR2)

NCT Number	NCT05178173
Document Date	03/25/2024

Clinical Intervention Study Protocol

Antiviral Efficacy 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 Research and Orthodontics, UNC Adams School of Dentistry

Supported by:

UNC Adams School of Dentistry

Study Intervention Provided by: *N/A*

Tool Revision History

Version Number: 3

Version Date: March 25, 2024

TABLE OF CONTENTS

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

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

Clare Bocklage, Study Coordinator, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 843-2596, eclare@live.unc.edu

Nare Ghaltakhchyan, Study Coordinator, 385 S. Columbia St., Chapel Hill, NC 27599, 1 (919) 962-2078, nare@live.unc.edu

Ellie Nanney, Study Coordinator, 385 S. Columbia St., Chapel Hill, NC 27599, (704) 651-4579, ellien@ad.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

Payton Mince, Research Assistant, GO Health Clinic, 2040 Old Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3418, pmince@ad.unc.edu

Carol Culver, GO Health Clinic Manager, 2040 Old Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3176, carol_culver@unc.edu

Kevin Moss, Statistician, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 573-3200, kevin_moss@unc.edu

Chris Wiesen, PhD. Statistician (survey data), Howard W. Odum Institute for Research in Social Science, Davis Library, 208 Raleigh St., Chapel Hill, NC 27514 USA, 1 (919) 357-3583, chris_wiesen@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

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

Clare Bocklage, Study Coordinator, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 843-2596, eclare@live.unc.edu

Nare Ghaltakhchyan, Study Coordinator, 385 S. Columbia St., Chapel Hill, NC 27599, 1 (919) 962-2078, nare@live.unc.edu

Ellie Nanney, Study Coordinator, 385 S. Columbia St., Chapel Hill, NC 27599, (704) 651-4579, ellien@ad.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

Payton Mince, Research Assistant, GO Health Clinic, 2040 Old Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3418, pmince@ad.unc.edu

Carol Culver, GO Health Clinic Manager 2040 Old Dental, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 537-3176, carol_culver@unc.edu

Kevin Moss, Statistician, 385 S. Columbia St., Chapel Hill, NC 27599, (919) 573-3200, kevin_moss@unc.edu

Chris Wiesen, PhD. Statistician (survey data), Howard W. Odum Institute for Research in Social Science, Davis Library, 208 Raleigh St., Chapel Hill, NC 27514 USA, 1 (919) 357-3583, chris_wiesen@unc.edu

PRÉCIS

Study Title

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

Objectives

The primary objective is to quantify the impact of antiseptic mouthwashes on salivary infectivity in COVID-19+ patients. The secondary objective is 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 two therapeutic, antiseptic mouth rinses to inactivate SARS-CoV-2 virus in saliva of COVID-19 positive patients aged 18-65 years old. Both mouth rinses are commercially available and will be used according to on-label instructions. Patients will be randomized to a mouth rinse 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 RT-PCR detection of viral SARS-CoV-2 RNA 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 84 COVID+ subjects per mouth rinse, with 3 mouth rinses (including a water control), requiring us to enroll 252 COVID+ patients. Patients will be

randomized to their selected mouth rinse assignment. These patients will have already had a confirmed COVID+ test within 10 days prior to enrollment. 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 the ingredients of the mouth rinses. Patients who are pregnant/ nursing/lactating, have a cognitive or developmental disability, 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 have xerostomia will be excluded from the study. There is no exclusion criteria based on gender or race.

1. STUDY OBJECTIVES

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 COVID-19+ patients will have a >95% reduction in SARS-CoV-2 viral infectivity following a 60 second oral rinse with an antiseptic mouth rinse and that this reduction will persist for at least 30 minutes.* This primary objective will focus on quantifying SARS-CoV-2 viral infectivity and RNA level using an *in vitro* infectivity assay and quantitative real time 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 COVID-19+ transmission.

1.2 Secondary Objectives

There is no secondary objective.

