*Investigating the effectiveness of oral antiseptic rinses on SARS-CoV-2 in-vivo - a randomised controlled trial.*

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**General Information:**

This document describes the study, defines the biological analysis, procedures and provides information about procedures for entering participants into the trial.

**Sponsor:**

York Teaching Hospitals NHS Foundation Trust.

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Funder: Johnson & Johnson £100,000

Authorisation

Mr Peter Nixon and Mr David Seymour are authorised to sign final protocol and protocol amendments.

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**Research information:**

* Title of research: *Investigating the effectiveness of oral antiseptic rinses on SARS-CoV-2 in-vivo - a randomised controlled trial.*
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* Proposed duration of study: 12 months

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**1. Summary and Study objectives**

Introduction / statement of the current problems requiring urgent research.

Dentistry is currently severely limited during the covid-19 pandemic.  Concern exists with regard to the potential risk from dental Aerosol Generating Procedures (AGP’s) spreading virus from an asymptomatic covid positive patient.1 SARS-CoV-2 has been shown to be present in saliva in significant quantities.2 The concern from this potential mode of transmission is currently mitigated for staff, through the use of appropriate Personal Protective Equipment (PPE3.).3 The concern however, is for the next patient to enter the surgery, as there could theoretically be viable virus circulating in the air from the previous patients AGP. As a consequence of this, patients are having great difficulty accessing appointments and care, which is leading to an ever-increasing backlog of cases awaiting care.

Unless a solution is found quickly for AGP’s in dentistry, to decrease or eliminate this ‘fallow time’, then oral health in the UK will undoubtedly suffer enormously. In addition, dental practices will soon become financially unviable or the cost of treatment will increase to several times pre-covid levels.

Several recent suggestions have been made for physically limiting the aerosol itself, such as multiple suction devices and physical barriers. Whilst these may have some merit in mitigating the aerosol spread, these methods have drawbacks, namely that some aerosol is always likely to escape, and barriers limit the clinicians access to the patient and field of view. The novel concept for this research is to approach the problem in by rendering any virus in the aerosol inactive. This approach could be adopted in combination with physical methods already employed in dentistry, such as high-volume suction to also limit the physical spread of the aerosol.

Study Objectives

To test methods of mitigating the risk of SARS-CoV-2 in the dental setting by rendering it non-infective prior to procedures. The methods for investigation will be the use of a pre-procedural mouthwash

1. **Literature review/Background**

Background to this approach

A number of researchers have already suggested the idea of using mouthwashes to inactivate the SARS-CoV-2 virus.4 This suggestion has been made not only for dental procedures but speculated as part of a broader measure to help control the spread of the pandemic in the population. Pre-procedural mouthwashes have been in common use in dentistry for many years and have proven efficacy reducing bacteria in aerosols, although not viruses.5,6,7 A number of mouthwashes have the potential to work against SARS-CoV-2, indeed one mouthwash has already been shown to be effective against the virus in vitro with a contract time of just 15 seconds.8 We have completed an investigation which identified four commercially available mouthwashes that inactivated SARS-CoV-2 in-vitro which is awaiting publication. Mouthwashes vary not only in their effectiveness against different microbes but also in their substantivity9 (the length of time they are effective for.)10

The mouthwashes are all commercially available and as such would have the appropriate certifications for that use.

It is important to know in this research if the virus in still infective, rather than just knowing its presence. Therefore, live culture of samples is necessary and not just sampling for genetic material.

It is currently not known how much virus is required to infect an individual.

1. **Research Questions:**

Can SARS-CoV-2 be rendered inactive in saliva by using a mouthwash? If so, how long does the effect last?

1. **Objectives of the three investigations within the study:**

Phase 1. In-vitro testing of mouthwashes for efficacy against SARS-CoV-2. **Phase 1 has been completed.** This initial aspect tested 5 types of mouthwash against SARS-CoV-2 for possible efficacy. The mouthwashes being tested are:

* Salvestan (hypochlorous acid)
* Orawise (hypochlorous acid)
* Listerine Advanced Defence Sensitive (Dipotassium Oxalate)
* Chlorhexidine
* Peroxyl (hydrogen peroxide)
* Povident (poviodine)

Results show Orawise, Listerine and Povident were all effective at inactivating SARS-CoV-2. The paper has recently been submitted to the Journal of General Virology for peer review but is available via pre-print server as per PHE protocol at <https://www.biorxiv.org/content/10.1101/2020.12.02.408047v1.full.pdf>

Phase 2. Randomised controlled clinical trial of mouthwashes in-vivo. Participants with a positive COVID-19 swab will be randomised to mouthwash or control group (bottled water.) Saliva samples will be collected prior to mouth washing, then at 1, 10, 30 and 60 minutes after mouth washing. These saliva samples will then be cultured for viable virus with PHE. The mouthwashes we propose to investigate are as follows:

- OraWize+ (hypochlorous acid).

