Study Protocol

Measurement of mouthwash anti-viral activity against COVID-19 (MOMA)

LAY SUMMARY

Short summary: We are proposing a clinical trial on coronavirus (COVID-19) patients to see if a mouthwash can reduce the amount of virus contained within saliva. The mouthwash could be used to reduce the risk of spreading the virus to healthcare workers during a procedure involving the mouth.

Background: Coronavirus (COVID-19) is a virus that can affect the lungs and lead to breathing difficulties. Currently there is no treatment or vaccine against the virus. Salivary spit from the mouth and throat of coronavirus patients contains a lot of viruses in early infection. The spread of virus by contaminated saliva is a major risk for healthcare workers caring for these patients.

Coronaviruses are surrounded by a "fatty" coating, which can be damaged by agents like hand gels and soaps, which is why regular hand washing is recommended to kill the virus and prevent the spread of the disease. These disinfectants cannot be swallowed, however, studies have shown that several mouthwashes are able to kill viruses similar to coronavirus in the mouth and throat. Laboratory tests have indicated that certain components in mouthwashes are able to affect the coronavirus "coat". We need to know whether this will work in patients.

What does the study involve? Three mouthwashes and a salt-water control will be included in this study and randomly allocated to each patient. Patients will be asked to "spit" a sample of saliva into a pot at the start of the trial. The clinician will then give patients one of the three mouthwashes (or salt water) to rinse in their mouth for 30 seconds. Patients will then spit out a sample of their saliva straight after rinsing, after 15 minutes, 30 minutes and after 60 minutes. Samples will be taken to the lab to test how much virus it contains. Personal details will not be used and the saliva samples will be disposed of and destroyed after testing according to HTA regulations.

Who can participate? Adults (≥18 years old) tested positive for COVID-19 in the last 14-days, who are able to give written informed consent.

What are the benefits and risks of this study? This study will not benefit patients directly or treat their coronavirus infection. This study is carried out to see if we can reduce the risk of healthcare staff catching the disease. In one of the mouthwashes, there is a small risk of sensitivity to iodine (<1:1,000 individuals), which would give a burning sensation in the mouth.

Where is the study run from? This study will be carried out at the University Hospital of Wales (Cardiff and Vale University health Board), Bristol Royal Infirmary (University Hospitals Bristol and Weston NHS Foundation Trust), both Wrexham Maelor and Glan Clwyd Hospitals at Betsi Cadwaladr University Health Board and the Royal Glamorgan Hospital (Cwm Taf Morgannwg University Health Board).

BACKGROUND TO THE PROPOSED PROJECT

The viral envelope: Coronaviruses, such as SARS-CoV-2, are surrounded by a phospholipid envelope, derived from the host-cell membrane, which can be readily disrupted by agents that dissolve lipids e.g. alcohols, detergents. Regular hand-washing and the use of alcohol solutions (60-70% v/v) have been recommended to kill the virus. We recently assembled an International group of experts (virologists, immunologists, lipid biologists) to undertake a literature review (10.1093/function/zqaa002) in which we reported that widely-available dental mouthwash components: cetylpyridinium chloride, and povidone-iodine have the potential ability to disrupt the SARS-CoV-2 lipid envelope. Based on the concentrations of these agents used, the study concludes that several deserve clinical evaluation. It is clear that systematic targeting of SARS-CoV-2 in the oral cavity is under-researched.

Proof of concept against COVID-19 *in vivo*: It is evident that whilst numerous antiseptic (and potentially anti-viral) compounds are components of mouthwashes, the existing data on activity (against a range of viruses including MERS, SARS and Herpes) is largely *in vitro.* It

is now clearly important to determine whether the virucidal activity described *in vitro* is observed in the oral cavity of COVID-19 patients *in vivo*. An **urgent need** now exists to determine whether mouthwashes could reduce the viral load of COVID-19 in the oral cavity as this has, perhaps surprisingly, not been investigated. Here, we describe a clinical trial of mouthwashes containing anti-viral agents at concentrations proven effective *in vitro*, in patients with coronavirus (COVID-19) infection who are in-patients at the University Hospital of Wales (Cardiff and Vale University health Board), Bristol Royal Infirmary (University Hospitals Bristol and Weston NHS Foundation Trust), both Wrexham Maelor and Glan Clwyd Hospitals at the Betsi Cadwaladr University Health Board and the Royal Glamorgan Hospital (Cwm Taf Morgannwg University Health Board).