1.3 Tertiary Objectives

The tertiary objective is to determine the acceptability of using antiseptic mouthwashes on reducing SARS-CoV-2 cellular infectivity by patients in various settings including healthcare contexts. *We hypothesize that patients will welcome the use of commercially available antiseptic mouth rinses into clinical practice.* All patients enrolled will be surveyed on the taste, appearance, sensation, and acceptability of using the mouth rinse in different settings. Understanding acceptability of a mouth rinse 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 mouth rinses to inactivate SARS-CoV2 holds great potential for quelling the virus' spread among healthcare workers, who comprised up to 10% of COVID-19 cases early in the pandemic.[1,2] Healthcare providers like dentists, who deliver care in and near the oropharynx, suffer the highest risk of occupational exposure, if using inadequate PPE.[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 mouth rinses to inactivate SARS-CoV-2 infectability in saliva. Several commercially available mouth rinses 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] Cetylpyridinium chloride

(CPC) has promising *in vitro* data for inactivating SARS-CoV-2 infectivity.[13] Minimal *in vivo* clinical data regarding SARS-COV-2 exists to-date for most oral rinses.

Despite promising *in vitro* data, there is a dearth of *in vivo* clinical trials interrogating the efficacy of mouth rinses 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] Our data from a smaller, prior study indicate that rinsing with Ethanol or CPC-containing products reduces salivary viral load by qPCR. As a result, we focus this investigation on evaluating the efficacy of an ethanol solution and a CPC solution to reduce salivary viral load and infectivity.

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. 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

2.2 Study Rationale

One promising strategy to reduce clinical spread is use of antiviral mouth rinses to inactivate SARS-CoV-2 infectability in saliva. Several commercially available mouth rinses 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] Cetylpyridinium chloride has promising *in vitro* data for inactivating SARS-CoV-2 infectivity.[13] Minimal *in vivo* clinical data regarding SARS-COV-2 exists to-date for most oral rinses.

Despite promising *in vitro* data, there is a dearth of *in vivo* clinical trials interrogating the efficacy of mouth rinses on reducing salivary SARS-CoV-2 viral infectivity. Given the rapid transmission and widespread distribution of the COVID-19 pandemic and its new variants, 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 our similar viral load studies evaluating mouth rinse efficacy against SARS-CoV-2 and studies about other enveloped viruses, we used power calculations to estimate effect size and determine sample size as a function of length of mouth rinse time course. With simultaneous comparisons across time points (T0-T1, T1-T2, T0-T2, T2-T3, T0-T3, T1-T3), the sample size n=43 for each mouth rinse can detect the effect size in Cohen's d =0.8 at 30 minutes, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction.[12] To account for 14% of patients having no virus in their saliva and 10% of patient producing insufficient saliva, we plan to enroll 55 participants per arm.

Subjects will be required to rinse their mouths with an unlabeled/blinded mouth rinse 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 mouth rinses. The mouth rinses 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 be 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 desktop 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 mouth rinses, 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 mouth rinse proposed in this study:

mouth rinse*	***Adverse effects***
26.9% ethanol plus essential oils	• If more than used for rinsing (20ml) is accidentally swallowed, get medical help or contact a Poison Control Center right away. The exact amount used for rinsing (20ml) will be dispensed to study participants, such that they cannot swallow more than 20ml, to reduce the risk of this adverse event. • Poison control: (800) 222-1222
0.1% Cetylpyridinium Chloride	• If more than used for rinsing (20ml) is accidentally swallowed, get medical help or contact a Poison Control Center right away. The exact amount used for rinsing (20ml) will be dispensed to study participants, such that they cannot swallow more than 20ml, to reduce the risk of this adverse event. • Poison control: (800) 222-1222
Sterile water	**No contraindications**

