Imperial College London

NHS Imperial College Healthcare NHS Trust

Study Protocol

Development of a SARS-CoV-2 Delta variant human infection challenge model (COVHIC002)

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Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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Funder

The Wellcome Trust has provided funding for this study. This protocol describes the COVHIC002 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 Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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2 STUDY SUMMARY

Title	**Development of a SARS-CoV-2 Delta variant human infection challenge model (COVHIC002)**

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Estimated Timeline	August 2022 – December 2024
Sites	A multi-centre study carried out in London and Oxford. London: A designated quarantine unit at Chelsea and Westminster Hospital NHS Trust, London. Screening and follow up visits will be primarily carried out by delegated members of the study team at the Imperial Clinical Research Facility (ICRF), Hammersmith Hospital, Imperial College Healthcare NHS Trust but could also be conducted by delegated members of the study team at the Imperial Clinical Respiratory Research Unit (ICRRU) at St Mary's Hospital, Imperial College Healthcare NHS Trust, or by delegated members of the study team at the Chelsea and Westminster Clinical Research Facility, Chelsea and Westminster Hospital NHS trust, dependent on capacity. Participants may also be seen in Imperial College Healthcare NHS Trust or Cheslea and Westminster Hospital NHS Trust for radiology procedures. Oxford: A designated quarantine unit in the Experimental Medicine Clinical Research Facility (EMCRF), University of Oxford. Screening and follow up visits will be primarily carried out by delegated members of the study team at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), or at the EMCRF within the University of Oxford. Participants may also be seen in Oxford University Hospital NHS Foundation Trust for radiology procedures.
Aims	This study aims to develop a safe, reproducible SARS-CoV-2 Delta variant human infection challenge model in adult volunteers to investigate factors associated with susceptibility and protection, and permit the future study of vaccines, antivirals and other interventions. To enable this, conditions (including pre-existing antibody level and inoculum dose) that result in reproducible infection in 50-70% of research participants (attack rate) will be identified. Study objectives are: 1. To establish the controlled human infection model in previously vaccinated seropositive participants, following nasal inoculation with escalating quantities of Delta-strain SARS-CoV-2 in order to reach an infection rate of 50-70%. 2. To characterise the clinical, virological and immunological responses following controlled inoculation of Delta variant SARS-CoV-2. 3. To identify determinants of breakthrough infection and correlates of protection in individuals with vaccine-induced immunity.
Design	The aim of this study is to inoculate successive cohorts with increasing titres of SARS-CoV-2 Delta variant in order to achieve target infection rates. A starting dose of $1x10^2$ $TCID_{50}$ will be given via intranasal drops to the initial cohort (n=5-10). If the ≥50% target attack rate is not achieved, escalating doses of SARS-CoV-2 will be admitted to subsequent groups and participants with low levels of anti-SARS-CoV-2 antibodies may be selected. If the ≥50% target attack rate is achieved after the first group of 5-10 at $1x10^2$ $TCID_{50}$ then there may be a dose de-escalation, and 6 participants may be given a dose of 1x10 $TCID_{50}$ after which the attack rate will be assessed and a decision on the dose level for expansion made. While the infection rate assessment should normally take place after at least 6 participants inoculated, if, due to unforeseen participant dropout, only 4 or 5 participants are enrolled in the initial quarantines, a preliminary assessment can be made. If <2/5 participants become infected and there are no safety concerns, dose escalation may take place. If only 4 participants are enrolled in the initial quarantines and no participants become infected and there are no safety concerns, a further 1-2 participants

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	will be enrolled at the lower dose to complete the cohort of 5-6 individuals but dose escalation may also take place within the same quarantine group as in both situations the target infection rate would still not be reached whatever the outcome of the additional 5th and 6th participant. At each dose escalation, a sentinel group of 4-10 participants will undergo assessment. Subsequent dose expansion quarantine groups are anticipated to include 6-12 participants depending on capacity, with a total group size of at least n=30. A Data Safety Monitoring Board will review safety and quantitative virology after every 4-12 participants and will make recommendations on continuation, dose escalation or de-escalation based on emergent data. A Trial Steering Committee will provide overall supervision of the project. Participants may be given an antiviral SARS-CoV-2 "rescue" therapy, using a treatment that has shown compelling evidence of efficacy in preventing risk of progression to severe COVID-19, if protocol-defined disease progression criteria are met. Decisions on treatment plans for cohorts of participants will be made by the CI in discussion with the DSMB if necessary. Participants will remain in the quarantine unit until the following criteria are met: • For participants with evidence of infection (defined as 2 consecutive viral detections by qPCR after day 2 post-inoculation): o A minimum of 14 days post-inoculation AND no viable virus in nose and throat swabs by overnight culture on two consecutive swabs AND a negative or overall decreasing viral load by quantitative PCR o Laboratory-performed lateral flow tests may be used in place of culture, if culture data are not available o A qualitative PCR may be used in place of quantitative PCR, if the latter is not available. o At the CI's discretion if protracted quarantine is deemed to be causing harm to the participant's mental or physical health and no viable virus is detected by culture or a lateral flow test is negative. • For participants who remain uninfected: o A minimum of 10 days post-inoculation AND 2 consecutive swabs with undetectable virus by PCR only prior to discharge.
Outcome Measures	**Primary Objective** • To identify a safe and infectious dose of SARS-CoV-2 Delta variant in healthy vaccinated volunteers, suitable for future intervention studies, that: o has an acceptable safety profile as measured by: ▪ occurrence of adverse events (AEs) from the viral challenge (Day 0) up to Day 28 follow up. ▪ occurrence of serious adverse events (SAEs) from the viral challenge (Day 0) up to Day 28 follow up. o induces laboratory confirmed infection in ≥50% of participants (ideally ≥70%). Laboratory confirmed infection is defined by:

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	▪ two quantifiable greater than lower limit of quantification (viral load ≥LLOQ) RT-PCR measurements from mid turbinate and/or throat samples, reported on 2 or more consecutive timepoints, starting from Day 2 hours post-inoculation and up to discharge from quarantine. **Secondary Objectives** • To further assess SARS-CoV-2 breakthrough infection rates in upper respiratory samples by qRT-PCR and cell culture • To assess the incidence of symptomatic SARS-CoV-2 breakthrough infection in vaccinated participants • To assess SARS-CoV-2 viral dynamics in upper respiratory samples (AUC, peak, duration, incubation period) • To assess SARS-CoV-2 induced symptoms (sum, AUC, peak, peak daily, frequency) **Tertiary/Exploratory Objectives** • To explore the safety of the SARS-CoV-2 Delta variant human challenge model (smell, cognition, spirometrysafety laboratory tests, concomitant medications) • To explore the SARS-CoV-2 viral dynamics in saliva, by qRT-PCR and cell culture (AUC, peak, duration, incubation period) • To explore SARS-CoV-2 viral infection rates in stool swabs, by qRT-PCR • To investigate the detection of SARS-CoV-2 breakthrough infection by lateral flow antigen tests • To explore the host-pathogen relationship in the SARS-CoV-2 Delta human challenge model (including humoral and cellular immunity, proteomics, transcriptomics, host and viral genomics, microbiome and systems biology) • To explore environmental contamination in SARS-CoV-2 Delta-infected participants (longitudinal quantitation and detection of virus in air sampling, exhaled breath, hand and surface swabbing), including during manoeuvres such as singing and reading aloud • To measure changes in the vasculature during SARS-CoV-2 breakthrough infection
Population	Sero-suitable healthy volunteers 18-30 years of age (inclusive) with no known risk factors for severe COVID-19
Number of Participants	up to 120
Eligibility	• Healthy volunteers, aged 18-30 with no underlying co-morbidities • Completed SARS-CoV-2 vaccination course • Sero-suitable as defined by anti-S and anti-N antibody detection • Non-smokers, normal BMI, no risk factors for severe COVID-19 • Normal FBC, U&Es, LFTs; negative for HIV, HBV, HCV; no evidence of immunosuppression; normal chest X-ray, spirometry and ECG
DURATION	The Sponsor estimates that for each participant, the duration of the study will be approximately 52 weeks from the time they sign the informed consent form until their last study related follow up.
KEYWORDS	SARS-CoV-2, COVID-19, immune, virus, viral challenge, viral lung disease, infection

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3 SCHEDULE OF ACTIVITIES

Study Day	Pre-screening	Screening -	D-2	D-1	Day 0 Pre	D0 Challenge	Day 0 Post	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11 (s)	D12 (s)	D13 (s)	D14 (s)	Extended stay days	Day 21 (t)	Day 28	Day 90	Day 180	Day 270	Day 360	Early withdrawal	COVID-19 Testing V1 (%)	COVID-19 Testing V2 (%)
Window		-90 to -3 days																					+/- 2 days	+/- 3 days	+/- 7 days	+/- 14 days	+/- 14 days	+/- 14 days			28 days after V1 +/- 7 days
Written informed consent	X	X																													
Eligibility criteria (a)	X	X		X[k]	X																										
Medical & medication history		X																													
Demographics	X	X																													
Height & weight, BMI (b)		X		X[k]																											
Patient Health Questionnaire (PHQ-9) (w)		X		(X[k])													(X)				(X)	(X)									
Generalised Anxiety Disorder Questionnaire (GAD-7) (w)		X		(X[k])													(X)				(X)	(X)									
Urinalysis		X		X[k]							X			X			X				X										
Urine drugs of abuse & nicotine screen		X		X[k]																											
Urine pregnancy test		X			X																										
Complete physical examination (w)		X		X[k]							X			X			X				X	(X)									
Directed physical examination					(X)		(X)	(X)	(X)	(X)		(X)	(X)		(X)		(X)	(X)	(X)	(X)		(X)		X	X	X	X	X	X	(X)	(X)
Vital signs (HR, RR, SBP, DBP, SpO_2 (c) (d) (x)		X	(X)	QDS	QDS			QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS		X	X	X	X	X	X	(X)	(X)
Temperature (d) (x)		X	(X)	QDS	QDS			QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS	QDS		X	X	X	X	X	X	(X)	(X)
Symptom diary cards (x)			(X)	TDS	TDS			TDS	TDS	TDS	TDS	TDS	TDS	TDS	TDS	TDS	TDS	TDS	TDS	TDS	TDS	TDS		X	X	X	X	X	X		
Smell Test (UPSIT) (u)				X				X			X			X			X			X		(X)		X	X	X	X	X			
Cognitive Tests (v)				X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X			
Chest X-ray		X																													
12-lead ECG (d)		X		X[k]	X			(X)	(X)	(X)	X	(X)	(X)	X	(X)	(X)	X	(X)	(X)	(X)	X	(X)		(X)	(X)	(X)	(X)	(X)	(X)		
Spirometry		X	(X)					(X)	(X)	(X)	X	(X)	(X)	X	(X)	(X)	X	(X)	(X)	(X)	X	(X)		(X)	(X)	(X)	(X)	(X)	(X)		
Challenge Virus inoculation						X																									
Administration of SARS-CoV-2 rescue treatment								(X)[y]																							
Serum β-HCG pregnancy test (all females) (e)				X[k*]																											
HIV, Hepatitis B & C		X																													
Thyroid function test		X																													
Coagulation (including d-dimer)		X		X[k]					X			X		X				X			X			X		X		X	X		
Haematology (f)		X		X[k]					X			X		X				X			X			X		X		X	X		
Biochemistry (f)		X		X[k]					X			X		X				X			X			X		X		X	X		
Troponin and creatine kinase		X		X[k]					X			X		X				X			X			X		X		X	X		
Blood - HLA typing (g)		X																													
Blood - Antibodies SARS-CoV-2 (h)	X			X[k]																	X			X	X	X	X	X	X	(X)	(X)
Blood - plasma markers (g)				X[k]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X		(X)	(X)
Blood paxgene RNA (g)				X[k]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X		(X)	(X)
Blood Lithium Heparin – PBMCs, other blood cells, +/- plasma (g)				X[k]				(X)		X		(X)		X[s]			X[s]				X[s]			X	X	X	X	X		(X)	(X)
Dried blood spot (fingerprick capillary blood sample) (g)	(X)																							(X)	(X)	(X)	(X)	(X)			

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Study Day	Pre-screening	Screening	D-2	D-1	Day 0 Pre	D0 Challenge	Day 0 Post	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11 (s)	D12 (s)	D13 (s)	D14 (s)	Extended stay days	Day 21 (t)	Day 28	Day 90	Day 180	Day 270	Day 360	Early withdrawal	COVID-19 Testing V1 (%)	COVID-19 Testing V2 (%)
Window		-90 to -3 days																					(+/- 2 days)	(+/- 3 days)	(+/- 7 days)	(+/- 14 days)	(+/- 14 days)	(+/- 14 days)			28 days after V1 +/- 7 days
Nasopharyngeal swab for respiratory pathogen screen - e.g. Biofire (i) (z)			X[k]																												
Throat FLOQ swab - Virology (g) (j) (x)		X	X					BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]		(X)	(X)	(X)			X	(X)	(X)
Mid turbinate FLOQ swab - Virology (g) (j) (x) (z)		X	X					BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]		(X)	(X)	(X)			X	(X)	(X)
Nasal curettage (Rhinopro) and/or nasopharyngeal swab for cells for RNA (g) (l) (z)		X	X					X		X		X		X			X				X			(X)						(X)	(X)
Saliva (g) (x)			X					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		(X)					X		
Self-performed lateral flow antigen test (g) (m) (z)			X					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X									
Nasosorption – immunology & virology (g) (n) (x) (z)		X	BD[1]		X			BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]	BD[1]		X	X	X	X	X	X	(X)	(X)
Mask wearing sampling (g) (o) (x)			X				(X)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		(X)							
Breath aerosol sampling (g) (p) (£)			(X)							(X)				(X)			(X)				(X)			(X)							
Environmental viral sampling (g) (q) (x)			X					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X									
Vascular measurements (EndoPATTM) (g) (r) (£)			(X)							(X)				(X)			(X)				(X)										
Stool & throat swabs for microbiome (g)			X					X	X	X	X	X	X	X	X	X	X	X	X	X	X			(X)	(X)	(X)	(X)	(X)			
AE recording			←----------																										X	X	X
SAE recording			←----------																										X	X	X
Concomitant medications		X	←----------																										X	X	X