HYPOTHESIS

The single administration of mouthwashes may effectively reduce the viral load in the oral cavity for up to 1 hour.

METHODOLOGIES

Trial Design: We propose a four-arm randomised controlled trial comparing the effectiveness of anti-microbial mouthwashes to reduce salivary viral load in patients with COVID-19, and test the substantivity of the effects

Procedures: The relevant agreement in principle from the Research & Development offices and clinical service across all four Health Boards has been obtained. Professor David Thomas and Professor Arvind Arya will lead the clinical trial at the University Hospital of Wales (Cardiff and Vale University Health Board) and Betsi Cadwaladr University Health Board respectively. Professor Nicola West, who runs the dental clinical trials unit, will recruit patients from the Bristol Royal Infirmary at the University Hospitals Bristol and Weston NHS Foundation Trust. Dr Ceri-Ann Lynch will lead the trial at the Royal Glamorgan Hospital (Cwm Taf Morgannwg University Health Board). The study will only involve in-patients with confirmed COVID-19 infection and approached on the designated COVID wards at Wrexham Maelor Hospital (currently Boney and the Prince of Wales Wards), Glan Clwyd Hospital (currently wards 5,7 and 13), University Hospital of Wales, Bristol Royal Infirmary (currently

wards 1 and 4) and the Royal Glamorgan Hospital. The PPE for the research clinician will include: visor, appropriate mask as indicated by current guidelines, apron and gloves in all settings and will adhere to National Guidelines (*vide infra*).

Consent process: Ethical approval for the study will be sought and all procedures will adhere to the *Declaration of Helsinki*. The potential participant will be approached by a member of the research team and the research discussed. Eligible participants will be given a *Participant Information Sheet* (see attached) and afforded the opportunity to ask questions about the study. Infection control precautions as appropriate will be taken at each stage. If the participant is happy to continue, informed consent will be received in accordance with infection control measures:

- If permissible, according to local infection control measures, the participant will sign the informed consent form themselves after washing/decontaminating their hands. The pen will remain with the participant.
- Alternatively, witnessed consent will be used by an independent member of the ward staff.

Once informed consent has been received, the member of the research team will perform an eligibility check to ensure the participant meets all of the inclusion and none of the exclusion criteria.

Randomisation and blinding: Participants will be assigned to one of the four arms; Dentyl® Mouthwash (Cetylperidium chloride 0.05% [v/v]); povidone-iodine (PVP-I 0.5% [v/v]); Ultradex® (Cetylperidium chloride 0.1% [v/v]) or Normasol® (sterile saline 0.9% [v/v]) using a balanced randomisation scheme designed by Dr Damian Farnell (Senior Lecturer in Applied Mathematics in Dentistry, Cardiff University School of Dentistry). The electronic randomisation programme will be installed on a PC at each study site. Since the mouthwashes used are different colours, it will not be possible for the PIs responsible for administering the intervention at NHS sites to be blinded. However, staff responsible for analysing the results will remain blinded to the allocations. Participants will not be informed of which allocation they have received.

Test agents and procedures: The three test (active) agents will all be used according to their current marketing approval. Baseline saliva will be collected before the participant rinses their mouth with 10 mL of one of the three active mouthwashes or saline mouthwash, depending on allocation. Saliva will again be collected after 1, 15, 30 and 60 minutes into sterile 30 mL Universal containers.