**All mouth rinses 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 determine 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 10 days of symptom onset and/or within 10 days of initial positive test; asymptomatic SARS-CoV-2+ subjects will be enrolled within 10 days of initial positive test. Positive test results will be accepted if they are provided by a medical professional or official testing site. Results can appear either in the UNC electronic medical record or be printed from an established testing site such as UNC, a local pharmacy, a medical practice, or an established lab. At-home positive test results will be accepted when they are confirmed by study

personnel with a rapid antigen test at the time of visit. Subjects will be seen and samples collected in the isolated Clinical Research Space associated with the GoHealth Clinic (Adams School of Dentistry, UNC Chapel Hill, North Carolina, USA). SARS-CoV-2+ subjects will be enrolled within 10 days positive test. Study participants who meet all inclusion criteria without any exclusion criteria will be randomized to either an experimental or control group using a block randomization tool.

Participants will be asked to give a baseline salivary sample (5ml over 5 minutes) and then asked to rinse with an unlabeled (blinded) antiseptic mouth rinse for 60 seconds according to the table below. The rinses and control will be similar in appearance visually, such that a double-blind can be maintained. The research assistant (Wendy Lamm) will be the sole unblinded staff member who will perform randomization and communicate the subject's group assignment to blinded study staff, in advance of the visit based on the block randomization schedule. The unblinded staff member will set-up the rinses for the participants appointment minimizing the possibility of study staff being able to identify the blinded rinses. In the instance that Ms. Lamm is unavailable, Ms. Payton Mince or Ms. Carol Culver will perform randomization and set-up the rinses. Blind will be strictly maintained except in the case of medical emergency requiring reveal of an individual's rinse identity. The product group key will be provided to the analyzing statistician at the conclusion of the clinical study.

mouth rinse*	***Number of participants (sample size)***	***Group***
26.9% ethanol plus essential oils	**84**	**Experimental**
0.1% Cetylpyridinium Chloride	**84**	**Experimental**
Sterile water	**84**	**Control**

**All mouth rinses 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 mouth rinse as part of routine dental and medical visits as well as in social settings. 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 and viral infectivity 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 inquire about their comfort. If participants exhibit signs of distress, 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 Maile) 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. All samples have labels with only alphanumeric identifiers thus maintaining the blind and protecting PHI through the entire laboratory analysis process.

1. Molecular analysis: For molecular 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, official testing site, or on-site rapid antigen test administered by study personnel at the time of visit. Either became symptomatic or initially tested positive in 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 mouth rinses. (self-reported)
    - Self-reported allergy to ethanol, essential oils (Eucalyptol, Menthol, Methyl salicylate, Thymol), Listerine, Cetylpyridinium Chloride, and other components in the mouth rinses.
    - 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 mouth rinse 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 Ethanol rinses 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.
    - Patients with sizable mucosal tears, abrasions, growths or burns in the mouth
- The following table lists exclusion criteria specific to each mouth rinse proposed in the study:

mouth rinse*	***Exclusions***
26.9% ethanol plus essential oils	• Do not use in children under 12
0.1% Cetylpyridinium Chloride	• Do not use in children under 6
Sterile Water	**No contraindications**

**All mouth rinses 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 Mouth rinse 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, mouth rinses), commercial cosmetics, iodine, and any ingredient in the mouth rinses (list will be provided).

Diagnosed COVID+ status by physician, official testing site, or on-site rapid antigen test administered by study personnel at the time of visit. Either became symptomatic or initially tested positive in the prior 10 days.	Established history of severe Xerostomia (drug-induced or autoimmune dry mouth), severe periodontal disease with actively bleeding gums, significant oral abrasions/ulcers or growths, alcohol abuse, and/or recreational drug abuse. 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 mouth rinse 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