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X	Once
( )	Parenthesis indicates the assessment is optional, or at the PI's discretion.
BD[1]	Performed once daily with an optional second sample. The timing of the baseline assessment will be the guide to establish the windows for subsequent measurements. For scheduling purposes, the baseline assessment will be defined as the first day when BD measurements are performed. Subsequent sampling/measures will be performed at a similar time.
TDS	Three times daily. The timing of the baseline assessment will be the guide to establish the windows for subsequent measurements. For scheduling purposes, the baseline assessment will be defined as the first day when TDS measurements are performed. Subsequent sampling/measures will be performed at a similar time.
QDS	Four times daily. The timing of the baseline assessment will be the guide to establish the windows for subsequent measurements. For scheduling purposes, the baseline assessment will be defined as the first day when QDS measurements are performed. Subsequent sampling/measures will be performed at a similar time.
a	Only the applicable Inclusion/Exclusion criteria will be reviewed at each time point.
b	Height will be taken at Screening only. Weight may be repeated in quarantine as required by local hospital policies or guidelines
c	During quarantine, vital signs will be taken at a similar time each day
d	Assessments may additionally be continuously monitored (e.g. core temperature; vital signs (HR, RR, SBP, DBP, SpO_2) as per Section 8.1.
e	Blood serum pregnancy test (ß-HCG) will be performed in all female participants.
f	Blood will be drawn under non-fasted conditions. Repeat bloods may be drawn under fasted conditions if a lipid profile (triglyceride) or glucose is required (at PI discretion).
g	Samples for related exploratory research. If a participant does not open their bowels on a given day, the stool swab sample will not be collected and this will not be considered a protocol deviation. If the plasma sample timepoints fall over a weekend or bank holiday, they may not be collected due to staffing capacity and this will not be considered a protocol deviation.
h	Virus serology will be performed to determine eligibility and seroconversion.
i	Nasopharyngeal swab (or other upper respiratory tract sample type, as per local SOP) for respiratory virus screen to assess for the presence of other respiratory viruses; if found positive the participant will not be eligible for the current quarantine and will be required to wait a minimum of 4 weeks before re-admission as per the inclusion/exclusion criteria.
j	Post inoculation nasal virology samples will be collected and used for RT-qPCR and viral culture assay (as appropriate). Samples may be used for related exploratory research
k + k*	Can be performed on Study Day -2 or Study Day -1. For Blood Lithium Heparin, this can be collected on Study Day -2 , Study Day -1 or Day 0 pre-inoculation. *Special Note:* k* Serum pregnancy test should be performed on either day -2 or -1, prior to Urine Pregnancy Test on Day 0 (pre-inoculation).
l	Nasal curettage or nasopharyngeal swab performed on alternate nostrils at each successive timepoint. If both performed on same day, these will be on different nostrils.
m	Participants will perform a lateral flow test themselves as per manufacturer's instructions, as per Section 8.10.2.. The participant will not be instructed by study staff and should follow written instructions from the manufacturer. Once performed, the lateral flow will be removed by study staff and the result read. The participant will not read the result.
n	One to two nasosorption will be taken at each time point and will be collected from the opposite nostril to that of the nasal swabs and alternated each day. Nasosorption will be the first sample collected, prior to any other nasal sampling and where two are collected, there will be at least a 5 minute pause between collection. Samples may be used for: cytokines/chemokines, sIgA, and virology, as well as stored for future usage.
o	Participant will be asked to wear one or two single-use facemasks for up to 60 minutes per mask, once a day to capture exhaled virus, as per Section 8.11.1. Participants may be asked to sing or speak during sampling. If throat and mid-turbinate swabs are negative, sampling may be withheld at discretion of the CI/PI.
p	Participants will be asked to wear a mask or breathe into a mouthpiece for 5-10 minutes, for quantification and sizing of exhaled aerosols, as per Section 8.11.2. Participants may be asked to perform respiratory manoeuvres e.g. vocalisation, coughing.
q	Environmental viral sampling from air, surfaces and hands. If throat and mid-turbinate swabs are negative, sampling may be withheld at discretion of the CI/PI.
r	Non-invasive measurements of endothelial function using an EndoPAT™ machine, as per Section 8.13. The Day 14 measurement will only be performed on participants who remain in the quarantine unit.
s	Participants who are uninfected may be eligible for discharge from the quarantine unit on day 10 (Section 6.3.2), at discretion of the CI/PI. They will be required to attend for daily visits on day 11, day 12, day 13 and day 14 for procedures stated in the SoA. Procedures will be performed at a maximum frequency of OD, at the time of the day 11-14 visit. ECG and complete physical examination are not required to be performed at these visits. Directed physical examination may be performed on day 14 at the PIs discretion. Participants who are infected will remain in the quarantine unit until at least Day 14 and continue with proceduces as stated in the SoA, until they meet the discharge criteria (Section 6.3.2).

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t	Telephone call with participant.
u	The UPSIT is designed to be self-administered after explanation of the test by study staff and will be performed once before virus inoculation and then at least every third day starting from Day 1, though the test can be conducted more frequently at the discretion of the PI/study physician. If at Day 28 anosmia has subsided and smell has returned the UPSIT test can stop. If anosmia is still present UPSIT should continue until resolution or the end of the study.
v	Cognitive tests performed once daily, at a similar time each day during quarantine and similar timing where possible during follow-up visits.
w	Before discharge from quarantine each participant will undergo a complete physical examination (the date of this may vary dependent upon length of stay) and may, at the CI/PIs discretion, undergo repeat GAD-7 and PHQ-9 questionnaires once.
x	Procedures should be performed at the appropriate scheduled timings, up until the point of discharge i.e. if the participant is being discharged at lunchtime, procedures that would normally be done in the evening will not be done
y	Participants may be given a SARS-CoV-2 rescue therapy, if disease progression criteria are met, as detailed in Section 9.1.
z	Where any nasal sampling time points occur together, the order of sampling will typically be (1) nasosorptions followed by (2) mid turbinate swab, (3) lateral flow test (4) nasopharyngeal swab and (5) nasal curettage.
£	These procedures may only be preferentially performed on participants after dose optimisation
$	Optional additional 20mL blood (lithium heparin) may be taken at one of these time points.
%	After discharge from the quarantine unit, participants who develop symptoms of COVID-19 will be requested to self-perform a lateral flow antigen test, as detailed in Section 6.4.1. Participants without symptoms who test positive for SARS-CoV-2 in the community (on a lateral flow test or RT-PCR) will also be requested to contact the study team. For those who test positive for SARS-CoV-2, the study team may request the participant attends for Testing Visit (Visit 1 within 5 days of symptom onset and Visit 2 28 days (+/-7 days) later). The participant may be asked to self-perform an RT-PCR swab before Testing Visit 1. If Testing Visit 2 falls within the window of a scheduled visit, the visits may be combined. If face-to-face visits are not possible, either onsite or at the participant's home, a telephone appointment may be arranged to collect information on symptoms, concomitant medications and COVID-19 test results
Notes:	For all participants QDS/TDS assessments on Day 0, the first assessment will be pre-virus challenge. The PI or delegated clinician may perform additional safety assessments as required.

Table 1 Key notes for SoA

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4 INTRODUCTION

4.1 BACKGROUND

Ninety-nine percent of the UK and a large proportion of the world's adult population is now SARS-CoV-2 seropositive due to a combination of vaccination and/or prior infection resulting in various levels of immunity. Along with high seroprevalence in children, the evolutionary selection pressure that this exerts is almost certainly driving development of new Variants of Concern (VOCs), which may emerge with the potential for greater transmission as well as more severe disease. With high levels of immunity to the Omicron variant following widespread community transmission and the relaxation of control measures, it is a real possibility that future VOCs will emerge that are distinct from Omicron and could exhibit characteristics more akin to pre-Omicron variants, such as Delta. A human infection challenge model with the Delta strain in seropositive participants would provide an important tool for early testing of therapeutic interventions that will be immediately available in the eventuality that a future VOC may arise from a Delta or similar lineage. Deliberate human infection of low-risk volunteers has previously been established to evaluate the measurement of viral dynamics, immunological responses, transmission dynamics and duration of infectious virus shedding after inoculation of sero-negative participants with an early wild-type Wuhan strain of SARS-CoV-2[1].

Until recently, all currently-approved COVID-19 vaccines were based on the original wild-type SARS-CoV-2 spike (S) protein, though recently bivalent booster vaccines that include an Omicron-specific component are being rolled out to vulnerable patient groups in the UK. In the face of newer antigenically-divergent variants, vaccine effectiveness has fallen. This, coupled with the fact that even in healthy people vaccine-induced antibody levels have been shown to wane rapidly, has necessitated the roll-out of booster vaccine doses. In addition, although first generation vaccines are disease-modifying, their transmission-blocking potential is limited, with high rates of vaccine breakthrough causing upper respiratory tract infections that continue to drive transmission, especially since the Delta and Omicron variants. Future vaccines should offer improvement in tolerability, length of protection, or ability to actually block infection and prevent transmission.

Over 85% of UK persons aged >12 years old have now received at least two doses of a first-generation vaccine and over 65% have received a 3rd dose[2]. While this has had a dramatic effect on reducing the level of serious illness and death from COVID-19, access to effective vaccines globally remains inconsistent and a large proportion of the world's population remain unvaccinated. Furthermore, substantial numbers of immunocompromised individuals who mount inferior responses to vaccination remain at risk of severe illness. The shortcomings of currently-approved vaccines highlight the pressing need to develop new and improved vaccines and interventions that will address the problems of new variants and avoid the need for repeated boosters. This relies on understanding the factors that influence how variants cause infection despite pre-existing vaccine-induced immunity. Identifying correlates and mechanisms of protection against infection and shedding of virus in vaccinated individuals, as well as understanding the durability of vaccine-induced protection are therefore key questions for research. SARS-CoV-2 human challenge studies can uniquely address these outstanding questions in a controlled setting and accelerate the clinical development of new interventions. Importantly, these studies going forward will have increased safety now that 99% of the volunteer population are seropositive from vaccination or infection; there is fuller

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understanding of the consequences of COVID-19; and highly effective antiviral treatments have been shown to significantly reduce the risk of progression to hospitalisation.

4.2 WHY DOES THE COVID-19 CHALLENGE MODEL REMAIN IMPORTANT?

Human challenge involves the deliberate infection of volunteers to allow detailed investigation of host–pathogen interactions and the effect of interventions. The strength of these studies lies in their controlled nature, with use of standardised amounts of well-characterised virus across carefully selected participant groups. This enables the exact longitudinal measurement of viral kinetics, immunological responses, transmission dynamics and duration of infectious shedding following experimental inoculation. By giving all study participants the same virus at the same dose and under the same conditions, confounding by virus strain, dose and exposure (which unavoidably limit the interpretation of all natural infection studies) is controlled. Host factors associated with inter-individual differences in clinical outcome as well as the effect of interventions can then be robustly inferred.

This contrasts with even the most well-controlled field trials, including household contact studies. There, the viral quasi-species (i.e. mixture of slightly-differing virus particles), dose, timing and conditions of exposure cannot be known, and contacts are only identified following diagnosis of the index case. At this time, secondary exposure has almost always already occurred, thus missing transmission events as well as the early phase of infection. Human challenge is therefore the only study design where the earliest pre-symptomatic changes post-infection may be studied. These early timepoints are critical to understanding how some people who are exposed to infection resist it and to delineate early infectiousness and transmissibility.

With the recognised limitations of existing vaccines and impact of novel variants, the development of next-generation vaccines remains a priority. However, as the virus moves towards endemicity, the path to licensure for novel vaccine and treatment candidates is becoming increasingly difficult. Maintaining unvaccinated placebo groups in field trials is now ethically impossible in most parts of the world and erratic transmission due to varying levels of pre-existing immunity and public health measures means that capacity for phase III trials to show efficacy in a timely fashion is increasingly limited. SARS-CoV-2 human challenge studies therefore remain an important strategy for efficacy testing to accelerate the clinical development of new antivirals and vaccines.

