Imperial College London

Assessment of wearable sensors during experimental human influenza infection (Sigma Plus)

RESEARCH PROTOCOL

Version 2.1 14th August 2020

MAIN SPONSOR: Imperial College London
FUNDER: United States Department of Defense DARPA & Duke University
STUDY COORDINATION CENTRE: Section of Infectious Diseases & Immunity, Department of Medicine, Imperial College London

NRES/REC reference: 19/LO/1441
IRAS ID: 269042
clinicaltrials.gov reference: NCT04204993

Protocol authorised by:

Name & Role	Date	Signature
Dr Christopher Chiu *Chief Investigator*	16th September 2020	

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Study Management Group

Chief Investigator: Dr Christopher Chiu
Co-investigators: Dr Chris Woods

Study Site

The study will take place at St Mary's & Hammersmith Hospitals, London (part of the Imperial College Healthcare NHS Trust).

Study Coordination Centre

For general queries, supply of study documentation, and collection of data, please contact:

Study Coordinator: Dr Christopher Chiu
Address: Section of Infectious Diseases & Immunity, Commonwealth Building, Hammersmith Campus, Du Cane Road, London W12 0NN

Position: Clinical Senior Lecturer

Tel: +44-20-8383 2301 E-mail: c.chiu@imperial.ac.uk

Clinical Queries

Clinical queries should be directed to Dr Christopher Chiu who will answer or direct the query to the appropriate person.

Sponsor

Imperial College London is the research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Research Governance and Integrity (Ruth Nicholson) at:

Room 221
Level 2, Medical School Building
Norfolk Place,
London W2 1PG
020 759 49832
r.nicholson@imperial.ac.uk

Funder

The United States Department of Defense DARPA in collaboration with RTI International and Duke University has provided funding for this study. This protocol describes the above study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK policy framework for health and social care (V3.3 07/11/17). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate including Good Clinical Practice.

STUDY SUMMARY

TITLE Assessment of wearable sensors during experimental human influenza infection

DESIGN Human viral challenge study in healthy volunteers

AIMS
1. Assess the utility of the Bittium Faros heart rate, physical activity and ECG wearable sensor in measuring changes during influenza infection
2. Assess the utility of the Biovotion Everion heart rate, physical activity and respiratory rate wearable sensor in measuring changes during influenza infection
3.
3. Assess the utility of the Profusa Lumee oxygen sensor in measuring changes during influenza infection
4. Correlate physiological sensor measurements with quantitative measures of viral load and influenza-specific antibodies, B cells and cell-mediated immunity
5. Correlate physiological sensor measurements with results of cognitive tests and questionnaires

POPULATION Healthy persons aged 18 to 55 years

ELIGIBILITY Healthy persons aged 18 to 55 years that fit the inclusion and exclusion criteria

DURATION 1 Year

GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
BAL	Bronchoalveolar Lavage
CMI	Cell Mediated Immunity
CRI	Centre for Respiratory Infection
CTL	Cytotoxic T cell
DC	Dendritic Cell
DNA	Deoxyribonucleic Acid
ELF	Epithelial Lining Fluid
FEV_1	Forced Expiratory Volume in One Second
FI-RSV	Formalin-inactivated RSV vaccine
GC-MS	Gas Chromatography with Mass Spectrometry
GCP	Good Clinical Practice
HA	Haemagglutinin
ICRRU	Imperial Clinical Respiratory Research Unit
LAIV	Live Attenuated Influenza Vaccine
NA	Neuraminidase
NHLI	National Heart and Lung Institute
NP	Nucleoprotein
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PEF	Peak Expiratory Flow
PIS	Participant Information Sheet
RSV	Respiratory Syncytial Virus
RV	Rhinovirus

SAE	Serious Adverse Event
SAM	Synthetic Absorptive Matrix
URT	Upper Respiratory Tract
VOC	Volatile Organic Compound
WHO	World Health Organisation

KEYWORDS
Influenza, immune, virus, viral challenge, viral lung disease, infection

INTRODUCTION

1.1. Background to research

Despite the availability of specific antivirals and vaccines, influenza remains one of the greatest causes of morbidity and mortality worldwide. Seasonal influenza affects between 10 and 46% of the population each year with mortality of up to around 12 deaths per 100,000 in developed countries[1]. Following the emergence of the pandemic 2009 H1N1 virus (pH1N1), many severe cases also occurred in previously healthy young adults. With every population therefore potentially at risk, the prevention and improved management of seasonal and pandemic influenza are of major continuing importance. This is accentuated by the high cost, variable efficacy and evidence of resistance that limit the usefulness of available antivirals, while current vaccines are poorly effective in their ability to provide long-term protection due to relatively weak immunogenicity and continual virus evolution that leads to immune evasion[2].

The unpredictability of timing and severity of epidemic and pandemic outbreaks additionally limit the effectiveness of both clinical and public health responses. Recent seasonal outbreaks dominated by H3N2 strains have caused unusually severe disease and unsustainable pressure on health services during winter seasons. Difficulties in ascertaining biomarkers that can predict susceptibility, protection and disease onset or severity have led to major bottlenecks in the improvement of diagnostics, vaccines and therapeutics as well as in patient management. Animal models, which may be informative when used to investigate immune mechanisms involved in immunopathology and protection against influenza (including innate, cell-mediated, and tissue-specific immunity), have been less useful in translation to humans. This has been particularly true when attempting to define physiological responses to infection, which are poorly replicated in animal models where disease is not fully recapitulated.

Recent experience in both natural infection and experimental challenge settings has highlighted the extensive heterogeneity between individuals on exposure to even identical inocula. A proportion of individuals remain completely resistant to infection, while other suffer a spectrum of disease severities from asymptomatic to life-threatening illness. While disease severity usually correlates with higher viral loads, more subtle understanding of the predictors and determinants of severe disease is lacking. Further investigation of the factors that determine why some individuals have minimal symptoms while others suffer severe disease will allow more accurate prediction of risk in individual patients and rational intensification of treatment, as well as identification of new targets for vaccine and therapeutic intervention. If it were possible to predict how severe an influenza-induced illness would be early following exposure, it might be possible to intervene more rapidly with isolation of highly infectious individuals or other public health interventions to prevent onward transmission.

Predicting influenza disease through physiologic monitoring: The wide range of clinical outcomes associated with influenza infection and inability to accurately stratify risk causes difficulties in the care of individual patients as well as management of limited health resources. This is complicated by the late presentation of patients, who generally only seek medical attention once symptoms have become intolerable. One approach to earlier detection of infection would be to identify physiological changes that occur prior to symptom onset that nevertheless predict individuals who go on to symptomatic disease. Previous studies have shown that heart rate variability (HRV) measurements could predict post-stroke infections[3] and sepsis[4,5] up to 60 hours before onset of clinical symptoms. HRV monitoring of high-risk

infants in a neonatal intensive care unit has also been used to predict sepsis and sepsis-like illness and is used in a commercially available HeRO system[6]. While these systems are reliant on continuous monitoring of hospitalised patients using hospital-grade electrocardiogram (ECG) equipment, they provide proof-of-principle that HRV measures can indicate relative risk of infection to guide clinical care and may provide timely warning of pending health changes. The addition of physical activity measures and sleep categorization will provide context to the HRV measures to enable assessment of active, ambulatory subjects. Tissue oxygen measures will aid in the classification of illness severity as oxygen saturation levels have been shown to correlate to with clinical outcomes in respiratory illnesses.

Cognitive changes associated with influenza infection:
Preliminary data suggest that cognitive changes may be detected during episodes of infection. Conversely, social aspects may impact on the response to infection. To investigate these, multiple cognitive testing modalities will be used to assess the subject's cognitive performance at baseline and additional time points throughout the course of influenza infection from flu exposure and recovery. Each instrument has been standardized and evaluated previously[7–9] . The social support questionnaire is a 27-item quantitative survey questionnaire intended to measure perceptions of social support and satisfaction with that social support[10]. The degree of social support has been shown to influence the onset and course of certain psychiatric disorders such as clinical depression[11]. The Reading the Mind in the Eyes test was originally developed for adults in 1997, to demonstrate the deficits of theory of mind in those with autism spectrum disorders[12]. The test involves describing the emotional/mental state of a person based on only an image of their eyes, in a fixed-choice paradigm.

Immune determinants of influenza severity: In animal models, a number of immune factors have been shown to both enhance and ameliorate influenza disease. In particular, the evocative term "cytokine storm" has been used to describe a situation in which inflammatory mediators induce severe life-threatening disease[13]. In mice, pro-inflammatory mediators are highly induced in the lung and contribute to immunopathology following infection with the 1918 H1N1 and avian H5N1 strains[14], but the relative role of soluble mediators in pathogenesis and protection in humans remains unclear. Whether extreme cytokine levels are the cause or effect of severe influenza has been difficult to determine, especially in the absence of samples from directly affected respiratory tissues. Although the respiratory tract is the primary site of influenza infection, easily and consistently accessible peripheral blood may also provide markers by which severity of disease can be predicted. Microarray analysis of whole blood by our collaborators has identified transcriptional signatures that distinguish different respiratory viruses prior to the peak of symptoms[15]. Transcriptomic analysis in both this and the nasal compartment are likely to identify signatures associated with infection and disease severity long before the onset of symptoms.