Subjects will be recruited from patients seen in the UNC Respiratory Diagnostic Clinic (RDC), UNC testing sites or UNC hospital who have tested positive for COVID+. The subjects are being approached by phone or email based off of the UNC RDC/EPIC COVID+ list. 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 hospital in-patients appearing on the EPIC COVID+ list, subjects will be approached by a study coordinator in their private hospital room. 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. If interested, consent forms will be left in the patient's room for review. Study team members will return later for final consent signatures and if consenting, the study visit can occur in their private room. 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 will be provided by the RDC clinic and UNC Epic, available via secure UNC servers and 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. Participants lacking a lab or physician confirmed COVID+ test will be administered a rapid antigen test by study personnel at the time of their visit to confirm their COVID infection. Any participants that produce a negative rapid antigen test (on-site) will be excluded. 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, 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 5 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. Intra-oral photographs of notable lesions may be taken to document oral manifestations of COVID-19. 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 salivary sample, and study participation will conclude. Participants will be provided with a gift card for their participation.

5.2 Handling of Study Interventions

Mouth rinses will be acquired from the following sources:

mouth rinse*	***Source:*** Online pharmacies and reputable marketplaces with over the counter mouth rinses
26.9% ethanol plus essential oils	Listerine's Original Mouth rinse- Amazon or CVS online store
0.1% Cetylpyridinium Chloride	Crest Pro-Health Mouth rinse- Amazon or CVS online store
Sterile water	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.*

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 mouth rinse and a short survey.

5.3.1 Allowed Interventions

There are no additional interventions, other than mouth rinsing with one of the 3 options previously described. If patients have an allergic reaction, EMS will be contacted for management and emergency medical procedures will be followed until EMS arrives. If a patient begins experiencing anaphylaxis, we will administer epinephrine via an epi-pen, as a rescue medication. No other rescue medications are indicated.

5.3.2 Required Interventions

The patient will rinse with a blinded mouth rinse 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 consumed alcohol within 12 hours of the study visit.

Patients who have used a mouth rinse within 24 hours of the study will be excluded.

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

Patients who are abusers of alcohol or recreational drugs.

5.4 Adherence Assessment

Adherence would be defined as rinsing with the mouth rinse for a full 60 seconds before expectorating, and then providing a baseline saliva sample followed by saliva samples every 15 minutes four times, until an hour post-rinse (15 min, 30min, 45min, 60min).

6. STUDY PROCEDURES

6.1 Schedule of Evaluations

Assessment	Phone Screening: (Day-14 to Day -1)	Final Screening, Baseline, Enrollment, Randomization, Intervention: Visit 1 (Day 0)
Informed Consent Form	**Provided for review**	**X**
Demographics		**X**
DXA	**X verbally asked**	**X**
Medical History		**X**
Current Medications		**X**
Rapid Antigen Test-COVID+		**X**

Urine Analysis-pregnancy		**X**
Vital Signs		**X**
Inclusion/Exclusion Criteria	**X reviewed**	**X**
Enrollment/Randomization		**X**
Treatment Administration Form		**X**
Concomitant Medications		**X**
Adverse Events		**X**

6.2 Description of Evaluations

Subjects are required to be onsite for a 70-90 minute, single visit appointment. After consenting and confirmation of meeting inclusion/exclusion criteria, 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 questions as part of a short survey about the rinsing experience and their willingness to rinse in different situations.

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 a study coordinator. 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 clinical isolation unit associated with 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. For hospital in-patients appearing on the EPIC COVID+ list, subjects will be approached by a study coordinator in their private room. 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. If interested, consent forms will be left in the patient's room for review. Study team members will return later for final consent signatures and if consenting, the study visit can occur in their hospital room. All patients are adults and have normal cognitive capacity, and therefore will be able to consent for themselves.

Screening

PreScreening is typically done through a phone call but may be supplemented with email correspondence. The screening process is finalized at Visit 1.

- During the prescreening conversation the patient will answer screening questions regarding inclusion and exclusion criteria and will be provided with the informed consent to read and review prior to consenting and enrollment process.