The first SARS-CoV-2 human challenge study was conducted in 2021 and showed no serious safety concerns in 36 healthy young adults (see Section 4.2.1). However, it is no longer possible in the UK to conduct SARS-CoV-2 human challenge studies in seronegative individuals, since an estimated >99% of the UK adult population now have antibodies against SARS-CoV-2[3]. However, the recent VOCs (Delta and Omicron) have shown high rates of vaccine breakthrough infection. This indicates that human challenge with a VOC may be optimised as a platform for the rapid testing of vaccines and therapeutics in small numbers of participants despite pre-existing immunity, especially between waves of the pandemic, when occurrence of natural disease is relatively uncommon.

Importantly, a model of vaccine breakthrough infection will have a substantially improved safety profile compared with the first (seronegative) study as:

1. **The risk of severe disease and protracted symptoms are reduced by vaccination**
2. **Highly effective antivirals are available as rescue therapy**

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3. Data from the earlier SARS-CoV-2 human challenge study will underpin study design and enable more robust informed consent

SARS-CoV-2 human challenge causing breakthrough infection will therefore enable efficient early-stage testing of the many vaccine and antiviral candidates in development so that the most promising can go forward quickly enough to large-scale field efficacy trials to meaningfully tackle the ongoing pandemic. In particular, human challenge will be the only way to provide timely efficacy readouts for products that:

- **Do not elicit circulating neutralising antibodies** and therefore cannot be approved on the basis of "immunobridging" studies where antibody levels are used as a predictor of efficacy;
- **Aim to block transmission** by reducing upper respiratory tract viral shedding, which can only be consistently measured in the controlled setting;
- **Seek to have cross-strain efficacy**, where demonstrating protection against multiple antigenically-distinct strains can only be achieved in the human challenge model where different strains are available.

The first human challenge study with wild-type (pre-Alpha, Wuhan-like) SARS-CoV-2 (COVHIC001)

1. Safety and tolerability of SARS-CoV-2 challenge of seronegative young adults

Between March and July 2021, the world's first human challenge study with wild-type SARS-CoV-2 was carried out at the containment unit of The Royal Free Hospital NHS Trust[1]. The primary objective was to identify an inoculum dose that was safe and well tolerated in a healthy young adult volunteer cohort with no pre-existing immunity, while resulting in an infection rate of at least 50%. By demonstrating the safety, tolerability and feasibility of the experimental approach, this study was the first step in establishing SARS-CoV-2 human challenge as a platform for efficacy testing of interventions as well as investigating correlates and mechanisms of protection against COVID-19.

Thirty-six participants aged 18-29 years were inoculated with a GMP SARS-CoV-2 (D614G-containing pre-Alpha "wild-type" virus) at the lowest inoculum dose quantifiable by viral culture (10 $TCID_{50}$) by intranasal drops. Eighteen participants developed PCR-detectable virus in the throat and nose. With 2 participants later found to have seroconverted between screening and inoculation, this gave an attack rate of 53% in seronegative volunteers.

Overall, SARS-CoV-2 human challenge was shown to be safe and well tolerated at this inoculum dose in this group of seronegative healthy young adults. There were no study-related serious adverse events and no criteria for commencing rescue therapy were met. Symptoms were reported by 16 (89%) infected individuals that began 2-4 days post-inoculation (Figure 1a). All symptoms were mild-to-moderate (Figure 1b). Symptoms were most frequent in the upper respiratory tract and included nasal stuffiness, rhinitis, sneezing and sore throat. Systemic symptoms of headache, muscle/joint aches, malaise and feverishness were also recorded. Other than temperature >37.8C in 7 (37% of infected) volunteers, there were no notable disturbances in any clinical assessments. There was no evidence of lung involvement based on normal thoracic CT scans at 5 and 10 days post-infection and normal spirometry throughout.

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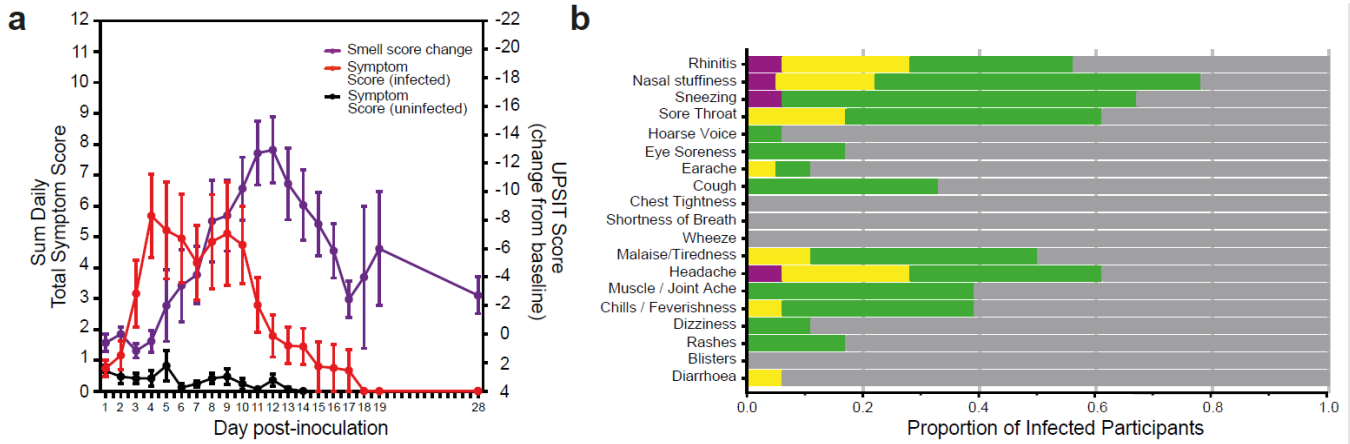

Figure 1: Mild-to-moderate symptoms occur following SARS-CoV-2 human challenge

A total of 13 adverse events deemed probably or possibly related to virus infection were noted. These were largely due to transient and non-clinically significant leukopenia and neutropenia, and mild muco-cutaneous abnormalities (mainly dry skin and rashes) during the quarantine period. One instance of epididymal discomfort occurred, which was deemed unrelated to the challenge agent and resolved completely. One participant was hospitalised ~6 months post-inoculation with left-sided loin pain and a presumed passed kidney stone. This SAE was deemed not related to the challenge infection.

Fourteen infected participants (78%) reported some degree of smell disturbance. Complete smell loss (anosmia) occurred in 9/18 infected individuals (50%), but most experienced rapid improvement before day 28. At day 28, some level of smell disturbance (mostly mild) was still reported by 11/18 participants (61%) and by day 180 this number had fallen to 5. Of these, measurable smell reduction persisted in only one individual after day 180 post-inoculation, although this was steadily improving both subjectively and objectively (UPSIT at baseline=31, day 11=9, day 28=11, day 90=17, day 180=23, day 270=26, with a difference from baseline of >4 considered abnormal). No other participants described smell-related AEs at day 270 follow-up. On direct questioning, all individuals with protracted smell-related symptoms tolerated their residual smell disturbances well and there was no impact on activities of daily living. Six individuals received smell training advice, including 2 who also received treatment with short courses of oral and intranasal steroids.

Thus, while smell disturbance was common in those with no prior immunity against SARS-CoV-2, complete recovery occurred rapidly in most individuals, with slower but steady recovery in a minority with more protracted partial anosmia. This was consistent with field data showing complete recovery in 96% of community cases by 12 months post-infection[4]. Volunteers had been specifically counselled regarding the risk of prolonged smell disturbance as part of the informed consent process, and those with prolonged smell reduction tolerated this well. There were no other prolonged symptoms or safety concerns.

Preliminary analysis of the cognitive tests performed daily during admission and at each follow-up visit showed divergence between those who became infected and those who remained uninfected post-challenge, with lower scores on average in the infected group for a small minority of tests. Participants did not describe “brain fog” or any other associated symptoms, so there was no clear functional association and these differences were deemed, by definition, sub-clinical. The changes were small in effect size and seen primarily in the Object Memory tests that were developed as highly sensitive assessments of short-term memory, at a precision beyond any tests commonly used in clinical practice. The statistical significance of differences within individual tests fluctuated substantially during late

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quarantine and follow-up, suggesting reduced signal:noise ratios at these later timepoints and limiting interpretability. Similar changes have been seen in other studies of young adults following mostly mild natural COVID-19, with test scores showing evidence of normalising with time in some key studies, including one that used the same memory task (Zhao et al. Brain Commun. 2022; 4(1): fcab295). They have also been observed in natural infection by other respiratory viruses (Smith AP. Brain, Behaviour and Immunity 2012. 26;1072 & Smith AP. Psychoneuroendocrinology 2013;38:744). However, the clinical relevance of these findings currently remains uncertain as these research tools have yet to be validated in larger clinical infection cohorts, where the scale of these differences, their impact and duration need to be tested. In preliminary data from the REACT community study, these effects are even less marked after Delta and Omicron breakthrough infections later during the pandemic, technically being within the negligible range, which given the larger population sample (n=120,000) suggests they may be less apparent in upcoming studies (Adam Hampshire, personal communication).

2. Viral dynamics in the upper respiratory tract – key findings

1. *Virus is detected earlier in the throat but peaks at higher levels in the nose*

   In the 18 individuals with PCR-confirmed infection, viral detection by qPCR became quantifiable in throat swabs from 40 hours (~1.67 days) post-inoculation, significantly earlier than in the nose, where initial viral detection occurred at 58 hours (~2.4 days) post-inoculation (Figure 2a & 2b). This was initially closely paralleled by viable virus measured by focus forming assay (FFA)(Figure 2a). Viral loads (VL) increased rapidly thereafter, with qPCR peaking in the throat at 112 hours (~4.2 days) post-inoculation and later at 148 hours (~6.2 days) post-inoculation in the nose (Figure 2a). At its peak, VL was significantly higher in nasal samples at 8.9 log10 copies/mL and 3.9 log10 FFU/mL than in the throat at 7.6 log10 copies/mL and 2.9 log10 FFU/mL (Figure 2c) for qPCR and FFA respectively.

2. *Prolonged viral detection in mild/asymptomatic infection of young healthy participants*

   In both nose and throat, viral shedding continued at high levels for several days and extremely high cumulative VLs by area under the curve (AUC) were therefore seen, particularly in the nose. In all infected participants, quantifiable virus by qPCR was still present at day 14 post-inoculation which necessitated prolonged quarantine of up to 5 extra days. At these later timepoints, VLs by qRT-PCR were more erratic, with low level qPCR positivity remaining in 15/18 (83%) at discharge. At day 28 post inoculation 6/18 (33%) remained qPCR positive but by day 90 these participants were all qPCR negative. Of the participants not meeting infection criteria, low level non-consecutive viral detections were observed by qPCR only in the nose of 3 participants and throat of 5 participants but were not associated with any rise in antibodies. In contrast, viable virus was detected by FFA for a more limited duration: 150 hours (6.5 days) in the nose and in the throat for 132 hours (6.25 days)(Figure 2d). Despite relatively high levels of late qPCR detection, the latest that viable virus could be detected was day 12 post-inoculation in the nose and day 11 in the throat.

   Importantly, despite the temporal association between VL and symptoms, there was no correlation between the amount of viral shedding by qPCR or FFA and symptoms (Figure 2e & f) with high VLs detected even in asymptomatically-infected individuals.

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3. *Lateral flow assays correlate with infectious virus and are reliable for shortening self-isolation "test-to-release"*

   Lateral flow antigen (LFA) tests are commonly used in the in UK and elsewhere to test for infectiousness in asymptomatic individuals or as a test-to-release from isolation. To assess the accuracy of LFAs in predicting viral detection by PCR and FFA, LFAs were performed on the same morning nose and throat swab samples assessed for VL. Results identified a delay of around 24 hours to LFA positivity relative to culture in 50% of infected participants. Towards the end of infection, the last LFA detection mainly occurred 24-72 hours after viable virus detection had ceased. Modelling showed that daily LFA testing would detect >90% of culturable virus and twice weekly LFA testing would capture 70-80%. This supported key UK public health policy decisions, demonstrating that LFA positivity is strongly associated with culturable virus and therefore contagiousness and is reliable as a trigger for interventions to interrupt transmission.