The major surface antigenic determinants of influenza, haemaglutinin (HA) and neuraminidase (NA), both elicit protective humoral responses. However rapid mutation and reassortment of the segmented genome leads to immune escape. Internal viral components, such as the nucleoprotein (NP), which are relatively conserved, do stimulate cross-reactive T cell responses[3] but while these may be associated with reduced disease severity, they do not appear to prevent infection with novel strains in the long term. The immune factors that determine whether an individual will develop symptomatic infection following exposure to a respiratory pathogen therefore remain poorly understood. Traditionally, serum antibody has been used as a correlate of protection against influenza and, indeed, high neutralising antibody titres do correlate somewhat with a decreased risk of influenza infection. However, it is clear that antibodies do not explain all aspects of immune protection as some individuals with no detectable neutralising antibody are still protected. Increasing evidence indicates that protective immunity is mediated by a number of factors including early innate immune mechanisms and cell-mediated immunity. Furthermore, these arms of the immune system are interlinked, with innate immunity critically influencing later adaptive responses. Thus a comprehensive understanding of the early innate responses and local mucosal immunity remains a high priority, as well as their association with physiological changes that occur with influenza disease.

Human challenge model at Imperial College London Experimental human infection studies are uniquely capable of addressing questions regarding early events that contribute to immune protection and pathology in influenza but few groups are currently able to carry them out. Additionally, the

controlled in-patient setting of these studies can reduce the variability in responses seen in community-based studies. The UK is world-class in having extensive recent experience using this model. We began conducting experimental human infections with RSV at Imperial College in 2010, with the goal of establishing challenge studies in an academic setting at lower cost, with greater flexibility and increased scientific focus. In 2015, we established the influenza challenge model at Imperial College London and have demonstrated its safety and usefulness in providing unique and consistent data for the study of immunity and immunopathogenesis during acute infection. Our group has now safely challenged >125 participants with respiratory viruses in our centre and are currently the only academic group in Europe with the capacity, experience and skills to conduct these studies. Recently, we have been given access to a new influenza H3N2 challenge strain derived from a 2015 clinical isolate (influenza A/Belgium/4217/2015). Not only does this new challenge agent better reflect recent seasonal outbreaks, but also recent studies have shown a 75% attack rate with moderate but consistent symptomatology. Using this challenge virus in our model will allow questions regarding the capacity of ambulatory physiological sensors to measure the response to influenza infection and how these responses correlate with cognitive and immune outcomes to be addressed.

This study will follow on from our previous Prometheus study, which systematically investigated the early pre-symptomatic period following exposure to influenza in humans and provided exploratory pilot data with non-commercial sensor devices that suggested that sleep disturbance occurred within 24 hours of influenza exposure. The data obtained in this study will be used to test the ability of novel wearable sensors to detect changes associated with infection and disease severity, thus allowing their use in general settings to predict which individuals will go on to more severe symptoms and higher viral shedding, enabling implementation of treatments and other interventions at an earlier stage. Ultimately these data will inform the development of a fully automated, network of human-based wearable sensors that detect biomarkers indicative of respiratory infection and level of severity, and transmit acquired signals to corresponding smartwatches or phones. Current public health networks track only patients who seek medical care. The total time associated with the development of infection symptoms, laboratory-based diagnostic tests, data compilation, and reporting results in a 3–5 week delay in the detection of the disease outbreak. By using human-based, wearable, networked sensors in concert with point-of-care diagnostics and automated data collection and reporting, the time required to detect disease outbreaks may be shortened by weeks. A required innovation is the development of algorithms for illness detection and severity estimation that distinguish host response from confounding factors such as increased mental or physical demands. This study will provide essential data for the development of these algorithms with the acquisition of sensor data from the pre-symptomatic phase of influenza through recovery. The collection of HRV data from multiple sensors will allow for comparison of ECG sensors to wrist and arm sensors that may be more convenient for long-term wear. The integration of physical activity measures will enable evaluation on subjects without activity restrictions.

1.2. Research Hypotheses

1. Novel wearable sensors (i.e, Bittium Faros, Biovotion Everion, and Profusa Lumee) can detect physiologic changes that are associated with influenza infection and disease severity.
2. Physiologic patterns of heart rate, physical activity, ECG, respiratory rate and oxygen saturation from wearable sensors can act as biomarkers that enhance current diagnostic practice for early detection of influenza infection and disease prediction.

STUDY OBJECTIVES

1.3. Primary Objective:

- To test the hypothesis that measurements of heart rate, physical activity, ECG, respiratory rate and oxygen saturation can detect influenza infection and disease

1.4. Secondary Objective:

- To identify early signatures using sensor data that predict influenza disease and severity, potentially enabling earlier medical or public health interventions

1.5. Exploratory Objectives:

- To test the associations between physiologic sensor measurements and cognitive function using objective tests
- To test the associations between physiologic sensor measurements and biological and immune readouts

PARTICIPANT ENTRY

1.6. Recruitment

Subjects will be recruited by research nurses and doctors by the following methods, advertisement in local newspapers, around College sites, and online: the Imperial Trust website, ICRF website, and on Social Media such as the Imperial CRF Twitter page, the ICRF have a volunteer database which we may utilise. Additionally, respondents to adverts for prior research projects in the department that were not subsequently enrolled, but were otherwise eligible for this study, will be contacted and invited to take part in our study (these have previously given their consent to be contacted). If interested, they will be invited for pre-screening by email and/or telephone call. We aim to enrol up to 20 participants to be challenged with influenza, in groups of 2-7 at a time depending upon recruitment (i.e. 7 volunteers are available at the same time).

1.7. Pre-registration evaluations

1.7.1. Pre-screening Visit

Since individuals with high levels of antibody against the influenza strain used to challenge are resistant to infection, participants first need to be pre-screened to ensure they are not already immune. Informed consent will be obtained prior to blood draw, after which 10ml of blood will be collected in a serum tube, which will be subsequently spun down and tested for anti-influenza antibodies by microneutralisation (MN) assay using the challenge strain. Serum from the pre-screening visit will be saved and used in this and other ethically approved studies, including future studies by UK and international collaborators.

1.7.2. Screening Visit (day -60 to day -7)

If the participant is found to meet serology criteria (i.e. microneutralisation titre of <1:20, unless a decision is made by the study steering committee to specifically investigate individuals with higher MN titres), they will be invited for a screening visit. The screening visit will involve each participant attending the Imperial Clinical Respiratory Research Unit (ICRRU) at St Mary's Hospital, Paddington or Imperial Clinical Research Facility (ICRF) at Hammersmith Hospital for a past medical history review and medical examination to find out if they are suitable for the study. Potential subjects will be given a participant information sheet (PIS) detailing the study and experimental procedures. These will also be discussed with them by the study doctor or nurse. When the subject has had enough time to consider their participation in this study, ask any questions they may have, and only when they have agreed to take part will they be asked to read, sign and date a consent form in the presence of the study doctor or nurse who will also sign the consent form. Consent will be obtained prior to any history-taking, examination or tests are carried out. A copy will be kept in the research file, a copy given to the patient and a copy put into their medical notes.

A medical history will then be taken and clinical examination, lung function tests, chest X-ray, ECG and blood tests performed by the study team. Women of childbearing potential will be asked about current contraceptive use, and be required to use effective contraception (barrier, oral contraceptive pill, depot injection, implant, or total abstinence) throughout the study. Blood tests include general screening for underlying illness, specifically full blood count, urea and electrolytes, liver function tests, coagulation C-reactive protein, lymphocyte subsets, immunoglobulins, HIV, hepatitis B and C serology. A urine drug screen for illicit drugs and pregnancy test will also be performed, a positive result from either will result in the volunteer being excluded from the study.

These will all be done in the ICRRU at St Mary's Hospital or ICRF at Hammersmith Hospital. If the evaluation and the results of these tests show no evidence of infection or any other problems with the participants' health, and matches the inclusion / exclusion criteria then they will continue to the main part of the study.

1.8. Inclusion criteria

Healthy persons aged 18 to 55 years, able to give informed consent

1.9. Exclusion criteria

Chronic respiratory disease (asthma, COPD, rhinitis, sinusitis) in adulthood

Inhaled bronchodilator or steroid use within the last 12 months

Use of any medication or other product (prescription or over-the-counter) for symptoms of rhinitis or nasal congestion within the last 3 months

Acute upper respiratory infection (URI or sinusitis) in the past 6 weeks

Smoking in the past 6 months OR >5 pack-year lifetime history

Subjects with allergic symptoms present at baseline

Clinically relevant abnormality on chest X-ray

Any ECG abnormality deemed clinically significant.