At the visit, participants lacking a lab or physician confirmed COVID+ test will be administered a rapid antigen test by study personnel at the time of their visit to confirm their COVID infection. Any participants that produce a negative rapid antigen test (on-site) will be excluded. Next, 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. If a participant meets enrollment criteria and consents to participate, their answers will be entered into the CDART research database managed by UNC.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

Outpatient subjects will be recruited from patients seen in the UNC Respiratory Diagnostic Clinic (RDC), UNC testing sites or UNC hospital who have tested positive for COVID-19. 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 in the associated clinical research isolation area. Consent documentation will be signed in person and in private in the Clinical Research Core. A list of COVID+ subjects will be provided by the RDC clinic and EPIC, accessible 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

Study personnel confirm enrollment qualifying criteria and perform a COVID rapid antigen test and pregnancy test on applicable individuals. Otherwise, there are no baseline assessments required as this study only requires a 70-90 minute single visit appointment.

Randomization

Our statistician will create a block randomization tool which will be provided to the unblinded study staff (Wendy Lamm) to perform the randomization. In the instance that Ms. Lamm is unavailable, Ms. Payton Mince or Ms. Carol Culver will perform randomization and set-up the rinses. The participant's group assignment will be communicated to the blinded clinical staff. All patients will be consented and then complete 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 mouth rinse 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 mouth rinse. 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 mouth rinse in an unlabeled storage syringe to provide to the patient for the rinse. Our statistical consultant, Kevin Moss, will set up a 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. Participants with at-home rapid antigen COVID+ tests will have their COVID infection confirmed with a rapid antigen test administered by study personnel at the time of visit. Results to the rapid antigen test will be available to participants and study personnel 15 minute after being administered. Therefore, no follow-up reporting is needed.

6.2.5 Completion/Final Evaluation

This study requires only one 70-90 minute, single visit appointment. After consenting and randomization, 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 severe 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. Severe xerostomia is an exclusion criteria during screening, yet we anticipate that some individuals may not know that they have severe xerostomia until they are participating in the study visit and then need to discontinue due to reduced salivary flow, as described.

7. SAFETY ASSESSMENTS

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

mouth rinse*	***Adverse effects***
26.9% ethanol plus essential oils	• If more than used for rinsing (20ml) is accidentally swallowed, get medical help or contact a Poison Control Center right away. The exact amount used for rinsing (20ml) will be dispensed to study participants, such that they cannot swallow more than 20ml, to reduce the risk of this adverse event. • Poison control: (800) 222-1222

0.1% Cetylpyridinium Chloride	• If more than used for rinsing (20ml) is accidentally swallowed, get medical help or contact a Poison Control Center right away. The exact amount used for rinsing (20ml) will be dispensed to study participants, such that they cannot swallow more than 20ml, to reduce the risk of this adverse event. • Poison control: (800) 222-1222
Sterile Water	**No contraindications**

**All mouth rinses 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 mouth rinses (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

Mouth rinse 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 mouth rinses (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 mouth rinses (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 mouth rinses on salivary infectivity and viral load. A crossover study was not pursued because the repeated use of different mouth rinses 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- mouth rinse.

Statistical Hypothesis

The central hypothesis of this study is that a 60 second antiseptic mouth rinse will effectively reduce viral infectivity (>50%) of SARS-CoV-2 for a minimum of 30 minutes duration.

The secondary hypothesis is that a 60 second mouth rinse will be well tolerated in a clinical care setting by a majority of patients and providers.

Sample Size Considerations

Based on our similar viral load studies evaluating mouth rinse efficacy against SARS-COV-2 and other enveloped viruses, we used power calculations to estimate effect size and determine sample size as a function of length of mouth rinse time course.[12] With simultaneous comparisons, the sample size n=43 for each mouth rinse can detect the effect size in Cohen's d =0.8, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction.[12] To account for 14% of patients having no virus in their saliva and 10% of patients producing insufficient saliva, we plan to enroll 55 participants per arm.

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

Primary Outcomes

The analyses will compare viral RNA level (qRT-PCR) and viral infectivity levels between mouth rinse types and across the time course

Data generated from the qRT-PCR 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.