The containers will be sealed by the patients and disinfected using 70% ethanol, each container will be wrapped in absorbent material, sealed in a zip lock bag, each bag being placed in a second bag. "Double-bagged" collection tubes will be placed in a sealable container for transport to the laboratory (Royal Glamorgan Hospital, Wrexham Maelor Hospital/ Glan Clwyd Hospital, Bristol Royal Infirmary in University of Bristol laboratory space where they will be stored at -80^{o}C, containment level2 prior to transport. Cardiff samples will be transferred to the approved cat3 facility at Cardiff University and stored at -80^{o}C. In the Royal Glamorgan Hospital, Betsi Cadwaladr University Health Board and Bristol Royal Infirmary (in University of Bristol laboratory space), due to the distance involved, the samples will be stored in the research facility prior to being transferred (with the consent forms) safely via a courier on dry-ice to Cardiff:

CL3 lab is 1F17A, 1st Floor Henry Wellcome Building,
Division of Infection & Immunity,
Cardiff University School of Medicine,
Henry Wellcome Building,
Heath Park,
Cardiff CF14 4XN.

The process for decontamination, transport and storage adheres to UK guidelines (https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories). The patients will not be reviewed again. Access to medical records is not required for those outside of the patient's immediate NHS care team.

Quantification of COVID-19 in salivary samples: Infectious virus in salivary samples will be quantitated by performing serial dilutions in DMEM/FBS, and inoculating Vero E6 monolayers for 1 hour, then overlaying with 2% Avicel. After 3 days, avicel will be removed, and plaques enumerated. Plaques will be stained with anti-spike (S2) antibody, to confirm

identity of isolated virus by immunofluorescence. Viral genomes will also be extracted from saliva and quantitated by RT-qPCR, using established primer sets. All samples will be destroyed and disposed of according to HTA regulations following being processed (hta.gov.uk).

Statistical analysis: 104 patients will be recruited in the 3 Centres that are approved first in a randomisation generated by AM. The primary endpoint will be viral load at 30 minutes and the primary analysis will be to compare each of the experimental treatments with the control treatment. The primary endpoint will be analysed using a mixed-effects regression model on the transformed scale with time and treatment as categorical covariates. There will be an interim analysis at the mid-point i.e. when 13 people have been allocated to each arm. Treatment arms are dropped for futility if the Z-test compared to the control arm is lower than -2.28 and are stopped for efficacy if above 2.28. Then the final analysis will calculate the treatment comparison Z-tests and are declared efficacious if the test statistics are above 2.28. This design controls the global null familywise error rate at 5% and has over 80% power when at least one treatment has a 1.8 difference in mean compared to the control mean. Since the endpoint is transformed on the log(x+1) scale the 1.8 difference in means is almost a two-fold reduction in viral load.

Estimated start date and length of study: The study has a proposed start date of July 30th 2020, and will be complete within 5 months (using a multi-centre approach will enable us to recruit the necessary patients).

ELIGIBILITY, RECRUITMENT AND CONSENT

Eligible patients will be approached and recruited from COVID in-patient wards within Cardiff and Vale University Health Board, Betsi Cadwaladr University Health Board, University Hospitals Bristol NHS Foundation Trust and Cwm Taf Morganwg University Health Board. Only patients who are deemed well enough and able to provide informed consent by their local clinical teams will be approached to take part in the study. When a member of the clinical team identifies a suitable patient, they will contact the local Principal Investigator (PI) at each site. The local PI will then visit the patient to provide information

about the study and answer any questions. Patients will be given a minimum of 30 minutes to consider taking part. For the safety of the research clinician, the entire process will be conducted in one visit in order to reduce their time on a COVID ward. Where a patient indicates that they do not wish to take part in the study, they will not be re-approached by the PI.

Where a patient indicates a willingness to take part, the PI will go through the Participant Information Sheet with them and take written consent. Three copies of the consent form will be completed- one copy will be provided to the patient to keep; one will be retained in the patient's hospital/medical records and one will be sent down securely with the sample via a courier to Cardiff University. On arrival at the School of Medicine, the tertiary packaging will be removed and the copy of the consent form taken, by DWT, to the School of Dentistry, where it will be stored with the site file (in the Chief investigators locked office).