Those in close domestic contact (i.e. sharing a household with, caring for, or daily face to face contact) with children under 3 years, the elderly (>65 years), immunosuppressed persons, or those with chronic respiratory disease

Subjects with known or suspected immune deficiency

Receipt of systemic glucocorticoids (in a dose ≥ 5 mg prednisone daily or equivalent) within one month, or any other cytotoxic or immunosuppressive drug within 6 months prior to challenge

Known IgA deficiency, immotile cilia syndrome, or Kartagener's syndrome

History of frequent nose bleeds

Any significant medical condition or prescribed drug deemed by the study doctor to make the participant unsuitable for the study

Pregnant or breastfeeding women

Positive urine drug screen

Detectable baseline antibody titres against influenza challenge strains

History of hypersensitivity to eggs, egg proteins, gentamicin, gelatin or arginine, or with life-threatening reactions to previous influenza vaccinations.

Participants may only recruited if they have previously been involved in research if they have completed the earlier study and are beyond the washout period of any administered drugs or period of effect of any intervention that would cause interference for either study.

All women of childbearing age will have a **pregnancy test performed prior to virus inoculation** to exclude pregnancy and be required to use contraception **throughout the study**.

1.10. Withdrawal criteria

Any subjects can withdraw from the study at any time if they wish to. Subjects can also be removed from the study if an investigator feels this is necessary or appropriate. Subjects will be closely monitored throughout by the study doctor. This is defined further in the section on 'Serious Adverse Events'.

If a participant loses capacity during the study, then the participant would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

The nature of this study design does not allow participants to be replaced after Day 0.

STUDY DESIGN

Table 1

Procedures			DAY (relative to viral inoculation)																					
	Pre-screen	Screen	-7	-3	-1	0 am	0 pm	1 am	1 pm	2 am	2 pm	3 am	3 pm	4 am	4 pm	5 am	5 pm	6	7	8	9	10	14	28	180
Consent	X	X																							
Virus inoculation						X[1]																			
PCR					X									X											
Physical Examination		X			X	X														X			X	X	
Chest X-ray		X																							
12 lead ECG		X						X				X				X			X		X				
Lung Function Tests		X			X	X		X		X		X		X		X		X	X	X	X	X	X	X	
Screen blood tests		40																							
Blood - serum	10					10																		10	10
Blood for HLA typing	5																								
Blood - PBMCs (mls)					20	20		30		10		30		10		10			40			30	10	30	20
Blood – plasma (mls)		6	6	6	10	10		10	6	10		10		10		10		6	6			6	6	6	6
Blood – RNA (mls)		5	5	5	7.5	7.5		7.5	7.5	7.5		7.5		7.5		7.5		5	5			5	5	5	7.5
Throat swabs			X		X	X		X		X		X		X		X		X	X	X	X	X	X	X	
Stool swabs					X	X		X		X		X		X		X		X	X	X	X	X			
Breath collection						X		X		X		X		X		X		X	X	X	X	X	X	X	
Oral swabs						X		X		X		X		X		X		X	X	X	X	X	X	X	

[1] Virus inoculation after sampling

Table 1 continued

Procedures			DAY (relative to viral inoculation)																					
	Pre-screen	Screen	-7	-3	-1	0 am	0 pm	1 am	1 pm	2 am	2 pm	3 am	3 pm	4 am	4 pm	5 am	5 pm	6	7	8	9	10	14	28	180
Nasal lavage (daily)		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nasosorption		X	X	X	X	X		X		X		X		X		X		X	X	X	X	X	X	X	X
Nasal curettage		X						X		X		X							X			X	X	X	X
Nasal brushing		X																						X	
Faros ECG sensor			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Biovotion Everion			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Insert Lumee			X																						
Lumee reader			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Symptom diary			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Urine tests		X				X																			
Cognitive test: Visual Arrays test					X							X									X				
Cognitive test: Sustained Attention to Cue task					X							X									X				
Cognitive test: Ant-Saccade test					X							X									X				
Mind in the eyes questionnaire					X							X									X				
Social Support questionnaire					X																				

1.11. Study visits

The study is divided into outpatient and confinement phases. Subjects will stay overnight for a period of 8-11 nights in total, from the morning before the viral challenge, to the 8-10th day after viral challenge. This period of confinement has been chosen to eliminate the possibility of subjects in the study transmitting the virus to anyone not involved in the study (i.e. family, household contacts, and the wider community). Confinement is not used to enable closer monitoring or to enhance safety for study subjects, although this may be an additional benefit in some circumstances. During the confinement period, all study procedures will take place in the confinement facility (Table 1). There will also be follow-up visits (1.11.3).

1.11.1. Pre-inoculation visits (ICRRU/ICRF/, day -7 and day -3)

On the day -7 (pre-inoculation) visit nursing or medical staff will perform lung function, blood tests, a throat swab, take washings from the participant's nose (nasal lavage), a nasal scrape, nasal brushing, and a nasal SAM. The Lumee device will be inserted and the Faros, and Biovotion sensors will also be given to the volunteer. At day -7 the participant will also be given a symptom diary card and asked to complete it (before any nasal procedures), detailing any respiratory symptoms they experience in their upper and lower airways. They will be asked to complete the symptom diary on a daily basis for one week prior to and two weeks after inoculation with the virus.

On the day -3 (pre-inoculation), we will perform lung function, blood tests, a throat swab, take washings from the participant's nose (nasal lavage), a nasal scrape, nasal brushing, and a nasal SAM. The wearable sensors will be checked for functionality and data will be downloaded if necessary.

Subject to a satisfactory assessment by the study doctor (brief interview and medical examination) and the taking of blood and nasal lavage samples, we will infect volunteers with influenza on day 0. Those challenged with influenza will then be confined as described (Section 1.10.2).

1.11.2. Confinement period (confinement, day -1 to day 8-10)

On the morning of the day before viral challenge, participants will attend the ICRRU, or ICRF. A single nasal lavage sample will be taken to exclude coincidental infection, breath collection, a nasal SAM and blood will be taken for PBMCs, plasma and RNA. Following this, subject to a satisfactory assessment by the study doctor (brief interview and symptom-directed medical examination), we will infect the volunteers with influenza A/Belgium/4217/2015 at a dose of $5x10^5$ $TCID_{50}$ in a volume of 0,5mL divided between nostrils. After infection, participants will be observed by a member of the study team or ICRF staff member for a period of one hour to ensure no adverse reactions have occurred. They will enter a residential research facility (confinement facility), where they will reside for the next 8-11 nights so we can monitor the development of cold symptoms and collect samples to evaluate immune responses and test the hypotheses. These will include daily blood samples, breath collection, throat swabs, nasal lavage, nasal SAM, nasal scrapes, and lung function tests. Participants will be seen daily by the study team. On the 8th day of residence, assuming all significant symptoms have resolved and at the discretion of the principle investigator, participants will leave the confinement facility and asked to return daily for assessment and sampling, at home they will need to continue their symptom diary only. If symptoms continue or the study doctors deems it necessary, the volunteer will be asked to remain until the 9th or 10th day of residence at which time they will be discharged, subject to a satisfactory assessment by the study doctor. If a subject is discharged before the 10th day or wishes to withdraw after virus inoculation but before the 10th day, we will strongly advise them to confine themselves in their own homes, and to strictly avoid any contact with young children, the elderly or other high-risk individuals for the remainder of the period during which viral shedding may occur. Additionally, subjects infected with influenza withdrawing before the 8th day post-inoculation may be treated with oseltamivir to reduce the risk of viral shedding having left confinement. This will be provided from pharmacy, as agreed with ICRF PRB.

1.11.3. Follow-up period (outpatient, day 11 – day 196)

Participants will return to the ICRRU or ICRF on days 14 and 28 post infection for assessment and sampling. During the 6 months after challenge, subjects will be asked to report episodes of upper respiratory tract symptoms by email to the study email address. If an episode of natural infection occurs, the subject will be invited to re-attend for blood (10mL clotted blood for serum, 40mL lithium heparin for PBMCs) and upper respiratory tract samples (nasal wash and SAMs) to be collected. After 6 months, subjects will be asked to return for further samples to be taken. Further samples will be collected at the peak of the secondary response 7 days post-infection. Table 1 summarises when each sample type will be taken.

STUDY PROCEDURES

A variety of procedures are carried out during the study period. The frequency and timing of these procedures are shown in Table 1 above. For the screening, and days -7, -3, -1, 14, 28 and 180 visits, procedures will take place in the procedures room on ICRRU, ICRF or CRC; for days -1 to 10 subjects will reside in the confinement facility and procedures will take place there (with the possible exception of virus inoculation).