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 mouth rinse ('remaining virus'). Therefore, the log10 titre and its (double) standard deviation (SD) were calculated as well as the variance of the RF.

Secondary Outcomes

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 mouth rinses 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 mouth rinse time course. The sample size is justified by a paired t-test on outcomes between time points. With simultaneous comparisons, the sample size n=43 for each mouth rinse can detect the effect size in Cohen's d =0.8 at 30 minutes, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction.[12] To account for 14% of patients having no virus in their saliva and 10% of patients producing insufficient saliva, we plan to enroll 55 participants per arm.

The statistical consultant on this project will create a block randomization tool for the study staff; the unblinded staff member will randomize a participant once enrollment compliance has been confirmed and consent has been given. The participant's group assignment will then be communicated to the blinded study staff. IRB approved study personnel will ask participants screening questions to confirm eligibility for enrollment. After consent and screening, the randomization tool will be used by study staff to assign the subject to a study group.

The researcher responsible for collecting the salivary samples will be blinded to the mouthwash solution, as they will be given a pre-aliquoted mouth rinse in an unlabeled 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 based on emergency medical necessity such as if a patient suffers an allergic or adverse reaction to one of the mouth rinses. Though highly unlikely as all mouth rinses are sold commercially at Walgreens and CVS, adverse or allergic reactions are possible. Using the numerical code associated with the participant and mouth rinse syringe, the study personnel responding to the emergency can reference the block randomization schedule to determine the mouth rinse type, to inform the emergency medical workers, poison control or the emergency room what type of mouth rinse 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 in the prior 10 days, and/or have symptom development in the prior 10 days (for symptomatic participants). Inclusion and exclusion criteria (described elsewhere) will be met. We are not treating patients for their COVID-19 infection.

9.4 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 or deviation will be documented in our CDART database system. All significant adverse events (SAEs) will be reported appropriately to the IRB.

The qRT-PCR and viral infectivity assays for our primary outcome will be documented in excel spreadsheets generated by the qRT-PCR and viral infectivity assay quantification devices, available weeks to months after sample collection when the experiments are conducted. Samples will be run in large batches. The survey results will also yield excel spreadsheets of data, available shortly after data collection.

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 mouth rinse is more effective.

9.4.1 Primary Outcome

The primary objective of this study is to determine the efficacy of antiseptic mouthwashes on reducing SARS-CoV-2 cellular infectivity in COVID+ patient saliva. *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 mouth rinse and that this reduction will persist for at least 30 minutes.* This primary objective will focus on quantifying SARS-CoV-2 viral infectivity and RNA level using an *in vitro* infectivity assay and quantitative real time 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 COVID-19+ transmission.

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

9.4.2 Secondary Outcomes

There is no secondary objective.

9.4.3 Tertiary Outcomes

The tertiary objective is to determine the acceptability of using antiseptic mouthwashes on reducing SARS-CoV-2 cellular infectivity by patients in settings including healthcare contexts. *We hypothesize that patients will welcome the use of commercially available antiseptic mouth rinses into clinical practice.* All patients will be surveyed on the taste, appearance, sensation, and acceptability of using the mouth rinse in healthcare settings. Understanding acceptability of a mouth rinse protocol is critical to implementation and therefore the ability to reduce COVID-19 transmission risk in clinical settings.

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.

9.5 Data Analyses All data analyzed will be deidentified.

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 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 mouth rinse ('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 our similar viral load studies evaluating mouth rinse efficacy against SARS-COV-2 and other enveloped viruses, we used power calculations to estimate effect size and determine sample size as a function of length of mouth rinse time course.[12] The sample size is justified by a paired t-test on outcomes between time points. With simultaneous comparisons, the sample size n=43 for each mouth rinse can detect the effect size in Cohen's d =0.8, which is between medium and large, under 80% power and 0.016 type-I error rate by Bonferroni correction.[12] To account for 14% of patients having no virus in their saliva, we plan to enroll 55 participants per arm. Analyses will compare viral RNA level (qRT-PCR) and 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 for participants ID and name will be set-up and maintained by the study coordinators and will be securely stored on UNC servers. This linkage file will be kept confidential. and not shared with anyone outside of the study coordinators. Clinical 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 by the study coordinators 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 and will never be shared beyond the study coordinators.