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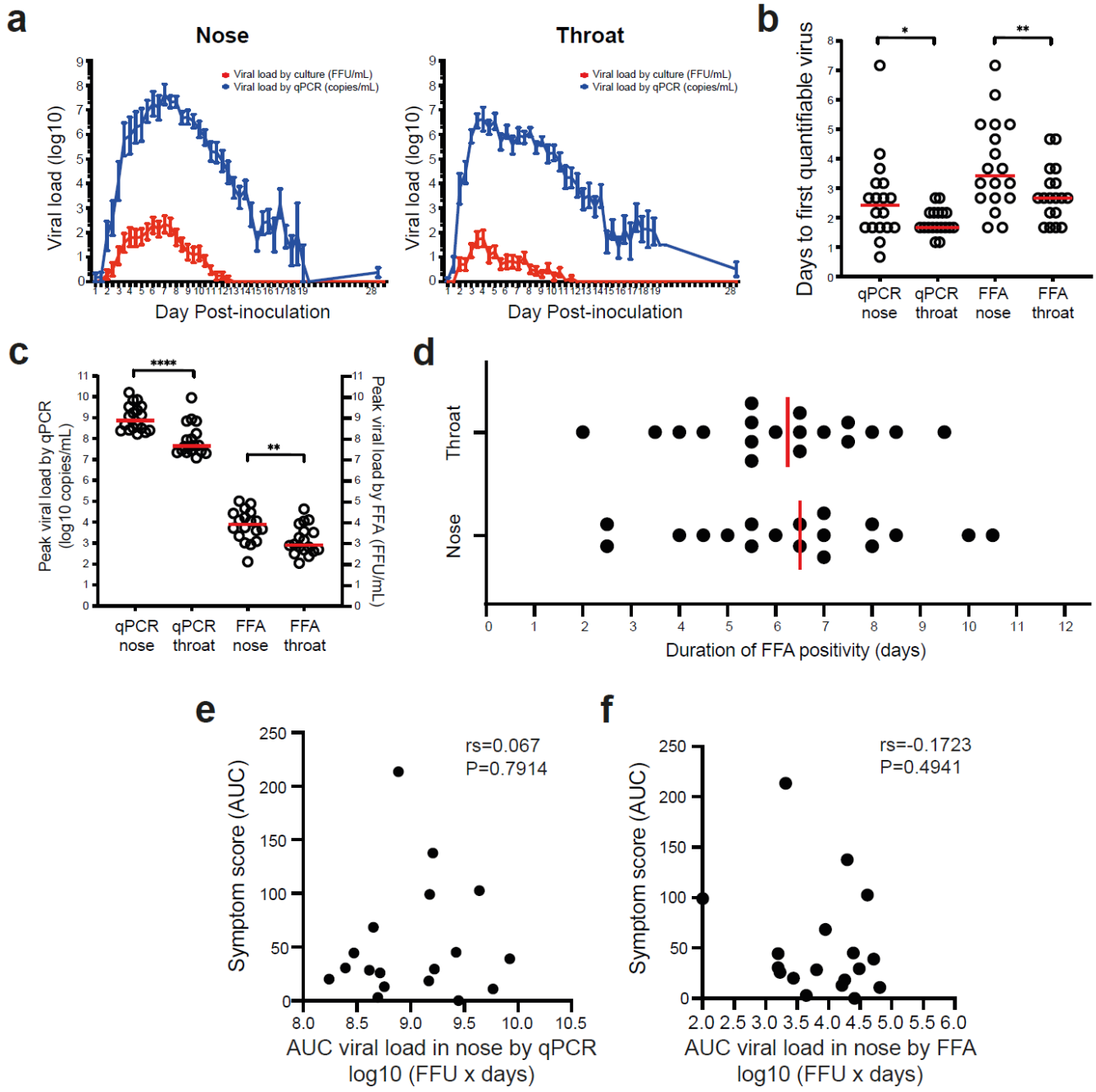

Figure 2: High levels and prolonged viral load do not correlate with symptoms during SARS-CoV-2 human challenge infection

Impact and future directions from the first human challenge study

3. What impact has COVHIC001 had so far?

These initial results have provided (for the first time) a robust and detailed characterisation of the entire course of infection, including the incubation period and duration of infectiousness, which had not previously been accurately defined, as well as the symptom profile and characteristics of mild and asymptomatic infection. The findings support the longitudinal data from natural infections, showing that SARS-CoV-2 human challenge can model mild infection that is the main driver for ongoing transmission in the community[5–7], but with greater accuracy and detail, due to the control of virus and timing. These data are unique in the information they

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have provided, detailing the early post-exposure, pre-symptomatic period and duration of viral shedding. They have also contributed importantly to public health decision-making through NERVTAG, SAGE and directly to the Department of Health and Social Care. In particular, our data supported key UK public health policy decisions, demonstrating that LFA positivity is strongly associated with culturable virus and therefore contagiousness and can be highly effective as a trigger for interventions to interrupt transmission as well as potentially shortening self-isolation periods.

4. Anticipated future outputs of COVHIC001

This study can never be repeated since almost all UK adults now have antibodies against SARS-CoV-2 and seronegative populations globally are rapidly decreasing. Ongoing analysis in this study of the local and systemic immune factors associated with susceptibility and protection against beta-coronaviruses such as SARS-CoV-2 will therefore be unique in providing understanding of how individuals with no pre-existing virus-specific immunity can nevertheless resist infection by a newly emergent pathogen.

Serial sampling of the blood and upper respiratory tract from these 36 volunteers has generated an extensive biobank of specimens, including PBMCs, serum and virology samples containing high titres of virus consistent with those seen after natural infection, as well as samples from those who were exposed but did not become infected (a group that are not identifiable in other settings). Further analysis of these samples to assess humoral, innate, B cell and T cell responses; soluble mediator expression in blood and upper respiratory tract; and transcriptional changes in blood and nose is in progress. Through this work, we seek to understand correlates and mechanisms of protection to enable improved vaccine development and identify new targets for intervention. Further work analysing viral emissions at short- and long- range into the environment and viral mutation within individuals will also increase understanding of routes and mechanisms of transmission in infected people.

4.3 RATIONALE FOR A DELTA VARIANT HUMAN CHALLENGE STUDY

Why conduct human challenge with a new variant?

The first SARS-CoV-2 human challenge study was established to answer outstanding questions about primary COVID-19 in seronegative adults that could not be addressed using field trials. By demonstrating the safety and feasibility of the approach, it was also the first step to developing a platform for rapid and early testing of interventions and diagnostics, including head-to-head efficacy trials. However, with the successful roll-out of vaccination and widespread infection during 2021 and 2022, it is estimated that 99% of the UK adult population now have some degree of immunity against SARS-CoV-2[3]. While a number of scientific questions remain the same, the high incidence of COVID-19 infection in fully vaccinated individuals has also identified new gaps in our understanding of how protection can be reliably achieved. Rapid vaccine testing with heterologous boosters and novel vaccines may be of critical importance should a pathogenic VOC emerge with predominant Delta strain characteristics. There is therefore a need to establish a model using a variant that causes breakthrough infection and generates sufficiently high attack rates in vaccinated people for future vaccine and therapeutic testing. Additionally, by developing an optimised human challenge model of breakthrough infection with a SARS-CoV-2 variant, not only can interactions between virus and host in the context of pre-existing immunity be investigated, but

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also determinants of heterologous (i.e. cross-strain) protection can be identified, to more rapidly establish targets for improved protection.

Why was the Delta SARS-CoV-2 variant chosen for GMP challenge agent manufacture?

The Delta variant became dominant in the UK in May 2021[8], outcompeting the previously dominant Alpha variant (B.1.1.7). It is defined by several mutations in the spike protein which have conferred greater transmissibility, estimated at approximately twice that of the wild-type (pre-Alpha) virus and up to 68% greater than the Alpha variant[9,10]. These spike mutations have also resulted in antigenic divergence, with serum antibodies elicited by previous infection or vaccination 3-5 fold less capable of neutralising Delta compared to Alpha, although antibody levels alone do not fully explain breakthrough infection[11,12]. A Delta isolate was therefore selected for manufacture under GMP conditions derived from a clinical specimen collected in the UK in mid-2021. Following *in vitro* characterisation, next-generation sequencing and extensive adventitious agent testing, this has now been released for human use.

The Delta variant was chosen for GMP manufacture to be representative of heterologous infection (i.e. infection in the face of pre-existing immunity against a dissimilar spike protein from the wild-type Wuhan-like virus in all approved vaccines and, more recently, from infection by the Omicron variant) as it was the dominant UK strain at the time and therefore of immediate relevance. In comparison with Alpha or other earlier VOCs, Delta caused increased rates of breakthrough infection in vaccinees and high viral loads (but similarly low rates of severe disease in healthy vaccinated populations). This provided supportive evidence that the model could be safely optimised using this strain to achieve the attack rate needed to power future vaccine efficacy studies with feasibly small participant numbers. Subsequent evidence has accrued that support these assertions.

Rationale for Delta challenge since the emergence of Omicron

Since the end of 2021, the highly transmissible Omicron variant has rapidly become dominant in the UK and elsewhere, the 5th VOC to have emerged globally in 2 years of the pandemic. Omicron displays increased antigenic divergence due to extensive changes to the receptor binding domain and other regions of the spike protein that permit immune evasion and causes high rates of vaccine-breakthrough infections and reinfections. VE against infection is lower for Omicron than Delta after 2 vaccine doses but a booster dose returns VE to a higher level, though waning of VE against symptomatic infection does occur. Omicron itself causes milder disease than Delta (estimated to be about as severe as seasonal influenza in those under 70 years of age), with around 50% reduction in risk of hospitalisation in adults[13,14].

Although Omicron is currently dominant in the UK and elsewhere, there is a real possibility that future VOCs could emerge that revert to more Delta-like characteristics. Recombinant viruses, which have a genetic mixture from both Delta and Omicron, have also been reported. Consequently, Delta itself remains relevant and related strains may again rise to global prominence. Which lineage will give rise to future variants is unpredictable and a key concern is that new variants will emerge with clinical features more similar to Delta but with the higher transmissibility of Omicron. Delta-like viruses therefore remain a potential cause of future pandemic surges and an optimised Delta challenge model to enable testing of antivirals, diagnostics and next-generation vaccines (particularly those seeking to induce cross-strain protection) will have immediate and ongoing value.

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A key goal of the human challenge model is to investigate protective immunity against SARS-CoV-2. Whilst strain-related differences do exist, we expect the Delta challenge model to enable identification of correlates of protection that can act against both existing and future variants independent of strain-specific antibodies. These findings will contribute to the development of "universal" SARS-CoV-2 vaccines. Use of the Delta agent, which is known to cause breakthrough infection, as a model to interrogate heterologous (i.e. cross-strain) immune factors that correlate with protection for comparison with homologous (same strain) protective readouts associated with pre-Alpha virus infection, is currently our best available option. We anticipate findings to be widely applicable across antigenically divergent strains, with Delta representing an equivalent and, in some respects, superior agent for human challenge than Omicron (Table 2).

Development of an Omicron challenge agent may be required for studies seeking to test the efficacy of strain-specific protection by novel vaccine candidates, particularly if Omicron-specific booster vaccines are developed. However, early animal data (available in pre-print) have suggested no greater effect of an Omicron booster than with the current vaccine[15], so future outcomes of Omicron-specific boosters remain uncertain. In any case, it will take time to manufacture a GMP Omicron virus, which would delay the testing of antivirals and vaccines that are currently in the development pipeline and being lined up for for human challenge trials. Nevertheless, the manufacture of an Omicron challenge agent is being pursued as a separate track via industry/academic collaboration. **Ultimately, having access to two widely divergent variants that can both cause breakthrough infection in seropositive volunteers will allow us to directly test the cross-strain protective efficacy of vaccines and monoclonal antibodies**. This Delta characterisation study will allow us to establish the optimal conditions that will underlie any new variant models that follow, thus accelerating their establishment and increasing the speed of our response to novel VOCs. In the meantime, by pairing efficacy data from a Delta challenge study with field efficacy data that shows protection against currently-circulating Omicron variants, compelling evidence of cross-strain protection can be achieved.

Strengths of Delta challenge	Limitations of Omicron challenge
Vaccine efficacy against severe disease remains extremely high with Delta infection	
The Delta challenge agent has already been manufactured with support by the Wellcome Trust to accelerate the availability of the platform	An Omicron challenge agent will take >6 months to manufacture, delaying access to a working human challenge model
Delta breakthrough infections may have higher peak viral load and longer duration of shedding than Omicron, providing more headroom for assessing the efficacy of interventions	Omicron infections might be so short that antivirals and other interventions may not have time to make any additional impact
Delta infection is likely to induce some symptoms even in vaccinated people, enabling clinical readouts that more accurately predict efficacy against disease endpoints	If most Omicron infections are asymptomatic, symptoms will not be useful as an endpoint
More long-term safety data has accumulated for Delta than Omicron	

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Data from Delta challenge is the only way to show cross-strain protection when paired with field data (currently entirely from Omicron)	Can only demonstrate Omicron-specific and not cross-strain specific protection
More data is available regarding efficacy of oral antivirals for disease progression with Delta than Omicron	
Limitations of Delta challenge	**Strengths of Omicron challenge**
More optimisation of prior immune status and inoculum dose may be necessary to maximise the breakthrough infection rate.	Rates of re-infection and vaccine breakthrough infection may be higher with Omicron at the same inoculum dose
	The risk of severe and protracted COVID-19 are even lower with Omicron
Strengths of having both Omicron and Delta available in the future	
Omicron and Delta are widely antigenically distant. Demonstrating efficacy against both Delta and Omicron in human infection challenge would provide the strongest possible evidence for cross-strain protection	
Omicron has mutations that may be responsible for functional differences the effect of which are poorly understood in humans but that could be investigated by controlled comparison between Delta and Omicron breakthrough infections	
By comparing breakthrough infection by Omicron and Delta, it will be possible to identify and further validate correlates of protection	

Table 2. Strengths and limitations of Delta vs Omicron challenge agents

Thus, in the short term, a Delta human challenge model will uniquely enable:

1. **A model of breakthrough infection that can be used to assess transmission-blocking potential of new vaccines and treatments.**
2. **Identification of the immune factors associated with susceptibility to breakthrough infection.**
3. **When paired with Omicron field data, or the pre-Alpha and planned future Omicron challenge models, studies that can uniquely provide proof-of-principle efficacy data for vaccine candidates that confer cross-strain protection.**

Clinical and virological outcomes from SARS-CoV-2 in healthy young adults

Even in the absence of pre-existing immunity, natural infection by SARS-CoV-2 frequently results in little or no symptoms. It is estimated that ~20-30% of SARS-CoV-2 infections are asymptomatic[16,17]. When symptoms occur, they include fatigue, cough, headache, body ache, fever, chills, loss of taste, loss of smell, diarrhoea, congestion, dyspnoea, nausea, sore throat, chest pain, abdominal pain, confusion and vomiting. Reductions in the sense of smell (anosmia) and taste (dysgeusia) are common, with one meta-analysis of >32,000 COVID-19 patients estimating prevalence at 38.2% for anosmia and 36.6% for dysgeusia[18]. In higher-risk individuals, these may be followed by a dramatic decline in clinical state, characterized by worsening dry cough, severe dyspnoea and profound malaise. Severe COVID-19 is strongly associated with older age and comorbidities.