1.12. Virus Inoculation

GMP A/Belgium/4217/2015 (H3N2) virus strain will be used for experimental infection of volunteers. On the day of inoculation (day 0), influenza virus stock will be removed from its storage in the -80°C freezer, transferred to the category 2 laboratory in the quarantine unit on dry ice, rapidly defrosted in a 37°C water-bath and dilutions prepared according to standard operating procedures (SOPs) for administration to subjects. Briefly, subjects will be inoculated with intra-nasal drops on a single occasion with diluted inoculum at a given dose divided equally between the two nostrils. This is carried out by the study or ICRF medical team. This will be done slowly with sufficient interval between each inoculation (2-3 minutes) to ensure maximum contact time between with the nasal and pharyngeal mucosa. Subjects will be asked not to swallow during the procedure to ensure maximal pharyngeal contact. The inoculation procedure will be performed using a negative pressure room or the quarantine ward in the ICRRU, or ICRF. Inoculations using intranasal drops will be done using a 1mL pipette with subjects supine. Following inoculation, advice regarding hand hygiene will be given and subjects will be provided with alcohol hand gel and face-masks if they are to move between the inoculation room and the quarantine ward.

1.13. Swabs for microbial analysis

1.13.1. Throat swab, and oral swab

A sterile dry cotton-headed swab is used to obtain samples from the pharynx for bacterial 16S gene analysis. This is performed with the subject sitting. Ensure adequate lighting and use a tongue depressor if required. Remove the swab from the container carefully to ensure the tip is not contaminated, and swab the dorsal aspect of the pharynx and soft palate, avoiding the tongue. Some subjects may experience a strong gag reflex. Two throat swabs will be taken by this method.; place one used swab into a dry container and freeze at -80°C prior to analysis and another into bacteriology culture medium.

The oral swab collects oral fluid and is run across the gums & inside of the cheek by the volunteer for 1-2 minutes, then kept at room temperature prior to processing.

1.13.2. Stool swab

Sterile dry cotton-headed swabs will be used to obtain stool samples for bacterial 16S gene analysis. These will be collected by the subject from the toilet paper after opening their bowels.

Procedure:

- Remove the swab from the collection tube by holding it firmly by the red cap. Do not touch the cotton part with your bare hands.

- Collect a small amount of fecal material by rubbing the cotton tip of the swab on a faecal sample: a piece of used bathroom tissue is the best material possible. A small amount is enough: it should cover half of the cotton tip. Do not try to collect too much biomass.
- Replace the swab in the collection tube and close it by pushing firmly on the red cap.
- Store the swab at -80°C within 48h. If it is not possible to store at -80°C, store the sample at 4°C until transfer into a cryogenic environment.

1.14. Nasal sampling procedures

1.14.1. Nasal scrape using Rhinopro®

Rhinopro® curettes will be used to obtain a sample of nasal epithelial cells from each nostril. This is a painless procedure and will not require local anaesthetic. The following technique is used:

Procedure:

- The subject should be sat comfortably, ideally with their head fixed, looking forward, while their chin rests on a support (if available)
- Tear bag and remove the flexible plastic Rhinopro® without contaminating the scoop end
- Place a speculum in the nose to keep the cavity open and employ good lighting
- Under direct visual inspection, insert the cupped probe onto the surface of the mid-inferior portion of the inferior turbinate. Note: Avoid the anterior bulb
- The Rhinopro® should be 3cm up the nose; the floor of the nostril can be used to rest on
- Have the cup of the Rhinopro® at the correct angle
- Gently press the cupped tip on mucosal surface and move out and in of nostril 3mm up to 3 times
- Note that this area has limited sensitivity and the subject should not find this procedure painful, although a nasolacrimal reaction usually occurs

The cell harvest is epithelial cells, goblet cells and mast cells. It does not contain deeper layers of the mucosa. The sample obtained should be placed immediately into a tube containing RNA Cell Protect® (Qiagen) or Trizol and frozen at -80°C for storage prior to analysis.

1.14.2. Nasosorption

Up to four strips of SAM will be used (2 per nostril, one after the other) for 2 minutes to obtain repeated samples of neat nasal ELF. This is a painless minimally invasive procedure that will not require any local anaesthetic. Following sampling, SAM will be placed in a 1mL microfuge spin filter tube containing 100µL of elution buffer (PBS/1% bovine serum albumin/0.05% azide/0.05% Triton®). Further details are given in the SOP Human Sampling Procedures.

The SAM will be transported on ice to the laboratory.

1.14.3. Nasal Lavage

Nasal lavage is performed using the following technique:

- 5-10mL of 0.9% saline is introduced into one nostril using a syringe attached to a nasal olive with the subject sitting with the head tilted forward
- The saline is then washed in and out of the nose approximately 10 times by alternately withdrawing and advancing the plunger of the syringe while the subject maintains a tight seal between the nasal olive and the nostril; the aim is to recover ~80% of the saline from the nose
- The fluid is then aliquoted into sterile microfuge tubes and centrifuged for analysis of cells

Lavage fluid will later be analysed to quantify the degree of RSV shedding. Multiplex and SARS-CoV-2 PCR will be performed on the pre-inoculation lavage and post-inoculation lavage collected during the study to exclude the presence of other respiratory viruses. Supernatants will be frozen and stored at -80°C. Further details are given in the SOP Human Sampling Procedures.

1.14.4. Nasopharyngeal Swab

The nasopharyngeal swab is performed using the following technique:

- Explain the procedure and gain consent from the participant.
- Ask them to tilt their head back.
- Inspect the nostrils for contraindications prior to preforming nasopharyngeal swab insertion.
- Measure the distance from the nostril to the earlobe. The swab will be inserted half this distance.
- Insert the swab horizontally, to the predetermined distance, into the nasopharynx.
- Leave the swab in place for a few seconds then rotate twice and remove.
- Place the swab into the culture medium provided, cutting the swab shaft to fit within the container.
- Label the sample (including site – left or right nostril) and place on wet ice for transfer to the laboratory.

The process should be repeated on the alternate nostril. Each swab should be placed immediately into its own container of culture medium.

On arrival to the laboratory the samples will be vortexed for five seconds to dislodge and suspend the cells. The swabs will then be rotated and squeezed to remove any excess liquid prior to being discarded.

Pool the left and right nostril sample from the same participant taken at the same time point. Centrifuge (400RCF, 10 min, +4oC), aliquot and freeze the supernatant at -80oC. If the remaining cell pellet is required it can be further processed, on ice, prior to storage.

1.14.5. Nasal Brushing

A nasal brush is used to obtain nasal epithelial cells from the nose for primary cell culture. A nasal brushing is performed as follows. Inspection is performed using a nasal speculum with a head lamp to assess for normal nasal anatomy prior to the procedure. A nasal brushing will be taken by inserting a cytology brush through the nostril and between the inferior nasal turbinate and the lateral wall of the nasopharynx and then removed with a twisting motion of the wrist. The brush will be placed in a 15ml falcon tube containing 2mls of media and transported to the laboratory for cell count and seeding. Complications include slight bleeding immediately following the procedure and controlled by simple finger pressure and nasal discomfort which should not require any analgesia.

1.15. Breath collection

Exhaled breath is believed to contain infectious virus during natural influenza infections. To collect potentially infectious particles in breath exhaled during infection, we will use an altered resuscitation mask with Teflon filter mouthpiece into which the participant will be asked to breath for 20 minutes.

1. The test will take a total of thirty minutes including twenty minutes of quiet breathing.
2. A study team member will explain what the device consists of and why the test is being performed.
3. The participant will hold the mask by the cardboard tubing and not touch any plastic surface.
4. They will gently press the mask against their face, covering the mouth and nose.
5. Then they test breathing through their nose and mouth while study staff check for leaks around the face.
6. If the participant experiences pinching of their nose, difficulty in nasal breathing or a leak is detected air can be removed with a 20 ml catheter tip syringe from the inflatable cuff on the facemask and the mask re-tested.
7. Once steps 4 and 5 have been optimised the participant will be asked to breathe in through their mouth and out through their nose (normal tidal volumes and rate).
8. If their nose is blocked due to symptomatic infection mouth breathing will be performed and this will be documented in the Breath Collection Log.
9. The participant will be asked not to speak, unless it is necessary.
10. They will be asked not to remove the mask from their face, unless absolutely necessary.
11. It will be explained that if the seal around their face is broken, e.g. if they remove the mask, the test will be restarted.

1.16. Blood sampling

Screening visit blood will be taken for full blood count, renal function, liver function tests, glucose, clotting, and CRP. These will be processed in the Haematology and Chemical Pathology Laboratories of Imperial College Healthcare NHS Trust. Serology will be performed at pre-screening, screen, and day 28 by microneutralisation assay. Serum will also be used in future serological tests for comparison purposes.

The total amount of blood taken at screening would amount to no more than 51mL. Blood for peripheral blood mononuclear cells (PBMCs) will also be taken (see table 1). On these occasions, 20-40mls of blood will be taken (see Table 1). In addition, blood for gene expression profiling, serum and plasma will be taken (see table 1). Blood for gene expression profiling will be taken up to 2 times per day. A maximum of 70mls of blood will be taken on any single day, with a total of no more than 500mls taken between screening and the 28 day challenge period (see Table 1).
DNA processing will be taken from the residual or additional blood samples provided by the subjects

1.17. Physical examination, physiological and symptom monitoring devices

1.17.1. Physical Examination will include ENT, respiratory and cardiac assessment, and will be performed by a study doctor at screening and days -7, -1, 0 (prior to inoculation), discharge, 14 and 28 days post inoculation.