-

- Listerine Total Care (Eucalyptol, thymol, menthol, sodium fluoride, zinc chloride).

- Listerine Cool Mint (Alcohol, eucalyptol, thymol, menthol).

We have sought advice from the MHRA and received confirmation that use of the above mouthwashes in this study would not require a Clinical Trial Authorisation or a notification for a device study.

**The objective of this phase is to assess the effectiveness of the mouthwashes in vivo, along with the duration of any effect.**

1. **Clinical Study flow chart:**

***Sampling***

*Inclusion –* SARS-CoV2-2 positive staff and/ or relative and/ or patient with mild to moderate symptoms, test in last 72 hours.

*Exclusion –* Staff member/ patient/ relative too unwell to participate, allergies, pregnant.

*Sample size –* 23 participants per group.

Randomisation

***Control group (water)***

Saliva sampling pre-mouth wash.

Saliva sampling 1, 10, 30 and 60 minutes following mouthwash use.

23 participants per group.

Anonymised study ID.

Samples frozen

***Test Groups***

Saliva sampling pre-mouth wash.

Saliva sampling 1, 10, 30 and 60 minutes following mouthwash use.

Participants split into one of three mouth wash test groups (23 participants per group). Anonymised study ID

***Testing***

Sample testing for SARS-CoV-2 at Public Health England.

Results linked to anonymised study ID.

***Data analysis***

Data analysis at York Teaching Hospital NHS Foundation Trust, in collaboration with Professor Hewitt (Statistician).

1. **Clinical study design:**
	1. **Type:**

The clinical study is a randomized controlled trial involving staff , members of the public and patients who have a combined nose and throat positive covid-19 swab within the last 72 hours.

**6.2. Disease/participants studied:**

Individuals will only be recruited if they are well enough, i.e. only showing mild to moderate symptoms/signs of Covid-19 infection.

**6.2.1.** **Inclusion criteria:** For inclusion in the study participants must meet the following criteria:

1) have had a positive covid-19 swab test result in the last 72 hours

**6.2.2. Exclusion criteria:**

1) Refusal of consent

2) Participants known to be pregnant

3) Have known allergies to any of the mouthwashes used in the trial

**6.3. Participant recruitment and consent**:

Participant recruitment

Participants will be recruited in the following way:

1. Staff members that receive a positive covid-19 swab result will be asked at the time of receiving their result if they mind being contacted about a research study. Providing the participant agrees to that, the research nurse will be passed the contact details and will invite the participant to take-part in the trial.
2. Members of the general public who attend for Covid PCR testing either at a local testing centre or University campus, may be provided with a brief leaflet about the study asking if they would consider taking part if they receive a positive covid test result and providing contact details for the research team.
3. Members of the research team may use local media or social media to raise awareness of the study.
4. In the event of local outbreaks (for example within an office or company), the research team may request that information about the study be distributed to those who may be affected.
5. Patients who test positive in hospital may, following discussion with their clinical team, be approached to take part.

Participant consent:

1. Staff members will be approached by Occupational Health following a positive swab result if they meet the inclusion criteria. They will be given the Patient Information Sheet (PIS) and have the project explained to them. If they agree to participate, they will be asked to complete the consent form. The participant can withdraw consent at any time. Withdrawing consent will not affect their medical care. As the trial needs to be conducted within 72 hours of the positive swab result, the participant will be able to consent to take part as soon as they have confirmed they have read and understood the PIS.
2. Any individual who makes contact with the research team following receipt a positive test result will have the study explained to them and will receive the PIS via email if possible (for speed). If they agree to participate, they will be asked to complete the consent form. The participant can withdraw consent at any time. Withdrawing consent will not affect their medical care. As the trial needs to be conducted within 72 hours of the positive swab result, the participant will be able to consent to take part as soon as they have confirmed they have read and understood the PIS.
3. Patients who test positive while in hospital will be notified to the research team by the hospital laboratory undertaking the test. Once the patient has been informed of their positive result, and following discussion with the clinical team, the patient will be given a PIS and have the project explained to them. If they agree to participate, they will be asked to complete the consent form. The patient can withdraw consent at any time. A decision not to take part or to withdraw consent will not affect their medical care and this will be made very clear to patients when they are approached initially. As the trial needs to be conducted within 72 hours of the positive swab result, patients will have up to 48 hours to consider taking part.
	1. **Recruitment Period:**

The period to recruit the required number of participants is difficult to anticipate as the number of eligible participants depends on the spread of the pandemic. The current rate of infection has dipped, due to the nationwide lockdown but the rate of infection is highly likely to rise again in the coming months.