In young adults <30 years old, severe outcomes are rare, even in the absence of pre-existing immunity[19]:

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- Risk of death in the absence of pre-existing immunity: 1.2-6.1 per 100,000 (0.0012-0.0061%)
- Risk of ICU in the absence of pre-existing immunity: 0.9-4.5 in 10,000 (0.009-0.045%)
- Risk of hospitalization in the absence of pre-existing immunity: 0.8-3.9 per 1,000 (0.08-0.39%)

For a screened healthy young adult group, these are likely to be over-estimates as these figures did not take risk factors or co-morbidities into account. With 99% of the UK adult population now seropositive from vaccination or infection, the risks of severe outcomes following infection will be further reduced substantially.

5. Clinical and virological outcomes in healthy vaccinated adults infected with the Delta variant

6. The impact of vaccination on clinical and virological outcomes from Delta

a) Vaccine effectiveness (VE) against severe illness

With the availability of highly effective vaccines, the risks associated with COVID-19 in vaccinated individuals have markedly decreased. COVID-19 vaccines approved in the UK have been consistently shown to be highly protective, substantially reducing the risks of severe outcomes following infection[20], with two doses of an mRNA vaccine (as recommended for all adults aged <30 years) conferring >90-95% protection against hospitalisation in young adults, including against the Delta variant [21–24].

Of note, following the emergence of the Delta variant, COVID-19 hospitalisation rates were observed to increase[25] and adults aged 18-49 years accounted for a larger proportion of hospitalised patients than in pre-Delta periods[26], raising concern about potential increased severity from Delta itself. This was driven by a greater proportion of unvaccinated hospitalised patients in the 18-49 age group (owing to the age-prioritised vaccine roll out programme). *In vitro* characterisation studies identified greater replicative ability of Delta compared to Alpha, with increased capacity for ACE2 receptor binding, cell entry and cell-cell fusion[11], suggestive of potential differences in pathogenicity. Some clinical studies that have assessed the risk of progression to severe illness (e.g. ICU admission, requirement for invasive ventilation or high flow oxygen, death) have detected higher risk of adverse outcome in unvaccinated Delta-infected people, compared to previous variants[27,28]. However, in other studies this increased risk of adverse outcome from Delta itself was not seen[26]. Importantly, data from several countries confirm that vaccination confers a strong protective effect against severe illness [26–28] with vaccinated people being 10 times less likely to be admitted to hospital and 11-16 times less likely to have fatal outcome from Delta variant SARS-CoV-2 infection[24].

b) VE against infection

In addition to protecting against hospitalisation/severe illness (VE against severe disease), vaccination may partially reduce the risk of people getting infected (VE against infection). VE against infection is usually calculated using surveillance data that identifies symptomatic infections defined via a country-specific case definition. The ability of vaccines to prevent asymptomatic infections is additionally reported in some studies but this is more limited.

For the Delta variant, the ability of vaccination to prevent infection is reduced compared to Alpha and pre-Alpha SARS-CoV-2[29,30]. In a UK study, VE against infection after 2 vaccine

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doses was 93.7% (BNT162b2, Pfizer mRNA vaccine) and 74.5% (ChAdOx1, Astra Zeneca adenoviral vector vaccine) for Alpha versus 88% and 67%, respectively, for Delta. Reports of VE against Delta infection have ranged between 40-80% [22–24,30,31]. VE point estimates are wide ranging because they are affected by time since vaccination (waning), whether asymptomatic infection is included in the estimate, vaccine type, as well as age and comorbidities. The ability of Delta to cause high rates of breakthrough infection is therefore likely contributed to by 1) its increased transmissibility, 2) waning of vaccine-mediated protection, and 3) antigenic divergence.

c) VE against transmission

Vaccines can reduce transmission by preventing infections, but also by reducing the infectiousness of breakthrough cases that occur. Once infected, field data suggest that Delta breakthrough infection in vaccinated people induces RNA viral loads as high as those found in unvaccinated individuals, although vaccinated people may clear infection more quickly and therefore may be infectious for less time[5,7,32]. In contrast, another study showed significantly reduced infectious viral load in Delta vaccine breakthrough infectious than unvaccinated individuals up to 5 days after symptom onset[33]. Consistent with this, studies have shown that Delta vaccine breakthrough infection does result in transmission to others but at lower rates than from the unvaccinated[34,35]. The effect of vaccination at preventing transmission is lower for the Delta than Alpha variant [34].

d) Waning of VE over time

The ability of vaccination to protect against Delta infection appears to wane considerably with time from around 6-12 weeks after vaccination, and by 5 months can have declined substantially to <40%[22,36]. In one study, a 22% relative reduction in vaccine effectiveness (VE) against all Delta infection was seen each month following second vaccine dose in 18-64 year olds[30], with waning more prominent against symptomatic infection (63% relative reduction in VE). Waning of VE is more pronounced with increased age[30,31]. VE and the effect of waning also differs depending on the vaccine type, with VE against infection for ChAdOx1 being lower than BNT162b2 after 2 doses in 18-64 year olds but waning less rapidly[30]. VE against Delta variant transmission has also been observed to decline with time after vaccination, reaching levels similar to an unvaccinated person by 12 weeks after ChAdOx1 and attenuating substantially after 12 weeks for BNT162b2[34].

However, protection against severe disease is well maintained, with VE against hospitalisation remaining at >90% up to 6 months after second dose in all ages and highest in the 16-44 year old age group (92% (95% CI: 88-95%))[22]. Where some waning of protection against severe disease has been observed, this has been in older adults and those in clinically extremely vulnerable groups[20,31].

Thus, the Delta variant can infect vaccinated people resulting in high viral loads with potential for transmission, thereby offering a good model for human challenge that allows for investigation of heterologous immunity and testing of novel vaccine candidates. With vaccination shown to be highly protective against severe disease, healthy young vaccinees are at very low risk of severe disease from Delta infection.

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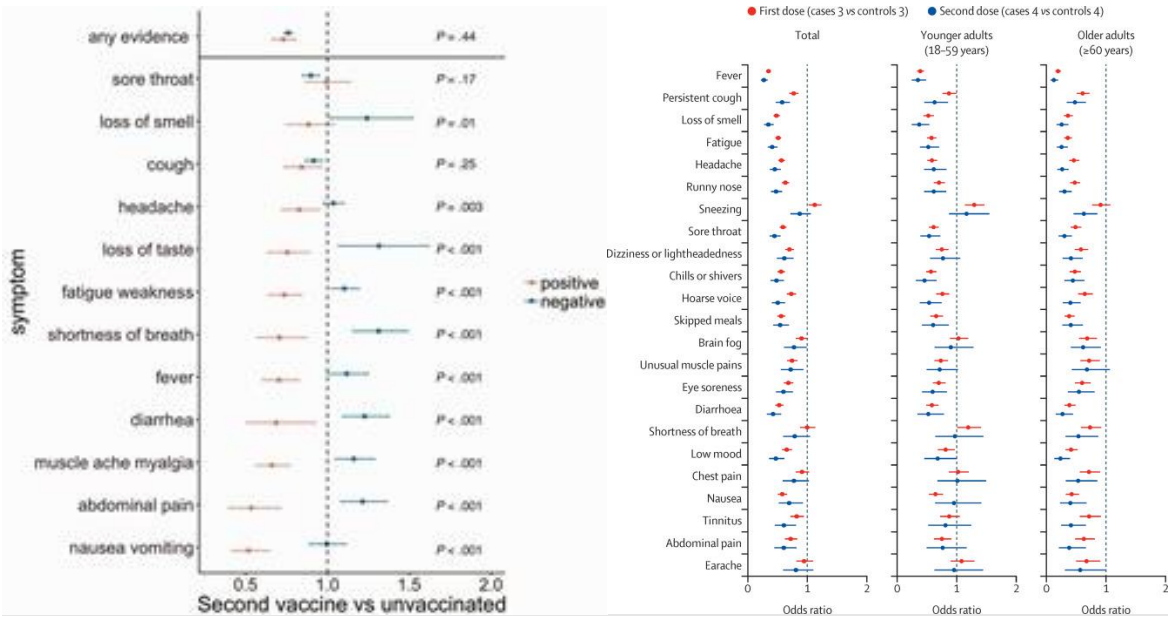
7. The impact of vaccination on symptom burden

Frequency and severity of symptoms in vaccine breakthrough infections are reduced compared to infections in the unvaccinated. The COVID Infection Survey (ONS) identified 10 of 12 symptoms that were reported less frequently in double vaccinated than unvaccinated PCR-positive cases (Figure 3a) [37]. The COVID Symptom Study (Zoe app) also reported that vaccination was associated with lower symptom reporting for almost all symptoms. Vaccinated 18-59 year olds were 1.5 times more likely to have asymptomatic infection and 0.7 times less likely to report >5 symptoms in the first week of illness(Figure 3b)[38].

Specifically, anosmia appears less prevalent in vaccinated people compared with those who are unvaccinated (25.9% of double vaccinated 18-59 years olds versus 47.8% who were unvaccinated in data from the COVID Symptom Study performed during Alpha and Delta circulation[38]).



A B

Figure 3: Symptoms after vaccination. a) taken from The COVID Infection Survey [37] and b) taken from The COVID Symptom Study[38].

8. The impact of vaccination on prolonged symptoms (long COVID)?

COVID-19 is known to be associated with protracted symptoms, but estimates of the prevalence of long-term symptoms following confirmed SARS-CoV-2 infection are varied, likely due to differences in methodology, classification and reporting[39]. Estimates of COVID-19 symptom prevalence at 12 weeks after SARS-CoV-2 infection have been wide ranging - reported at 2.3% (COVID Symptom Study[40]), 2.72% (2.64-2.80%) (COVID Infection Survey[41]), 14.8% (REACT-2 for >=3 symptoms[42]) and 37.7% (REACT-2 for >=1 symptom[42]). In a large US Veterans database, the estimate was 4.1% in non-hospitalised individuals[43]. Issues with many of these observational studies include the lack of a control group, that symptoms that may have been present before SARS-CoV-2 infection are often not recorded or assessed by recall, and issues around patients who are lost to follow up.

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In 18-30 year olds specifically (per inclusion criteria for this study), the COVID Symptom Study reported rates of persisting symptoms at 12 weeks of 1.4% in females and 0.9% in males (Prof Claire Stevens, personal communication). In the COVID Infection Survey, the proportion of 17-24 year olds self reporting long COVID who first had (or suspected they had) COVID-19 at least 12 weeks previously was 1.59% (1.31-1.86) and 2.56% (2.27-2.86) for 25-34 year olds. When considering only functionally limiting symptoms at 12 weeks in a study using primary care records, estimated prevalence in 20 year olds was 1.2%[44]. Prevalence of self-reported long-COVID with subsequent activity limitation was 0.53% (0.37-0.69) in 17-24 year old and 0.6% (0.46-0.74) in 25-34 year olds in the COVID Infection Survey[45].

Risk factors for long COVID include older age, female sex, poor general health, asthma, obesity, white ethnicity, and more severe symptoms/higher viral load during the acute episode of COVID-19[39,40,43–45]. While anosmia is a common symptom, a systematic review of studies investigating anosmia showed that on average smell disturbance lasted for 7-14 days, with one study in which 97 patients with anosmia were followed up for 12 months with objective testing showing that 96% made a full recovery, with partial recovery of the remaining patients[4]. In a study from Israel where electronic health records of those with and without COVID-19 were compared, the hazard ratio for anosmia and dysgeusia in **unvaccinated** patients during wild-type and alpha waves was highest around 4-6 months, peaking at 5.58 (4.02-7.76) then slowly declining, to 2.37 (1.52-3.70) at 12 months. In the 19-40 age group who were unvaccinated, the risk difference per 10,000 persons for anosmia and dysgeusia at 6-12 months was 13.2 (8.5-17.9)[46].