Inclusion criteria

1. Adults aged ≥18 years
2. With positive SARS-CoV-2 carriage by RT-PCR within 14 days
3. Capable of using the mouthwash required by the trial
4. Capacity and capability to give informed consent to take part in the trial

Exclusion criteria

1. Known or suspected intolerance or hypersensitivity to povidone-iodine or other study materials (or closely related compounds) or any of their stated ingredients.
2. Known pregnancy, currently breastfeeding or women of childbearing age without effective contraception
3. Current requirement for invasive or non-invasive ventilation; or planned within next 6 hours
4. Known dermatitis herpetiformis (Duhring's disease)
5. Inability to communicate in English or read English

COMPLAINTS PROCESS

The patient information sheet provides 2 direct contact numbers should the participants be unhappy about the conduct of the study and/or investigators. These contact numbers are of the PI (DWT) 02920-742416 and the Study Sponsor/Research Governance Team 02920-879277.

CLINICAL IMPLICATIONS AND IMPACT

If this is shown to work, it is an easily administered, low-cost intervention, which may be adopted on a population basis. A simple mouthwash could safely and immediately be employed in routine practice in this and other viral pandemics to reduce the viral load exposure to health care professionals during procedures involving the oral cavity. The membrane model will allow variations in formulation to be screened *in vitro* in not only anti-viral but also potentially in anti-bacterial applications of the mouthwash.

Publication and dissemination plan: The impact of this work may be significant in managing patients and there is a genuine desire to see and employ the results of the study (if positive) in the NHS and Welsh Assembly Government. Once the data is analysed, **if positive**, the results will initially be disseminated via the Chief Dental and Chief Medical Officers and submitted for publication with minimum delay.

RESEARCH TEAM AND SUPPORT

Professor David Thomas leads the Advanced Therapies Group in translational research, having transferred novel antimicrobial therapies from the lab into human clinical studies and subsequent approval by the FDA and EMA. Professor Arvind Arya is a Visiting Professor at Wrexham Glyndwr University and Consultant in ENT surgery at Betsi Cadwaladr University Health Board, and will lead the sample collection at the North Wales site. Professor Nicola West runs a clinical trials unit in Bristol Dental hospital. Dr Ceri-Ann Lynch is a Consultant anaesthetist at Cwm Taf Morgannwg University Health Board. In virology, Dr Richard Stanton has established a live virus culture system in our Cat3 facilities in Cardiff, enabling local access to virus, infectivity assays, antibody and RT-qPCR testing. Mr David Owen is a Consultant ENT surgeon at the University Hospital of Wales. Dr Manon Pritchard is a dentist and a Sêr Cymru EU Precision Medicine Fellow. Dr Nick Claydon has successfully

completed >80 studies of oral-health products, including those conforming to ADA guidelines on chemotherapeutics (10.1016/s0002-8177(86)24021-0). Dr Jonathan Underwood is a Consultant in Acute Medicine and Infectious Diseases at University Hospital of Wales and is an MRC-NIHR Clinical Academic Research Fellow. Mr Robert McLeod is a Specialty registrar in otorhinolaryngology and WCAT Fellow at the University Hospital of Wales. Mr James Heyman is a Specialty Registrar in Otorhinolaryngology at the University Hospital of Wales.

Study design and analysis of data will be conducted by Dr Damian Farnell (Senior Lecturer in Medical Statistics) and Professor Adrian Mander (Professor in Medical Statistics and Director on Statistics) at Cardiff University.

Contact details:

Cardiff and Vale health board (Chief investigator):
Professor David W Thomas (ThomasDW2@cardiff.ac.uk)

Betsi Cadwaladr University Health board:
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University Hospitals Bristol and Weston NHS Foundation Trust:
Professor Nicola West (N.X.West@bristol.ac.uk)

Cwm Taf University Health Board:
Dr Ceri-Ann Lynch (ceri.lynch5@wales.nhs.uk)

Funding source: Cost for the study is being met by a grant from Venture Life Sciences (manufacturers of Dentyl®). The company will arrange delivery of Venture products to each health board; the remaining funds will support PPE, sample pots, purchase of Normasol® and povidone iodine, samples transport and sample testing. No staff working on the study will be paid anything in addition to their normal NHS/University salaries. All conflicts of interest have been appropriately declared. The project costings (staff, consumables, assay

systems, transport costs etc) have been peer-reviewed and formally approved by Cardiff University.