**6.5.** **Link-anonymisation:**

On recruitment to the study each participant will be assigned a unique Trial ID. The member of the research team taking consent will assign the next sequential number and then document in the Trial Master File the Trial ID and corresponding NHS number of the participant. This will allow reconciliation of trial results, if required at the end of the trial.

**6.6. Saliva sample collection, storage and transport:**

Participants will be randomised by random number generator or sealed envelope randomisation (<https://www.sealedenvelope.com/>) to mouthwash or control group (bottled water).

York hospital has a ‘drive-through’ service for testing staff for SARS-CoV-2. This will be used as the main preferred location for sample collection. Participants will be asked to drive (or be driven by a household contact) to the hospital and will remain in the car throughout the process. The first saliva sample will be obtained before mouth washing. Five millilitres will be collected by participant spitting in to a 60ml sterile universal container. Following that the participant will perform mouth washing. 10ml of mouthwash will be vigorously swilled around the mouth, timed for one-minute duration. The mouth wash will then be spat out into a cardboard bowl and disposed of as infectious waste. There will be no gargling, as this might generate aerosols. Further saliva samples will be collected at 1, 10, 30 and 60 minutes after mouth washing.

The collection containers will be wiped down with a disinfectant wipe (Clinell Universal wipe) for 30 seconds, allowed to dry and then labelled. The samples will be linked-anonymised by writing the participants unique Trial ID on the tube by the research staff. Samples containers will then be sealed within two clear plastic bags for transport to the laboratory. The outside bag will be attached to a specimen request form that will be labelled with the participant’s study number and the timing of the sample.

At all times when within 2 metres of participants, or handling samples, research staff will wear level 2 personal protective equipment (i.e., eye protection, fluid-repellent surgical face mask, apron (or lab coat) and gloves) in accordance with local and national infection prevention guidance for COVID-19.12 Samples will be transported to the lab in a zip sealed sample pouch.

To minimise viral decay saliva samples will be taken by the research nurse to the laboratory and frozen at -80oC within 30 min of collection. The exact times of sample collection and freezing will be documented. The samples will be batched for transport and sent on dry ice in Category B Biological Substances in UN3373 compliant packaging to the PHE laboratory.

In the event of a local outbreak the research team may move to the site of the outbreak and ask participants to undertake the sampling in their own accommodation. Samples would be flash frozen in liquid nitrogen and maintained at that temperature before being transferred to -80 storage at the earliest opportunity.Patients will be approached in their hospital room by staff wearing PPE in line with Trust infection control requirements.

1. **Virology Work:**

**7.1. Saliva sample preparation and testing:**

Levels of SARS-CoV-2 in saliva samples will be assessed quantitatively by determining the 50% tissue culture infectious dose (TCID50), and will report the infectious virus titre in TCID50/ml. In addition, samples will be inoculated onto SARS-CoV-2 susceptible cells *in vitro* and passaged to amplify low levels of residual virus that may be below the level of detection of the TCID50 assay; this test gives a qualitative result that reports either the presence or absence of infectious virus.

**7.2. Virology results analysis:**

Saliva samples taken prior to mouth washing will be used to indicate the levels infectious virus present in the saliva of that individual (baseline sample). The difference in virus titre and at various times after mouth washing will be evaluated by subtracting the logarithmic virus titre at each time point from the logarithmic virus titre of the baseline sample. For individuals in which infectious virus is present but not quantifiable (i.e. positive by passage but not by TCID50), results for each mouth washing time point will be reported as either infectious virus recovered or not recovered.

**7.3. Statistical Analysis:**

Power calculation of 23 participants per group would give 90% power to show a difference of 1 standard deviation in the viral load and a 35% difference (0% to 35%) clearance rate.

1. **Overall study analysis:**

The aim of the clinical study is to determine whether a pre-procedural mouthwash can significantly lower the quantity of viable virus in saliva. The measurement of / change in viable virus titre is the main outcome variable. This testing regime of taking samples over a period of 1 hour, will not only test for effectiveness in-vivo but will also provide evidence of substantivity for up to an hour.