Importantly, emerging data shows a reduction in the risk of long COVID and prolonged anosmia/parosmia in vaccinated people. The COVID Symptom Study showed that vaccination reduced the risk of self-reported long COVID (persistent symptoms for >28d after infection) by about half (11.4% vs 5.2% in all ages and 7.2% to 3.1% in those aged 18-59 years)[38]. Data from the COVID Infection Survey also showed a reduced likelihood of self-reported long COVID (symptoms >12 weeks) in 18-69 year olds after 2 doses of vaccine, of around 22%[47]. After a first vaccination dose, the largest numeric decreases in the odds of individual long COVID symptoms were for loss of smell (12.5% decrease (95% CI 21.5-2.5%) and loss of taste (9.2% decrease (95% CI 19.8-2.7%), although confidence intervals were large with uncertainty around these estimates.[47] In a large US Veterans cohort, which included predominantly older individuals (mean age=66 years), vaccination had a more modest effect, reducing long COVID (symptoms at 6 months) by around 15% (HR=0.85, 0.82-0.89).[48]. The effect of vaccination on long COVID symptoms appears to be greater in younger people (age <60) than older people (age >60) [49,50]. In a small group of double vaccinated 18-30 year olds with no comorbidities who provided longitudinal data to The COVID Symptom Study (Claire Stevens and Michela Antonelli, personal communication), the mean duration of altered smell (parosmia) was 1.84 days (SD 4.3 days) and anosmia was 3.17 days (SD 6.95), with very few vaccinated participants reporting parosmia/anosmia after 28 days (n=2 for parosmia, n=4 for anosmia) (Figure 4).

a b

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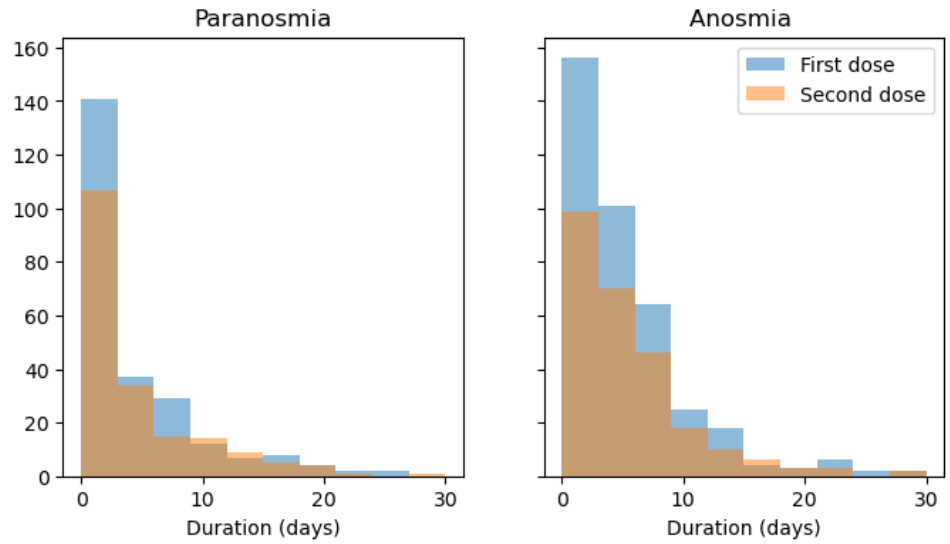

Figure 4. Duration of a) parosmia and b) anosmia in healthy vaccinated 18-30 year olds infected with the Delta variant who had received one (blue bars) or two (brown bars) doses of a COVID-19 vaccine. Unpublished data from the COVID Symptom Study. Unvaccinated group was too small to include for comparison.

Therefore, vaccination reduces the risk of prolonged symptoms (long COVID) but does not completely prevent it, suggesting that strategies to limit breakthrough infections may be required to reduce the burden of long COVID.

4.4 MITIGATING RISK IN EXPERIMENTAL HUMAN SARS-COV-2 INFECTION

Data from the first SARS-CoV-2 human challenge study along with >2 years' accumulated data from community COVID-19 cases in unvaccinated and vaccinated people have provided an extensive clinical database with which risk to participants in future SARS-CoV-2 human challenge studies can now be assessed by both investigators and volunteers. Importantly, all future SARS-CoV-2 challenge studies in the UK will by necessity take place with volunteers who have pre-existing immunity either by vaccination or infection (or both). The risk profile of these studies is therefore substantially lower now than when the first study in seronegative volunteers was conducted. By recruiting only vaccinated individuals in this study, the risks of severe illness, anosmia and long COVID will all be reduced.

However, to further minimise the likelihood of injury to participants, rigorous risk mitigation strategies will continue to apply:

1. The study will be performed by an **expert team** with extensive respiratory virus human challenge experience
2. **Inclusion/exclusion criteria** will specify young age (18-30 years) and exclude anyone with known risk factors such as obesity and any comorbidity detected on screening
3. A robust **informed consent** process will make clear the potential risks based on the most relevant safety data
4. Starting with a **low infecting dose** before incremental optimisation of conditions
5. **Intranasal administration** of inoculum to avoid direct instillation to the lung
6. **In-patient quarantine** in individual negative pressure en-suite rooms, with clear criteria for discharge to prevent onward transmission
7. Early recognition of features associated with progression to more severe disease through **close in-patient monitoring**

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8. Clear criteria for **rescue treatment** of infected participants using effective antivirals, when indicated
9. Full support by local acute medicine and **critical care** services
10. **One year follow-up** with specialist referral as necessary

Experience of SARS-CoV-2 human challenge of the team and in the UK

The UK is world-class in having extensive recent experience using models of respiratory viral challenge, the study team have extensive and direct experience of SARS-CoV-2 human challenge in particular. At Imperial College London, >200 participants have been challenged with influenza virus and RSV. Professor Chris Chiu was the Chief Investigator of the first SARS-CoV-2 human challenge study and his team have safely run human challenge studies at Imperial for >12 years. The Delta challenge study will take place in a high containment unit at the Chelsea and Westminster Hospital NHS Foundation Trust, undertaken by Professor Chiu's experienced team with the full support of local acute medicine and critical care services.

At the University of Oxford, Professor Helen McShane is the Chief Investigator of COV-CHIM01 (NCT04864548), the first SARS-CoV-2 human challenge study in seropositive volunteers. Her group has been leading novel tuberculosis (TB) vaccine development and is experienced in delivering BCG human challenge studies, endeavouring to tackle this major infectious disease of high burden at global scale. At the Oxford site, the Delta challenge study will be delivered by Professor McShane's group in the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) or Experimental Medicine Clinical Research Facility (EMCRF), University of Oxford, supported by NHS colleagues at Oxford University Hospitals NHS Foundation Trust.

Rescue therapy

Given the potential for harm due to deliberate inoculation of individuals with SARS-CoV-2, availability of an effective rescue therapy was an important ethical consideration highlighted by the World Health Organisation in their regulatory framework for challenge models. Several antiviral therapies have shown efficacy in preventing the progression of mild COVID-19 to severe disease, which may be used as rescue therapy in this study depending on availability (in order of preference): Paxlovid, Molnupiravir, and Remdesivir (Table 3). In clinical trials, these therapies were primarily tested on populations who have risk factors for progression to severe COVID-19, or on those who are unvaccinated. While it is not possible to directly extrapolate these data to our volunteer population, trial findings show these all to be safe and well-tolerated treatments with evidence of clinical effect. It is also expected that a targeted treatment against SARS-CoV-2 infection would have greatest impact when given early in the course of infection, which will be the case in a human challenge setting. Given all antivirals have demonstrable efficacy, order of preference is based on route of administration (oral over intravenous). **A stock of Paxlovid has been provided by Pfizer for this study so as not to impact on NHS stocks. This will be accessible to the study team 24 hours a day, 7 days a week. Local treatment guidelines may be used for access to other treatments if Paxlovid is not available or not appropriate (as determined by the CI/PI). A drug supply agreement will be in place between Pfizer and the Sponsor.**

	Administration	**Population tested**	**Clinical effect - Reduction in**	**Antiviral effect – reduction in viral load**

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			hospital admissions or death	
Paxlovid	Oral, 5-day course BD	Unvaccinated, at least 1 risk factor	89% within 3 days of symptom onset 88% within 5 days of symptom onset	0.87log$_{10}$ copies/mL at day 5 when given within 3 days of symptom onset
		Unvaccinated, standard risk OR Vaccinated with at least 1 risk factor	70% within 5 days of symptom onset	Approximately 1 log$_{10}$ copies/mL
Molnupiravir	Oral, 5-day course BD	Unvaccinated, at least 1 risk factor	30% within 5 days of symptom onset	Not yet published May have reduced effect on Delta than other variants
Remdesevir	IV infusion, 3-day course OD	Unvaccinated, at least 1 risk factor	87% within 7 days of symptom onset	No significant effect

Table 3. Comparison of rescue therapies

9. PAXLOVID (nirmatrelvir with ritonavir)

PAXLOVID is comprised of nirmatrelvir (PF-07321332), a SARS-CoV-2 3CL protease inhibitor, co-packaged with ritonavir (an HIV-1 protease inhibitor and CYP3A inhibitor). Ritonavir does not have activity against SARS-CoV-2 but is included to inhibit CYP3A-mediated metabolism of nirmatrelvir and increase plasma concentrations to levels anticipated to inhibit SARS-CoV-2 replication. PAXLOVID is orally-administered, every 12 hours for 5 days. PAXLOVID has been issued a temporary authorisation from the UK MHRA and an EUA from the US FDA.

The **EPIC-HR** (NC04960202), phase 2/3 randomised, double blind, placebo-controlled trial included n=2246 symptomatic, unvaccinated, non-hospitalised adults with COVID-19 at high risk of progression to severe illness[51]. The trial reported that PAXLOVID significantly reduced hospital admissions and deaths by 89% (within three days of symptom onset) and 88% (within five days of symptom onset) compared to placebo. Of those who were treated within three days of symptom onset, 0.7% (5/697) of patients who received PAXLOVID were admitted to hospital up to day 28 after randomisation, with no deaths. In comparison, 6.5% (44/682) of patients who received placebo were admitted, with nine deaths ($p<0.0001$). Similar reductions were seen in people treated within five days of symptom onset, with 0.8% (8/1039) in the paxlovid group admitted up to day 28 (no deaths) and 6.3% (66/1046) in the placebo group (12 deaths), ($p<0.0001$). 1.1% of patients who received PAXLOVID were hospitalized through Day 28 (1/94 hospitalized with no deaths), compared to 16.3% of patients who received placebo (16/98 hospitalized with 6 deaths), with high statistical significance ($p<0.0001$). In the overall study population through Day 28, no deaths were reported in patients who received PAXLOVID as compared to 12 (1.2%) deaths in patients who received placebo.
In the EPIC-HR trial, in a secondary endpoint, SARS-CoV-2 viral load at baseline and Day 5 were evaluated for 1574 patients. After accounting for baseline viral load, geographic region, and serology status, PAXLOVID reduced viral load at day 5 by approximately 10-fold, or 0.87 log$_{10}$ copies/mL, relative to placebo, when initiated within 3 days after symptom onset, indicating robust activity against SARS-CoV-2.

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Treatment-emergent adverse events were comparable between PAXLOVID (23%) and placebo (24%), most of which were mild in intensity. Fewer serious adverse events (1.6% vs. 6.6%) and discontinuation of study drug due to adverse events (2.1% vs. 4.2%) were observed in patients dosed with PAXLOVID, compared to placebo, respectively.

Interim analyses of **EPIC-SR**[52], a phase 2/3 study that included unvaccinated adults who were at standard risk (i.e., low risk of hospitalization or death) as well as vaccinated adults who had one or more risk factors for progressing to severe illness, showed that the novel primary endpoint of self-reported, sustained alleviation of all symptoms for four consecutive days, as compared to placebo, was not met. However, the key secondary endpoint showed a 70% reduction in hospitalization and no deaths in the treated population for any cause compared to placebo. Additionally, there was approximately a 10-fold, or 1 $\log_{10}$ copies/mL, decrease in viral load compared to placebo, consistent with results from the EPIC-HR study. This interim analysis included n=673 adults (45% of the trial's planned enrolment), 0.6% of those who received PAXLOVID were hospitalized following randomization (2/333 hospitalized with no deaths), compared to 2.4% of patients who received placebo and were hospitalized or died (8/329 hospitalized with no deaths). A follow-on analysis at 80% of enrolled patients was consistent with these findings. In this analysis, 0.7% of those who received PAXLOVID were hospitalized following randomization (3/428 hospitalized with no deaths), compared to 2.4% of patients who received placebo and were hospitalized or died (10/426 hospitalized with no deaths); p=0.051. Treatment-emergent adverse events were comparable between PAXLOVID (22%) and placebo (21%), most of which were mild in intensity. Rates of serious adverse events (1.4% vs. 1.9%) and discontinuation of study drug due to adverse events (2.1% vs. 1.2%) were also comparable between PAXLOVID and placebo.

10. LAGEVRIO (molnupiravir)

Molnupiravir is a small-molecule ribonucleoside prodrug of N-hydroxyctidine (NHC) which has activity against SARS-CoV-2 acts by introducing mutations into the viral genome during viral replication. After oral administration of molnupiravir, NHC circulates systemically and is phosphorylated intracellularly to NHC triphosphate. NHC triphosphate is incorporated into viral RNA by viral RNA polymerase and subsequently misdirects the viral polymerase to incorporate either guanosine or adenosine during viral replication. This leads to an accumulation of deleterious errors throughout the viral genome that ultimately render the virus non-infectious and unable to replicate. LAGEVRIO is orally-administered, every 12 hours for 5 days. The UK MHRA and US FDA have issued temporary authorisation for treatment of mild-to-moderate COVID-19 in adults with at least one risk factor for severe illness.