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 mouth rinse experience (taste, color, sensation) and willingness to use a mouth rinse in various 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

The Clinical Quality Management Plan (CQMP) establishes the quality management guidelines this study, also referred to as *MOR2*. The purpose of the CQMP is to identify and document the ongoing processes and activities that will be used to monitor and facilitate quality protocol execution following study initiation. Quality measures related to study development and start-up are not included in this plan. An individual who has no other role on the study team will be responsible for execution of this plan.

QM Activity Schedule and Tools

Quality Management (QM) activities will be conducted for each enrolled study subject. Additional QM activities and reviews will be conducted on an as-needed basis in response to staff or process changes.

The site Quality Assurance Coordinator (QAC) will use the Quality Management Subject Data Review Tool to review completion and accuracy of the source documents, eCRFs and Delegation of Responsibility (DOR).

The following outlines the QM process:

- The QAC will maintain the CQMP and QM tools/logs in electronic folder.
- The QAC will review 100% of the executed consents using the QM Subject Data Review Tool. The QAC will verify form completion and accuracy and verify that the most current IRB-approved study consent documents are being used.
- The QAC will review 100% of CDART eCRF data. This includes all eCRF records containing CRF and/or source documentation. The QAC will verify completion of study data entry, subject dispositions and if any unanticipated problems, adverse events,

promptly reportable events and/or protocol deviation have been documented and reported to local IRB as required by the UNC Office of Research Ethics.

- The QAC will review completion of the Delegation of Responsibilities (DOR) prior to study start and upon new study personnel.
- At an interval of 10 enrolled subjects, the QAC will randomly select one subject and review all source documentation and any other corroboration documentation for eCRF data entry, all eCRF's and pregnancy log (if applicable).
- This CQMP will be a living document and will be reviewed for applicability and accuracy and updated as necessary by the QAC. Upon each review, the QAC will provide the study team feedback and report items covered by the review, identification of problem areas and trends, suggest corrective action plan(s) (if applicable), and if/when there is a need for revision to CQMP. Additional QM needs identified will be communicated to the study team where needed and revisions made upon request of the Investigator.

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

Findings of the site Quality Assurance Coordinator (QAC) will be presented to the investigators and study coordinators (as appropriate for the findings) at selected weekly meetings for discussions and remediation actions as appropriate. There is only one site for this study. The use of on-label, widely used mouth rinses (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. 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 RT-PCR and viral infectivity 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.

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

Findings of the site Quality Assurance Coordinator (QAC) will be presented to the investigators and study coordinators (as appropriate for the findings) at selected weekly meetings for discussions and remediation actions as appropriate. The QAC and the lead study coordinator will make recommendations to the PI if needed outside of the regularly weekly study team meetings.

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

Patients who have tested positive for COVID-19 in the UNC Respiratory Diagnostic Clinic (RDC), UNC testing sites or UNC hospital will be contacted to participate in this study by research personnel. 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 clinical isolation area associated with 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 isolation area adjacent to the Go Health Clinical Research Unit at Adams School of Dentistry.

For hospital in-patients appearing on the EPIC COVID+ list, subjects will be approached by a study coordinator in their private room. 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. If interested, consent forms will be left in the patient's room for review. Study team members will return later for final consent signatures and if consenting, the study visit can occur in the patient's room.

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 offered 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 tool and the unblinded staff member will randomize participants. All patients will be consented and then answer 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, by the unblinded staff member. 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 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

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. 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

15. SUPPLEMENTS/APPENDICES

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