The Bittium Faros, Biovotion, and Lumee devices will be given to each participant at the day -7 pre-inoculation visit. They will be fitted as required and the participant will be counselled regarding their positioning, care and data downloads (if appropriate). A mobile telephone (without SIM card) will be provided to each participant with applications for the relevant sensor devices pre-loaded. After completion of the study, all Faros, Biovotion, Lumee patches, study mobile devices and mobile device charging cables will be returned to study personnel.

1.17.2. Bittium Faros sensor

The Bittium (formerly eMotion) 180° Faros sensor monitors heart rate variability, electrocardiography and physical activity. The sensor box (Figure 1) will be attached to the chest with two disposable, adhesive electrodes positioned on the chest. The device is CE-marked (93/42/EEC) and is in accordance with MDD Class IIa. The sensor and electrodes are not waterproof and will need to be removed before showers etc. They are also not suitable to use in an MRI environment. The device is not intended to be used along high frequency surgical equipment or a defibrillator. The device will require charging via docking station and data will be downloaded intermittently via USB cable to a computer by study personnel at study visits. After successful connection of the sensor, study personnel will explain and train subjects on how to use the Faros as well as ensuring study subjects understand usage, handling and maintaining of the device and electrodes. An initial data collection process will be undertaken to check that the Faros is working correctly prior to the end of the study visit.

Figure 1: Bittium Faros sensor and positioning.

1.17.3. Biovotion Everion sensor

The Biovotion Everion (Figure 2) is a CE marked, non-invasive wearable multi-sensor device that monitors heart rate, skin temperature, respiration rate, steps, activity, and energy expenditure from the skin's surface. It will attached to the left or right upper arm using an armband and will be in contact with intact skin only. All measurements are taken on the surface of the skin. Data from the device is collected via the Biovotion application, which will be pre-loaded to the mobile device provided. Each participant will have a study user account created with identification only by subject study ID. After successful connection of the sensor and mobile device, study personnel will explain and train subjects on how to use the Biovotion Everion and the features of the Biovotion application as well as ensuring study subjects understand usage, handling and maintaining of the Biovotion Everion. An initial data collection process will be undertaken to check that the Biovotion Everion is working correctly prior to the end of the study visit. The following parameters will be collected: activity, barometric pressure barometer temperature, skin blood perfusion index, blood pulse wave, energy expenditure, galvanic skin response, heart rate, heart rate variability, local temperature, skin temperature, respiration rate, blood oxygenation and steps per day.

Figure 2: Biovotion Everion device, charger/docking station and armband positioning.

1.17.4. Profusa Lumee™ Oxygen Platform device

The Profusa Lumee oxygen monitoring device is designed to reliably report tissue oxygen levels and is currently used in clinical practice to monitor the severity of artery narrowing and oxygen delivery to the limbs. The device is in two parts, the sensor and the reader. The sensor is a soft, flexible fibre approximately 5 mm long and 0.5 mm in diameter and is made a material shown to be biologically compatible with body tissue. It will be injected under the skin at depth of 2-6 mm from the skin surface using a single-use, disposable Lumee Pen injectable device by trained study staff. Previous studies have shown that the Lumee sensor is biologically inert, causes no reaction in the tissues and once inserted can neither be seen nor felt. The sensor reacts with oxygen in the tissue and emits invisible light proportional to the amount of surrounding oxygen. In total, 2 sensors will be inserted (one in the skin of the upper arm and one on the side of the chest). A wireless patch reader is placed on top of the skin over the area where the sensor has been placed to measure local oxygen content. These data are then transferred to the Lumee Patch App on the dedicated mobile device via Bluetooth. When the Lumee is first given to the participant, it will be paired with the Lumee Patch app via Bluetooth. After that, the device will automatically synchronise via Bluetooth with the mobile device. After successful connection of the sensor and mobile device, study personnel will explain and train subjects on how to use the Lumee and the features of the Profusa Lumee application as well as ensuring study subjects understand usage, handling and maintaining of the Lumee. An initial data collection process will be undertaken to check that the Lumee is working correctly prior to the end of the study visit.

The sensor and current version of the wired reader devices are CE Marked for use in the European Union and other countries that accept the CE Mark. The wireless reader that will be used in this study has not yet been received the CE Mark. This certification is in process but this study and data generated will not be used in any CE marking application.

Of note:

- Extensive pre-clinical and clinical studies show that the device causes minimal foreign body, scarring or allergic reactions
- The oxygen sensor contains no electronic components and does not store data
- Once inserted, the oxygen sensor is not visible by the naked eye and is usually not palpable
- Light is emitted from the sensor but is undetectable by eye and readable only by the patch reader
- Data is stored on the patch reader until it is downloaded to the Lumee Patch App, which will be installed on a mobile phone device
- Resulting data will be anonymised before transfer via a desktop computer to a storage server

Please see the Instructions for Use for full information about this device.

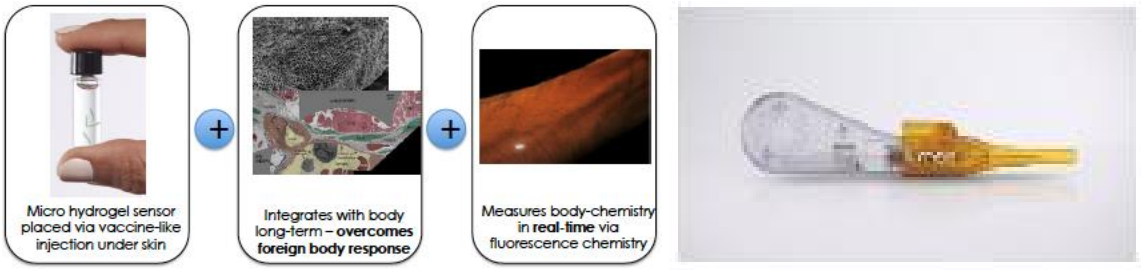

Figure 4: Profusa Lumee and Lumee Pen insertion device.

1.18. Lung Function Tests

At the screening visit and at other visits as described in table 1 in, measurements of FEV1 and FVC will be made as outlined below.

Predicted Values for Lung Function Measurements = the predicted or reference values for lung function measurements are those recommended by the Report Working Party for the European Community for Coal and Steel. Also incorporated are the recommendations of the British Thoracic Society and the Association of Respiratory Technicians and Physiologists.

1.18.1. Measuring FEV_1 and FVC by Spirometry

Performing the measurement:

- Posture must be consistent during a study, either standing or sitting, with no breathing limitation
- The subject should breathe in to total lung capacity. A good tight seal by the lips round the mouthpiece is essential. The subject should then exhale forcibly into the spirometer, blowing as hard as possible and continue to residual volume
- The best value of 3 attempts will be recorded
- Calibration: Vitalograph dry wedge bellows spirometers will be serviced and calibrated at least once every year by an engineer from Vitalograph. However, the calibration will be checked at least once a month using a calibrated one litre syringe.

1.19. Clinical Scores and Cognitive Tests

To assess the impact of infection, a self-completed diary card of upper and lower respiratory tract and systemic symptoms will be provided between day -7 and day 14, and participants will be instructed to complete this prior to inoculation and daily sampling. During the course of the study, 3 computerised

tests of attention will also be performed. Finally, to estimate the impact of social support on their response to infection, participants will be asked to complete two paper questionnaires to assess their social support and impacts, which will be given to them at the beginning of the quarantine period and completed by the participants as soon as possible during their in-patient stay.

1.19.1. Upper Respiratory Tract Symptoms

A total 'upper respiratory clinical symptom score' will be derived using a four-point scale (0-3 for absent, mild, moderate and severe) for each of the following eight respiratory symptoms: sneezing, headache, malaise, fever/chills, nasal discharge, nasal obstruction, sore throat and cough according to established methods, giving a maximum clinical severity score of 24. This is an established method for studies of common cold illnesses[44]. Symptoms will be recorded at the same time of day and before any procedures such as nasal lavage are performed.

A diary card of lower respiratory tract symptoms will be completed with a scoring system outlined below.

0 = Absent, 1 = Mild, 2 = Moderate, 3 = Severe

An example is shown below:

Systemic & upper respiratory tract symptoms assessment & scoring																							
Symptoms	**D-7**	**D-6**	**D-5**	**D-4**	**D-3**	**D-2**	**D-1**	**D0**		**D1**		**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**
Time	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**6am**	**5pm**	**8am**	**5pm**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**	**8am**
Muscle aches																								
Fatigue																								
Headache																								
Fever/feeling hot																								
Total of above																								
Stuffy/blocked nose																								
Earache/pressure																								
Runny nose																								
Sore throat																								
Sneezing																								
Total of above																								

1.19.2. Systemic, Lower Respiratory Tract and Other Symptoms

A diary card of lower respiratory tract symptoms will be completed with a scoring system outlined below.