The **MOVe-OUT** trial[53], a phase 3 double-blind, parallel-group, randomized, placebo-controlled trial evaluated the safety and efficacy of molnupiravir 800 mg twice-daily for 5 days in non-hospitalized adults with Covid-19. N=1433 patients underwent randomisation who were unvaccinated against SARS-CoV-2, had laboratory-confirmed SARS-CoV-2 infection, symptom onset within five days of study randomization, and at least one risk factor associated with poor disease outcomes (e.g., heart disease, diabetes). Molnupiravir reduced the risk of hospitalization or death at day 29: 9.7% (68/699) of patients in the placebo group were hospitalized or died compared to 6.8% (48/709) of patients who received molnupiravir, for an absolute risk reduction of 3.0% (95% confidence interval [CI]: 0.1, 5.9). Nine deaths were reported in the placebo group, and one in the molnupiravir group (29-day all-cause mortality, 1.3%), a risk of death that was lower by 89% (95% CI, 14 to 99) with molnupiravir than with

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placebo. All 10 participants had been hospitalized before death, and all the deaths were considered by the investigators to be Covid-19–related.

Results from this final analysis were more modest than reported from the pre-specified interim analysis, on which MHRA authorisation was based (30% vs 50% reduced risk of hospitalisation). Potential reasons reported by the investigators include differences in pre-existing SARS-CoV-2 nucleocapsid antibodies, infecting SARS-CoV-2 variants, and lower viral load at enrolment, which require further evaluation. In most prespecified subgroups, the percentage of participants who were hospitalized or died was lower with molnupiravir than placebo, but the associated confidence intervals indicate substantial uncertainty about the magnitude of these effects. Analyses of the effect of molnupiravir on viral load are ongoing and sequencing to confirm variant status is incomplete. Within 55.7% of the cohort in whom baseline variant status was complete, the effect on hospitalisation or death from Delta was limited (18/237 for molnpiravir vs 22/221 for placebo, ARR -2.4% (-7.8 to 2.9)) and lower than other variants (ARR -19.1% for Gamma, -7.9% for Mu and -7.8% for other clades). In phase 2 trial reports, molnupiravir was less beneficial when administered late in the disease course (>3-5 days after symptom onset or after hospitaliation)[54,55].

Adverse events were reported in 216 of 710 participants (30.4%) in the molnupiravir group and 231 of 701 (33.0%) in the placebo group. The percentage of participants with adverse events considered by the investigators to be related to the trial regimen was similar: 8.0% vs. 8.4%. Deaths resulting from adverse events, none of which were deemed by the investigators to be related to the trial regimen, were reported less frequently in the molnupiravir group than in the placebo group. After day 29, three additional deaths resulting from adverse events occurred in the placebo group, as compared with one additional death reported in the molnupiravir group. The most common adverse reactions for molnupiravir (incidence ≥1%) were diarrhoea (1.7% for molnupiravir, 2.1% for placebo), nausea (1.4% for molnupiravir, 10.7% for placebo) and dizziness (1% for molnupiravir, 0.7% for placebo). Discontinuation of study intervention due to an adverse event (AE) occurred in 1% of participants receiving molnupiravir and 3% of participants receiving placebo. One participant each in the molnupiravir and placebo groups met the prespecified criteria for a postbaseline platelet count below 50,000 per microliter; the low platelet count in the molnupiravir-treated participant was reported on day 12 and was not deemed to be related to treatment.

In the **MOVe-IN** phase 2/3 randomized, placebo-controlled, double-blind trial in patients 18 years old and older requiring in-hospital treatment for laboratory-confirmed Covid-19 with symptom onset 10 or fewer days, a 5-day course of molnupiravir up to 800 mg twice daily was not associated with dose-limiting side effects or adverse events, but did not demonstrate clinical benefit and the study was therefore discontinued after interim analysis. Of 304 randomly assigned participants, 218 received at least one dose of molnupiravir (200 mg, 400 mg, or 800 mg) and 75 of placebo (1:1:1:1 ratio). Adverse events were reported in 121 of 218 (55.5%) molnupiravir-treated and 46 of 75 (61.3%) placebo-treated participants, with no apparent dose effect on adverse event rates and no evidence of hematologic toxicity based on prespecified adverse events. Of 16 confirmed deaths, most were in participants with severe Covid-19 (75.0%), with underlying comorbidities (87.5%), older than 60 years of age (81.3%), and/or symptom duration longer than 5 days (75.0%) at randomization. Median time to sustained recovery was 9 days in all groups, with similar day 29 recovery rates ranging from 81.5% to 85.2%.

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Potential mutagenic toxicity of molnupiravir has been raised as a concern, following a report of mutational activity in mammalian cell culture (CHO-K1, Chinese hamster ovary cells)[56]. However, further report from Merck[57] indicates a lack translation to in vivo mammalian systems, using two rodent models. Given the totality of data, regulatory authorities in the United Kingdom have stated that the risk of mutagenicity or genotoxicity in the clinical use of molnupiravir is low. However, molnupiravir is not recommended for women who are pregnant or breast-feeding or for those who might become pregnant during treatment.

Further clinical data is expected from the RECOVERY trial, which will compare molnupiravir (800 mg twice daily for five days) with the usual standard of hospital care in adult patients who are hospitalised because of COVID-19 and the PANORAMIC trial in community-based patients with COVID-19 and <5 days of symptoms who are aged 50 or over, or aged 18 or over with a pre-existing condition.

11. Remdesivir

Remdesevir is a nucleotide prodrug inhibitor of the SARS-CoV-2 RNA-dependent RNA polymerase. Remdesevir is administered IV once daily. It has been available to UK clinicians treating hospitalised patients with COVID-19 pneumonia requiring supplemental oxygen, since May 2020. A recent study[58] investigated the use of early remdesevir in non-hospitalised patients to prevent progression to severe disease. In this randomised, double blind, placebo controlled trial, 562 patients who had symptom onset within 7 days and at least one risk factor for disease progression, were randomised to receive a 3 day course of remdesevir or placebo. COVID-19–related hospitalization or death from any cause occurred in 2 patients (0.7%) in the remdesivir group and in 15 (5.3%) in the placebo group (hazard ratio, 0.13; 95% confidence interval [CI], 0.03 to 0.59; P=0.008). A total of 4 of 246 patients (1.6%) in the remdesivir group and 21 of 252 (8.3%) in the placebo group had a COVID-19–related medically attended visit by day 28 (hazard ratio, 0.19; 95% CI, 0.07 to 0.56). No patients had died by day 28. The risk of COVID-19 related hospitalisation or death from any cause was 87% lower in the remdesevir group than placebo. However, the was no significant difference in viral load from baseline to day 7 between the remdesevir treated and placebo groups.

Remdesevir had an acceptable safety profile. Adverse events occurred in 42.3% of the patients in the remdesivir group and in 46.3% of those in the placebo group. Fewer patients in the remdesivir group than in the placebo group had serious adverse events (5 of 279 patients [1.8%] vs. 19 of 283 patients [6.7%]). By day 28, laboratory abnormalities of grade 3 or higher had occurred in 29 of 279 patients (10.4%) in the remdesivir group and in 23 of 283 (8.1%) in the placebo group. At day 14, the mean (±SD) change from baseline in creatinine clearance was minimal (0.26±21.2 ml per minute in the remdesivir group and 1.9±18.6 ml per minute in the placebo group). Similarly, at day 14, the mean change from baseline in alanine aminotransferase levels was minimal (−3.0±21.6 U per liter in the remdesivir group and −1.0±27.4 U per liter in the placebo group).

4.5 RESEARCH QUESTIONS

This study will identify optimised conditions to achieve a >50% infection rate using a GMP Delta challenge virus in previously vaccinated volunteers. In addition, it will address key questions about Delta variant infections in the context of vaccine-induced heterologous immunity and how they compare with primary pre-Alpha infection (comparison to previous

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human challenge study) and secondary pre-Alpha infection (homologous vaccine-induced immunity).

1. How susceptible are healthy young, vaccinated adults to Delta variant infection, and how does this compare with pre-Alpha infected participants?
2. What are the clinical, virologic and immunologic features of vaccine breakthrough infection by the Delta variant in the context of partial heterologous immunity compared with homologous immunity against pre-Alpha challenge?
3. What immune, transcriptomic and genomic markers correlate with susceptibility or resistance to Delta variant compared with pre-Alpha infection?

4.6 Research Strategy

This project forms part of a wider programme of work funded by the Wellcome Trust, UK Vaccine Taskforce (VTF), Department of Business, Energy and Industrial Strategy (BEIS) and Department of Health and Social Care (DHSC) that aligns protocols and procedures from all SARS-CoV-2 human challenge studies performed to date, as well as sharing samples and data to enable cross-comparison and integrative analysis.

To maximise comparability of data, similar inclusion/exclusion criteria as the previous studies (aside from vaccination and sero-status) will be used (see Section 6.5).

For SARS-CoV-2 human challenge to be maximally useful for vaccine testing, a relatively high infection rate (ideally ~70%) should be achieved so that placebo-controlled efficacy trials can be powered with feasibly small numbers of participants. Data from the first human challenge study (COVHIC001) showed that intranasal administration of 10 $TCID_{50}$ pre-Alpha SARS-CoV-2 safely caused infection in 53% of seronegative volunteers. However, data from the ongoing COV-CHIM01 (NCT04864548) study showed that dose escalations up to and including 10^5 $TCID_{50}$ of pre-Alpha SARS-CoV-2 did not cause sustained infection in seropositive (from previous infection + vaccination) volunteers. Based on the findings of COV-CHIM01 and existing field data[5], we anticipate Delta challenge of vaccinated volunteers to result in substantially lower attack rate than pre-Alpha challenge of seronegative volunteers. It is therefore likely that conditions will need to be optimised in order to achieve this target infection rate.

The first cohort of 5-10 vaccinated volunteers will be challenged by intranasal drops with 10^2 $TCID_{50}$ of the Delta challenge agent. This starting dose has been selected with knowledge of the favourable safety profile of the COV-CHIM01 (NCT04864548) study, where 10^5 $TCID_{50}$ has not caused any adverse safety signals in 5 volunteers. (Helen McShane, personal communication). Field epidemiological data also show that in a vaccinated young population, severe outcomes due to Delta infection are extremely rare.

An initial 5-10 participants will be challenged, either all at one site or split across the two sites. Daily nose and throat swabs will be taken and infection defined as 2 consecutive viral detections by qPCR from day 2 post-inoculation.

If the target infection rate ($\geq$50%) is not observed in the first cohort of 5-10 volunteers, we will take a stepwise approach to increase the attack rate (Figure 6). Our stepwise approach to increase the attack rate will include inoculating with higher doses of SARS-CoV-2 and may

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also incorporate pre-selecting participants with lower baseline antibody levels if volunteer numbers allow. While the infection rate assessment should normally take place after at least 6 participants inoculated, if, due to unforeseen participant dropout, only 4 or 5 participants are enrolled in the initial quarantines, a preliminary assessment can be made. If <2/5 participants become infected and there are no safety concerns, dose escalation may take place. If only 4 participants are enrolled in the initial quarantines and no participants become infected and there are no safety concerns, a further 1-2 participants will be enrolled at the lower dose to complete the cohort of 5-6 individuals but dose escalation may also take place within the same quarantine group as in both situations the target infection rate would still not be reached whatever the outcome of the additional 5th and 6th participant.

If an infection rate of ≥50% is observed (i.e. 3/6, 4/8 or 5/10 participants or more are infected) following inoculation of the initial cohort with 10^2 $TCID_{50}$, a subsequent cohort of n=6 may be inoculated with a lower dose of 10 $TCID_{50}$. Based on the infection rate achieved, we will proceed to expand the sample size at either 10^2 or 10 $TCID_{50}$ (Figure 6).

This will therefore establish the clinical and virological outcomes of the Delta variant at the lowest dose that gives rise to an attack rate of 50-70% in individuals with heterologous vaccine-induced immunity (Figure 6).

If the de-escalated dose of 10 TCID50 is used and the infection rate is <70% after the n=30 group, we will consider dose escalating to 10^2 in consultation with the DSMB/TSC.

Whilst we aim to recruit vaccinated participants with no previous SARS-CoV-2 infection, with the ongoing high rates of Omicron transmission in the community, it is likely that very low numbers will be found (i.e. anti-S positive, anti-N negative). Therefore, enrolment of those with both anti-S and anti-N antibodies will occur. These individuals are anticipated to be at even lower risk of infection or severe symptoms due to their pre-existing immunity.

Approach to increasing the attack rate in vaccinated volunteers challenged with Delta variant SARS-CoV-2

1. Increasing infection rate by dose escalation

If the pre-defined attack rate is not reached at the starting dose, the inoculum dose will be increased to 10^3 $TCID_{50}$. We will then conduct a series of dose increment cycles until we identify the lowest dose which achieves the objective of viral replication in the upper respiratory tract of ≥50-70% of participants. All changes in conditions and inoculum dose will be carried out with the advice of the independent Data and Safety Monitoring Board (DSMB). Once an attack rate of ≥50% is observed, further dose escalation to optimise attack rate may be conducted with the advice of the DSMB and TSC.