1. **Project Milestones:**

December 2020 - Complete regularity authority approval, and funding application

May 2021 - Start participant recruitment

October 2021 - Complete participant recruitment

January 2022 - Complete data analysis and write-up for publication

1. **The research Team**

The chief investigators will be Mr David Seymour, with Mr Peter Nixon as Co-Investigator, they will be responsible for organising the trial, compiling results and writing-up. Dr Marian Killip is responsible for virology at PHE. Dr Damien Mawer will be responsible for overseeing the storage and transport of samples from York Hospital. Research nurse time has been identified to conduct the study. Trial management will be provided by York Teaching Hospitals and trial design, methodological input and statistical support is provided by Prof Torgerson, Dr Adamson and Miss Ada Keding of the University of York.

Mr David Seymour and Mr Peter Nixon have significant experience in clinical trials including NIHR randomised controlled clinical trials. Dr Marian Killip is scientific lead in high containment microbiology and has expertise in the laboratory aspects of the virology. Damien Mawer is consultant microbiologist, experienced in clinical trials, infection control and handling of microbiological samples. Professor Torgerson, Dr Adamson and Miss Keding have extensive experience of undertaking and advising on high quality and scientifically rigorous randomised controlled trials.

1. **The research environment**

The clinical research environment will be the York Teaching Hospital swabbing centre. Samples will be stored and transported from the microbiology lab at the Hospital to PHE in London.

The biological research environment where saliva samples will be processed and cultured will be the High containment Unit, National Infection Service,Public Health England*, 6*1 Colindale Avenue*,* Colindale*,* London.

1. **Ethical considerations:**

Full medical confidentiality will be maintained according to UK law and the study will be conducted according to ICH GCP guidelines. HRA approval has been obtained via an IRAS application. The biological side of the study will be carried under BEC approval.

The trial information will be held by the Research Nurse team in a locked filing cabinet during the study period. After that, it will be archived and retained for a period of 5 years. Following this period, it will be destroyed.

The trial will be monitored according to York Teaching Hospital’s Research and Development Unit guidelines.

The Investigators do not intend to inform the General Practitioner of the participant’s involvement in the study as the care the participant will be receiving will not be significantly different to that they would have had if they were not in the trial.

1. **Data Handling and record keeping:**

Participants will be allocated a unique Trial ID number upon recruitment to the study and all personal identifiers will be removed at the earliest opportunity. No identifiable information will be leaving the Trust premises at any stage. Non-identifiable clinical data together with the Trial Master File will be held on a trust password protected computer or in a locked filing cabinet. During the pilot study, biological data collected from the linked-anonymised samples will be held on a YTH password protected computer, located in a password protected folder, on a dedicated research drive with restricted access to the research investigators.

The Chief Investigator will allow trial-related monitoring and audits, providing direct access to source data/documents. Full confidentiality will be maintained in accordance with UK law.

**14. Financial details:**

To be confirmed

 **Clinical work related:**

|  |  |  |
| --- | --- | --- |
| **York Teaching Hospital**  | Mr Peter NixonMr David SeymourDr Damian Mawer | £21000(2 x PA’s/ year) |
| **University of York Clinical Trials Unit** | Professor David Torgerson Dr Joy AdamsonProfessor Martin BlandDr Victoria Allgar | 14834 |
| **University of York** |  |
| **Research Nurse** | Band 6 (233 hours) | £6412 (330 hours) |
| **NHS Pharmacy technician** | 2 hours | £1613 (75 hours) |
| **NHS laboratory technician** | 10 hours | £2,295 (150 hours) |
| **Study Manager** | Band 6 (0.2 WTE 6 months) | £20,915 (1.0 WTE) |
| **Band 4** | Band 4 (0.2 WTE 6 months) | £3012 (0.2 WTE) |
| **Consumables** |  |  |
| **Saliva Collection Tubes** | 10ml x 500 | £226 |
|  |  |  |
|  |  |  |
| **Randomisation** | Sealed envelope | £95 |
| **Freezer storage** |  | £49 |
| **Sample shipment**  | Category B Biological | £396 |
| **Public Health England** | Virology analysis | £26,213 |
| **Sponsorship** |  | £1000 |
| **QA** |  | £2606 |
|  |  |  |
| **TOTAL** |  | **£100,666** |
|  |  |  |

**15. Outcomes and dissemination:**

The aim of the clinical study is to determine whether a pre-procedural mouthwash can significantly lower the quantity of viable virus in saliva. The measurement of / change in viable virus titre is the main outcome variable.

The intention is for publication in a scientific/clinical journal of best possible impact, of the informative results. Such publication may take the form of a series of articles, to present each stage of the trial as soon as results are available.

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