0 = Absent, 1 = Mild, 2 = Moderate, 3 = Severe

1.19.3. Definition of a clinical cold

A clinical cold is diagnosed if **two or more** of the following are present:

- A cumulative clinical symptom score of 14 or greater over a 6 day period
- Nasal discharge is present on three or more days over the six-day period post viral inoculation
- A subjective impression of a cold developing. This latter criterion is used because there are a few subjects who have had a very strong subjective impression of a clinical cold but the cumulative clinical score does not reach the arbitrary cut-off level

1.19.4. Computerised Attention tests

Attention control tests assess the ability to concentrate on a task and distractibility[16]. We hypothesise that the onset of influenza infection leads to changes in ability to concentrate that occur before symptoms are noticeable. Three computerised tests will be administered. Following explanation of how the tests will be conducted, a laptop computer with tests pre-loaded will be given to the participant and how the tests are done will be explained to them. They will self-administer the test. Data will be labelled only the participant identification number only and stored on the laptop until the end of the study.

Visual Arrays test: Each trial begins with a reminder to subjects of how to record their responses. Afterward, subjects see a fixation cross displayed on the screen for 1000 ms, which they are asked to focus on, followed by the words “RED” or “BLUE” for 300 ms. This instructs participants to pay attention to only the red or the blue rectangles on the next screen. For example, “BLUE” tells participants that they will later be asked about the blue rectangles. Next, an array of blue and red rectangles is presented for 250 ms. It consists of either 5 or 7 rectangles per colour (10 and 14 total) scattered around the screen.

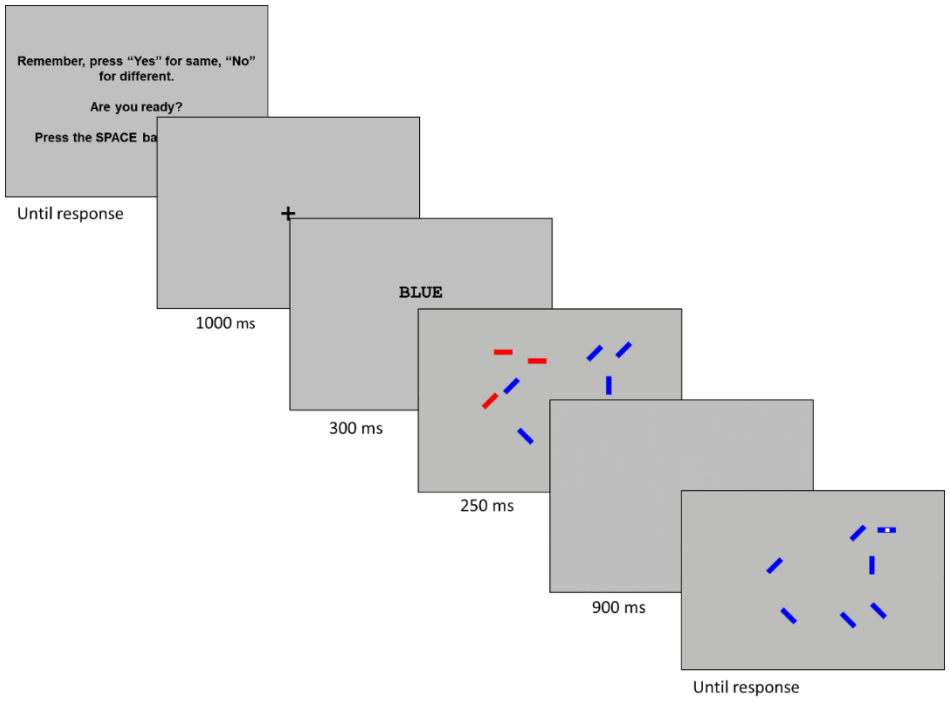

Figure 5: Visual Arrays test

After a delay of 900 ms, the array is presented again, this time with only the bars of the relevant colour. One of the rectangles is cued with a white dot. Subjects must indicate whether the indicated rectangle has changed orientations from the first presentation of the array. Cued rectangles change orientation on 50% of all trials. There are 48 trials of both the 10- and 14-item arrays, for a total of 96 trials. The dependent variable is a capacity score (k), one for each array size. The final dependent variable is the mean of these two k scores.

Administration of this task takes approximately five minutes and requires a standard computer mouse and QWERTY keyboard. The test will be administered three times during the study – at entry, at 3-4 days post exposure, and at pre-discharge.

Sustained Attention to Cue task: In this task, subjects need to focus on a visual circle cue and identify a target letter presented briefly at its centre. Each trial begins by focusing on a central fixation cross for 2000 ms or 3000 ms. After the fixation, following a 300 ms tone, a large circle cue appears in a random location on either the left or right side of the screen. To orient the subject on the circle cue, the large circle begins to shrink until it reaches a fixed size after 1500 ms. It remains onscreen for either 2, 4, 8, or 12 seconds, at which time a white asterisk appears at the centre of the screen.

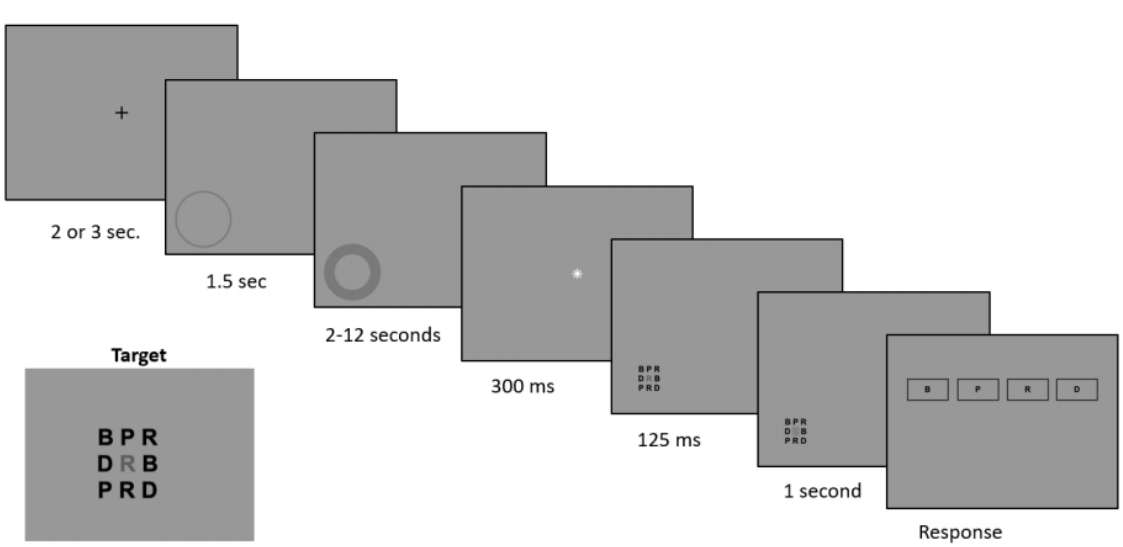

Figure 6: Sustained Attention to Cue task

The asterisk blinks on and off in 100 ms intervals for 300 ms. Then, a 3x3 array of letters appears at the location cued by the circle. The letters in the array consists of B, D, P, and R. The central letter is the target letter and appears in a dark grey font. The non-target letters appear in black font with each letter occurring twice. After 125 ms, the central letter is masked with a # for 1000 ms. Afterward, the response options are displayed in boxes across the upper half of the screen. The subject uses the mouse to select whether the target was a B, D, P, or R. Feedback is given during the practice trials but not the experimental trials. Target identification accuracy is the dependent variable.

Administration of this task takes approximately fifteen minutes and requires a standard computer mouse, QWERTY keyboard, and earphones. The test will be administered three times during the study – at entry, at 3-4 days post exposure, and at pre-discharge.

Ant-Saccade test: Subjects see a fixation cross lasting either 2000 or 3000 ms followed by an alerting tone for 300 ms. After the alerting tone, an asterisk appeared for 300 ms on either the left or the right of the screen followed immediately by a target “Q” or an “O” for 100 ms on the opposite side of the screen from the asterisk. The target letter is masked by “##” until they respond, or until 5000ms pass. The subject’s goal is to ignore the asterisk and instead look away to the other side of the screen to catch the target “Q” or “O.”

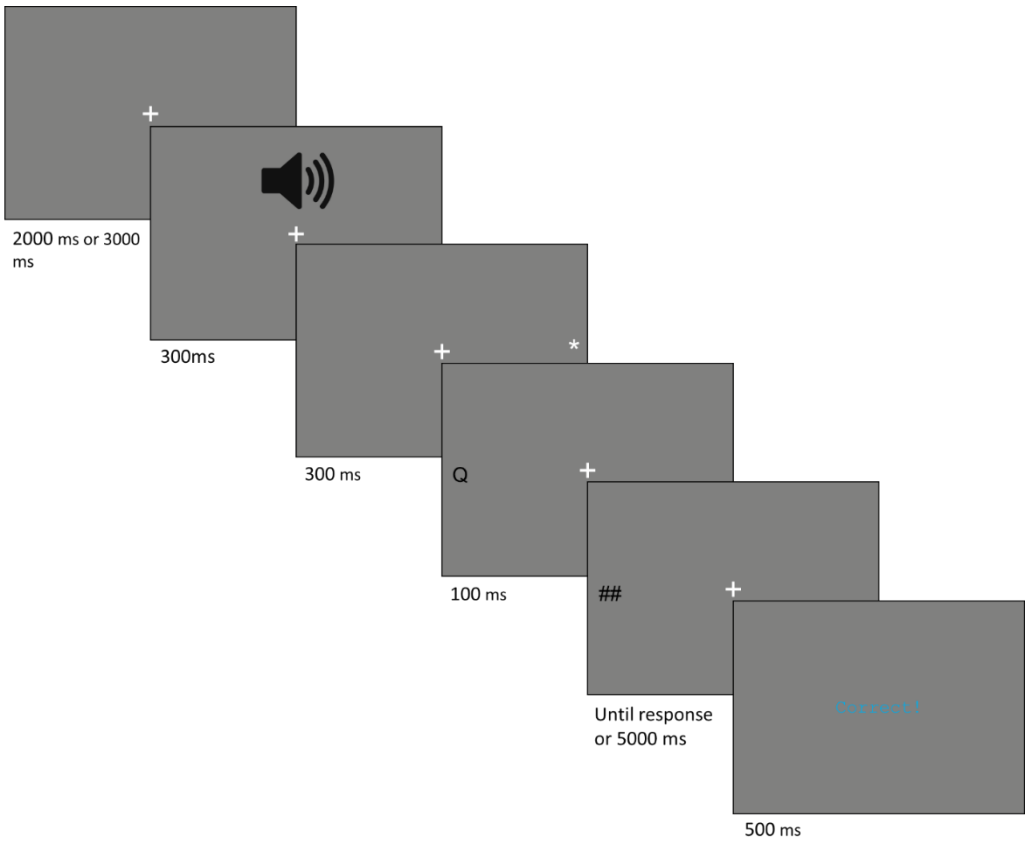

Figure 7: Anti-Saccade test

Subjects have as much time as needed to identify the letter that appears on that trial and do so via key press. Subjects will complete 72 trials, with trial-by-trial feedback for 500 ms following each response. A 1000 ms precedes the next fixation, which indicates the start of the next trial. The dependent variable is the number of correctly identified target letters. Administration of this task takes approximately five minutes and requires a standard computer mouse, QWERTY keyboard, and earphones. The test will be administered three times during the study – at entry, at 3-4 days post exposure, and then at pre-discharge.

1.19.5. Reading the Mind in the Eyes test

This task involves describing the emotional/mental state of a person based on only an image of their eyes, in a fixed-choice paradigm. In the Reading the Mind in the Eyes test, images are presented, followed with forced choice between four emotion terms. Only 1 term is correct, and the test should take ~20-30 minutes to complete. The test will be administered by paper questionnaire identified only with the participant identification number. The test will be administered at entry, mid challenge (~3-4 days post exposure), and then pre-discharge.

Instructions to the participant are given below:

- For each set of eyes, choose and circle which word best describes what the person in the picture is thinking or feeling.
- You may feel that more than one word is applicable but please choose just one word, the word which you consider to be most suitable.
- Before making your choice, make sure that you have read all 4 words.
- You should try to do the task as quickly as possible but you will not be timed.
- If you really don't know what a word means you can look it up in the definition handout.

Please see Appendix I for the Mind in the Eyes questionnaire.

1.19.6. Social Support Questionnaire and Interpersonal Support Evaluation List

The Social Support Questionnaire is a 27-item questionnaire designed to measure perceptions of social support and satisfaction with that social support. Each item is a question that solicits a two-part answer: Part 1 asks participants to list all the people that fit the description of the question, and Part 2 asks participants to indicate how satisfied they are, in general, with these people. The test is administered as a paper questionnaire (identified only with the participant identification number) and should take approximately 20 to 30 minutes to complete. The test will be administered once during the study, at entry to the quarantine unit. Please see Appendix II for the Social Support questionnaire.

The Interpersonal Support Evaluation List is a 40 point scale made up of 4 sub-scales (tangible support; belonging support; self-esteem support; appraisal support). Participants rate each item's statement on how true or false they believe it is for themselves. All answers are given on a 4-point scale ranging from "Definitely True" to "Definitely False". Please see Appendix III for the Interpersonal Support Evaluation List questionnaire.

ADVERSE EVENTS

1.20. Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- **Requires hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

1.21. Risks and expected adverse events

1.21.1. Risk Determination

All study procedures involve no more than minimal risk to participants. Similar procedures have been used for many years without severe adverse effects. These include blood and nasal sampling; participants will be counselled as follows:

Blood draws: risks include discomfort as the needle goes through the skin and/or bruising. Infection, excess bleeding, clotting, or fainting are also possible, although unlikely.

Nasal lavage (washings), SAM strips (a soft strip placed up the nose for two minutes), and nasal curettage (scrape): may tickle, make their eyes water or be slightly uncomfortable. They should not be painful. The throat and oral swabs are not painful.

Four devices are to be used within the study. All will be connected to a mobile device, which will have dedicated applications for data collection. All data collection will be via Bluetooth, with other connection methods deactivated, to reduce the risk of data loss or breach.

Bittium Faros device: risks are similar to typical wearable devices, the only known physical risk related to wearing these types of sensors relates to skin irritation caused by the electrodes. The device is not intended to be used at the same time with high frequency (HF) surgical equipment or with defibrillator, of which neither situation should arise during the study. If such a situation arises, the device will be removed. Participants will be counselled about this specifically.

Lumee oxygen sensor insertion using the Lumee Pen: risks include possible local bruising, minor discomfort during skin injection, allergic reaction, redness, bleeding, infection, itching, irritation, fibrosis and discolouration of skin at insertion site. The sensor is not removed following the study but is invisible and rarely palpable, with no long-term risks having been identified in pre-clinical and clinical testing.

Lumee Patch reader: temporary skin irritation and temporary redness are possible where the patch is in contact with skin. Minor eye injury or irritation (comparable to looking directly into sunlight) may occur if the reader is not used properly and the light from the reader shone directly into the eyes. Participants will be counselled specifically to avoid this.

Lumee sensor removal: the sensor is not intended to be removed following the study but can be removed if the participant specifically requests it. Removal of the sensor is a minor surgical procedure and as such may cause scarring, pain, bleeding, infection, and allergic reactions to local anaesthetic. Participants will be counselled about these specific risks if they request sensor removal.

Biovotion Everion device: risks are similar to typical wearable devices; the only known physical risk related to wearing these types of sensors relates to skin irritation. The device will be removed once per day for around an hour for charging, allowing the skin to breathe and to permit washing and showering.

For the study protocol as a whole, there is no more than minimal risk for minor side-effects or adverse reactions aside from the expected adverse events related to influenza infection.

1.21.2. Potential adverse events related to influenza infection

We would expect subjects to experience typical symptoms of a common cold (including, but not limited to: fever, headache, malaise, rhinorrhoea, nasal congestion, sneezing, sore throat, and cough). These would not be deemed adverse events, unless in the opinion of the study doctor. However, fever greater than 38ºC for more than three consecutive days or withdrawal from the study due to intolerable symptoms in more than two subjects will lead to a suspension of the study. The safety monitoring committee will be convened to determine any systematic cause for unexpectedly severe symptoms.

Any influenza-like illness resulting in

- **Sustained elevated heart rate >120bpm AND sustained low blood pressure SBP<100**
- **Sustained elevated respiratory rate >30/min AND sustained low blood oxygen SaO2<94%**
- **Evidence of pneumonia on clinical examination**
- **New ECG abnormalities (compared to baseline)**

will lead to discussion with the Principal Investigator and possible referral for assessment in Accident and Emergency. Hospitalisation of any subject will lead to immediate suspension of the trial. The safety monitoring committee will be convened to assess the clinical evidence in order to determine whether the study may proceed.

1.21.3. Potential adverse effects of chest x-ray

Each volunteer who is eligible to enter into the study will have a single chest X-ray at baseline prior to inoculation, which is entirely for research purposes. The estimated dose will be 0.014mSv (table 11, HPA-CRCE-012 2010 dose review) which is approximately equivalent to 2.5 days natural background radiation and carries risk of inducing a cancer of approximately 1:1,400,000 based on risk factors for a healthy adult. This is classified as a trivial risk level (ICRP 62).

1.22. Reporting procedures

A Safety Monitoring Committee (SMC) will be established to advise the trial team on study progression in the event of a safety issue. The SMC will be informed of all unexpected AEs at least monthly during the clinical study period and convene during the study if there are any serious adverse events, protocol deviations, or other safety issues. The SMC will be appointed and meet once (in person or by teleconference) prior to commencement of the study. It will consist of at least three members independent of the study team experienced in clinical trials and experimental medicine, with at least one member familiar with human challenge studies and one member experienced in respiratory infections. If any pausing rules or other safety issues arise, they will be convened to assess the clinical data and make a judgment on whether the study can proceed. In addition, study conduct will be monitored by a non-medical study monitor and medical monitor appointed by Duke University.

All adverse events should be recorded in the clinical record form. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance. In the event of an SAE, an SAE form should be completed and emailed to the Chief Investigator and the Sponsor within 24 hours of the event having been discovered by or notified to the study team. The safety monitoring committee, Duke medical monitor and co-investigator will also be informed within 24 hours and a meeting of the SMC convened as soon as possible.

All SAEs should be reported to the West London REC 2 Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures AND
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted to ethics, the sponsor and the R&D office within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies.

All non-compliance/unanticipated problems, adverse events, audits and investigation reports will be reported to DON HRPP and SSC Pacific.

The US Research Monitor (appointed by Duke University and approved by the Human Research Protection Office [HRPO] of the US Department of Defense) will be Emily Ko. The US Research Monitor is responsible for overseeing the safety of the research subjects and, when unanticipated problems involving risks to subjects or others associated with the protocol, for reporting of findings to the Institutional Review Board (IRB) or a designated institutional official. The US Research Monitor may discuss the research protocol with the investigators and may recommend that the PI/Sponsor stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects pending review by the IRB and/or SMC (see Monitoring SOP). Furthermore, they shall have the responsibility to promptly report their observations and findings to the ethics committee or other designated official and the HRPO.

1.22.1. Non serious AEs

All such events, whether expected or not, should be recorded. These will be discussed by the safety monitoring committee (see Section 12)

1.22.2. Serious AEs

An SAE form should be completed and faxed to the Chief Investigator and the Sponsor within 24 hours. The safety monitoring committee (see Section 12) will also be informed and a meeting convened as soon as possible.

All SAEs should be reported to the NRES London-Fulham Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted to ethics, the sponsor and the R&D office within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies.

Contact details for reporting SAEs
Fax: 020 7262 8913 for the attention of Dr Christopher Chiu
Please send SAE forms to: Infectious Diseases & Immunity, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN
Tel: 020 8383 2301 (Mon to Fri 09.00 – 17.00)

ASSESSMENT AND FOLLOW-UP

Study participants will be seen frequently during the study period, and at least daily during the confinement period following infection. They will be given details to contact the study doctor and research nurses at any time and will be offered daily telephone contact. In this way, participants will be assessed regularly by the investigating team and any adverse events detected rapidly; subjects meeting the criteria for a serious adverse event will be offered prompt treatment as appropriate.

Subjects will have completed the main study when they have had final convalescence investigations, expected to be 28 days after the initial inoculation with the influenza virus. Additionally, they will be invited to return for a further visit at 6 months for sampling to assess their longer-term immunity. The overall study will be completed when sufficient numbers of subjects have been recruited. The end of the study is defined as the last visit of the last participant.

When the study is completed they will not be routinely followed-up. Subjects will return to the care of their GP following completion of the study.
If a participant loses capacity during the study, then the participant would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.
The study is a low-risk non-CTIMP, carried out by an experienced PI and study team. The study has appropriate risk assessments in place and will be checked through the PRB system at ICRF.
Local monitoring will be carried out by study staff to ensure that CRFs are being completed, with no key data missing. Duke University will appoint one of their in house monitors to check the data produced, paper CRFs will be uploaded to their eCRF system. The monitor will check consent forms and data logs.

STATISTICS AND DATA ANALYSIS

Sample size estimation: the goal of this study is a proof-of-concept demonstration that physiological measures can detect influenza infection in the pre-symptomatic phase. As such, it is not possible to carry out a formal power analysis to determine the sample size, since no preliminary data on the effect size in influenza infection using the sensors in this study are available. However, heart rate variability (HRV) parameters have been shown to enable detection of sepsis with 86% sensitivity and 100% specificity in a study of 17 subjects (14 positive and 3 negative for sepsis)[5]. Therefore, we aim for a similar target for numbers of symptomatically infected individuals (13-15), which is likely to be achieved with a sample size of 20 subjects based on the previously established attack rate of 75% using this challenge virus. Larger studies would be required in the future to generate generalizable detection statistics, but the 20-subject sample size proposed here should be sufficient for concept demonstration.

Quantitative assessments of symptom scores, lung function, virus load, leukocyte numbers and inflammatory markers will be compared within subjects to determine differences between baseline and during infection. Intra-subject differences will be analysed using ANOVA and 2-tailed paired Student's t-tests or Wilcoxon signed rank test as appropriate. Correlations between inflammatory cell, illness severity, viral load and leukocyte counts will be examined using Spearman's rank correlations to investigate possible causal relationships.
We aim to determine if the different sensors in the study can measure changes associated with influenza infection. For each sensor we will define and compute multiple metrics from the raw sensor output. Each of these metrics will be compared in the healthy versus sick states for each subject. We will consider a metric to be a potential predictor of illness in a single subject if there is a significant ($p< 0.05$) difference between the mean value in the sick and healthy state. Control comparisons will be made by computing the significance of differences between two different healthy periods (e.g. prior to infection and post recovery).
The potential predictors of illness from the within subject analysis will be compared across all subjects to determine if any sensor metrics provide generalizable indicators of illness. A metric will be considered a valid indicator if it is present in at least 85% of the within subject sick/healthy comparisons and less than 25% of the of the within subject control comparisons. This odds ratio is sufficient to achieve 80% power and a 5% error rate with a sample size of 13 subjects. Based on an established attack rate of 75% with this challenge virus, we estimate that will have 15 of our 20 subjects develop influenza. Therefore, the sample size of this study is sufficient to determine the feasibility of using the selected sensors to measure changes associated with influenza.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period according to Imperial College London policy.

REGULATORY ISSUES

1.23. Ethics approval

The Study Coordination Centre has obtained approval from the Fulham Research Ethics Committee (REC) and Health Regulator Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

1.24. Consent

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered, time allowed for consideration, and any questions participants may have answered. Signed participant consent will be obtained prior to any screening tests being carried out. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

1.25. Confidentiality

The Chief Investigator and all of the research team will preserve the confidentiality of participants taking part in the study and abide by the Data Protection Act. All personal/identifying information will be stored in password protected files, accessed via on password protected computer accounts. Only the study research nurses and doctors will have access to this information. PID (Personal Identifiable Data) will be stored for 10 years as per College retention policy. Paper medical records will be stored as per Trust policy, within a key-coded and fireproof door medical records storage facility within the ICRF. The site

file and paper CRFs will be kept in a locked filing cabinet, in a locked office within the Commonwealth building at Hammersmith campus.
The investigators will adhere to the GDPR and Imperial College London policies (UK Policy Frame Work for Health and Social Care Research) for handling any data.

Indemnity
Imperial College London as sponsor of this study holds negligent and non-negligent harm insurance policies which apply to this study.

Sponsor
Imperial College London will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trust taking part in this study. Sensor data analysis will be carried out by RTI International.

Funding
This study is funded by the United States Department of Defense DARPA via a collaboration with Duke University. They are acting as sole funders and this agreement is in place. The investigators will not receive any additional payment above their normal salaries. Participants in the study will have their travel costs refunded up to £20 per visit. They will also be given a donation of up to £2500 to compensate for the time and inconvenience of taking part in the study. These expenses will be paid at the end of the main study period, 28 days post-influenza inoculation. Where participants have completed the confinement period but fail to attend further follow-up appointments, they will be paid on a *pro rata* basis according to the number of visits they have attended.

1.26. Audits and inspections

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

1.29. Sample storage and usage

Samples of tissue, cells and fluids will be stored at the Commonwealth building and Medical School building sites at Imperial College London. Samples will be fully anonymised. These may be used for further assays or in other ethically approved studies. Samples and data may be shared with UK and international collaborators in studies that have been approved by local ethics committee and subject to a valid Materials Transfer Agreement. Data and samples sent outside the UK will be fully anonymised with no patient identifiable data transferred. As part of the current study, samples and associated clinical, immunological and virological data will be sent to collaborators at Duke University for transcriptomics, proteomics and metabolomics analysis.

STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr Christopher Chiu, Clinical Senior Lecturer and Honorary Consultant in Infectious Diseases. In addition, a safety monitoring committee will convene monthly during the study to discuss all adverse events, protocol deviations, and other safety issues.

PUBLICATION POLICY

Our expectation is that after analysis the data from this study will be widely distributed in the medical and scientific community. Facilitated with presentations at local, national and international meetings, we hope to publish widely in the medical literature. In addition we have an excellent media department at Imperial College and will publicise research that has public interest when it is published. No identifying participant information will be published. The study will be registered on clinicaltrialls.gov.

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