It is intuitive that the concentration of virus to which a participant is exposed is likely to determine the chances of successful infection, but may also affect the severity of the inflammatory response and, potentially, the disease. This has been shown in a limited fashion in the Syrian hamster model of COVID-19[59] as well as in animal models of other viral infections. A dose-response has been shown in mouse models with several strains of SARS-CoV-1. The infectivity varies between different strains of the virus, which modifies the shape of the dose-response curve, but nevertheless consistent dose-response relations are observed

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with the severity of the infection[60]. Human influenza infection challenge studies have also demonstrated higher infection rates related to dose[61]. Data from the COV-CHIM01(NCT04864548) study (Helen McShane, University of Oxford) has shown that dose escalation up to 10^5 $TCID_{50}$ was safely performed in seropositive volunteers inoculated with the pre-Alpha SARS-CoV-2 virus.

2. Increasing infection rate by screening for low anti-spike antibody levels

Several studies have shown that serum antibody levels (anti-spike, anti-RBD and neutralising antibody) correlate with reduced risk of symptomatic infection[62–65] though no specific protective threshold has been defined. Spike antibody levels are known to wane with time, from as early as 1-2 months after vaccination[66–68] and this coincides with the timing of increasing susceptibility to infection detected in exposed contacts[34]. This supports selecting volunteers with lower serum spike antibody levels as a strategy to increase symptomatic infection rate. In young healthy adults, the protection against severe disease induced by vaccination is not subject to the same rate of waning. In those with lower post-vaccination spike antibody levels (who are likely to have other existing immune protection mechanisms induced by vaccination), the risk of severe illness remains very low (and certainly lower than risks estimated in the first challenge study in anti-spike negative young healthy volunteers).

Also of note, whilst serum antibody may correlate with protection from symptomatic infection, no serological measurement has been shown to correlate with protection from infection itself (i.e. including asymptomatic or pauci-symptomatic infections) and even those with higher levels of serum neutralising antibody may still suffer Delta breakthrough infection. This implies that other immune mechanisms are also important in preventing infection and highlights the importance of understanding how breakthrough infections occur, correlates of protection beyond serum antibodies, and the immunological consequences of these infections in antigenically-experienced individuals.

As a protective serum antibody threshold is not yet identified, we propose, where feasible depending on recruitment, to incorporate selection of volunteers within the lower range of S antibody titres based on data in the young adult population from UKHSA. Figure 5 below depicts UKHSA weekly data on the distribution of S antibody levels in young healthy adults aged 18-30 years old who are N antibody negative and N antibody positive between Sept 14th-Oct 6th 2022 (week 37). Of note, 87.7% of 18-30 year olds in the cohort were N antibody positive during week 37. Of those N antibody negative participants included here, it is not possible to completely rule out that a proportion could have had previous infection but with negative N antibody titres due to waning or lack of N seroconversion.

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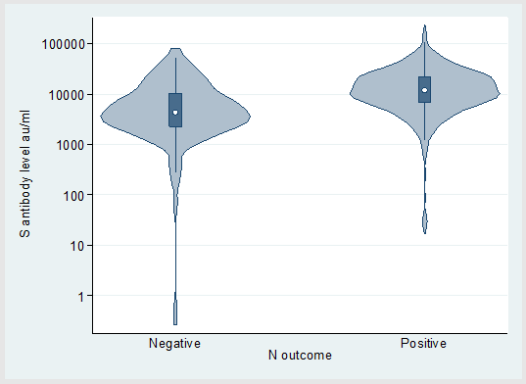

Percentiles	Roche total S (IgM/IgG) titre	
	N antibody negative (n=84)	N antibody positive (n=568)
1%	0.4	35
5%	350	2077
10%	1471	3627
25%	2219	6894
50%	4332	11853
75%	10511	22073
90%	23098	34831
95%	27929	50010
99%	52012	95149

Figure 5. Distribution of serum spike antibody titres in adults aged 18-30 years between March 28-April 3rd 2022.

Data from UKHSA shows anti-S protein serum antibody levels using the Roche total S antibody (IgM/IgG) quantitative assay in young adults with (n=568) and without (N=84) anti-N antibodies. The table reports data from week 37 (14th March to 6th April).

These data may be updated during the course of the study.

Comparison with homologous pre-Alpha challenge

The early response to infection with a novel pathogen such as SARS-CoV-2 is characterised by reliance on intrinsic and innate immunity that acts in advance of B cell and T cell activation, proliferation and mobilisation. This contrasts with secondary viral exposures, where antibodies and memory cells elicited by previous infection or vaccination make important contributions to both immediate prevention of virus entry and later viral clearance. Adaptive immunity is usually highly effective at preventing homologous re-infection, but antigenic divergence can lead to immune evasion, as has been seen with the Delta and Omicron variants. Understanding of how the host response is still able to provide protection in this context and reduce the risk of infection as well as severity of disease remains incomplete. Furthermore, the impact of partial immunity on viral kinetics and potential pressure on in-host mutation is uncertain.

Homologous protection against SARS-CoV-2 is more robust than heterologous immunity but it remains unclear how this is mediated in terms of quantity or quality of the adaptive immune response. Furthermore, a major outstanding question for ongoing vaccine development is how the phenomenon of original antigenic sin (OAS) may negatively impact our ability to stimulate new antibody responses that optimally recognise antigenically-divergent VOCs. OAS describes how first exposure to a virus can shape the outcome of subsequent exposures to antigenically related strains, particularly referring to an antibody recall response that shows higher affinity to the original strain, thus limiting efficacy of boost exposure against the newer variant. This phenomenon has been observed in relation to immunological imprinting by previous seasonal coronavirus infections[69].

To test how first-generation vaccination differentially impacts the response to subsequent homologous and heterologous infection, we intend to challenge a further vaccinated cohort with the pre-Alpha virus once conditions have been optimised with the Delta virus and directly compare viral shedding, infectiousness and the magnitude and quality of immune responses

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(antibody and T cell responses) with Delta variant infection. We anticipate that the infection rate and quantity of viral shedding in vaccinees infected with pre-Alpha will be significantly lower than with Delta and correlate with differential levels and specificities of immune effectors, thus enabling quantification of the determinants of differential clinical outcome associated with antigenic divergence.

In light of the ongoing COV-CHIM01 study (NCT04864548) carried out at University of Oxford (Helen McShane, personal communication), in which sustained infection has so far not resulted in seropositive participants up to an inoculation dose of $1x10^5$ TCID50, a decision on the scientific value of proceeding with the pre-Alpha comparison arm will be taken with the DSMB and TSC. This will take into consideration the available data from COV-CHIM01 and the COVHIC002 (Delta inoculated participants) at the time.

5 STUDY OBJECTIVES

- To test the safety of a GMP SARS-CoV-2 Delta variant challenge virus in vaccinated healthy young adult volunteers and establish the attack rate (ideally >=70%) at an optimised dose.

- To define the clinical, virological and immunological readouts that characterise Delta variant breakthrough infection and contagiousness, compared with those following challenge with the D614G pre-Alpha strain to define the influence of homologous and heterologous immunity.

- To identify viral and host factors associated with differences in attack rate and clinical outcome after
    - Delta variant challenge, correlating with heterologous vaccine-mediated protection;

Thus, we will establish a safe and well-tolerated human challenge model with the SARS-CoV-2 Delta variant for use as a platform to accelerate next-generation vaccine testing and to determine the factors associated with altered clinical and virological outcomes for use as readouts in the model; correlates of protection; and targets for the development of novel vaccines, therapeutics and diagnostics.

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6 STUDY DESIGN

6.1 OVERALL DESIGN

A schematic overview of the study design is presented in Figure 6.

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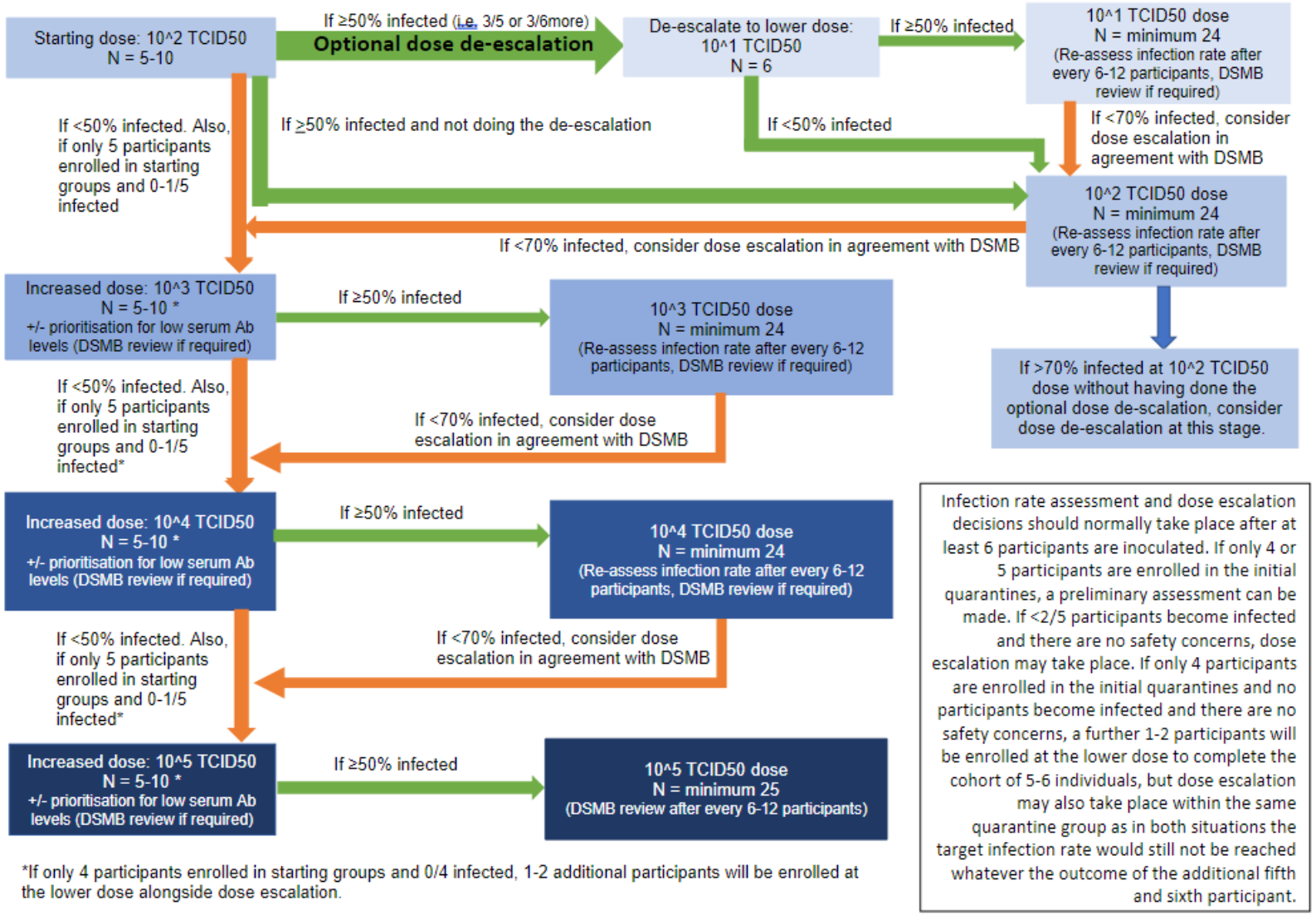

Figure 6. Schematic overview of the study design.

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This is a human experimental infection study in healthy adults 18 to 30 years of age inclusive with no known risk factors for severe COVID-19, using a GMP-produced SARS-CoV-2 Delta variant strain.

Following dose optimisation to identify the minimum dose that results in an infection rate of 50-70% with the SARS-CoV-2 Delta variant, at least n=30 will be tested at the same "dose optimised" conditions.

Pre-selection of participants who have low serum antibody levels may occur where feasible depending on recruitment.

Dose de-escalation to 10 TCID50 may optionally occur depending on the infection rate at 10^2 and in consultation with the DSMB/TSC.

If the de-escalated dose of 10 TCID50 is used and the infection rate is <70% after the n=30 group, we will consider dose escalating to 10^2 in consultation with the DSMB/TSC.

For volunteers, the study is divided into three phases, the screening phase, quarantine phase (confinement phase) and follow-up phase.

At the London site, the screening and follow-up phases will take place in an outpatient setting at the Imperial Clinical Research Facility (ICRF), Imperial Clinical Respiratory Research Unit (ICRRU) and/or Chelsea and Westminster Clinical Research Facility and the quarantine phase will take place in negative pressure rooms at Chelsea and Westminster Hospital.

At the Oxford site, the screening and follow-up phases will take place in an outpatient setting at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) or the Experimental Medicine Clinical Research Facility (EMCRF) and the quarantine phase will take place in a designated quarantine unit in the Experimental Medicine Clinical Research Facility (EMCRF) at the University of Oxford. Chest X-Rays will take place at The Churchill Hospital, Oxford.

Specific procedures to be performed during the study, as well as their prescribed time points and associated visit windows, are outlined in the SoA in Section 3. Details of each procedure are provided in Section 7.

Where multiple assessments are scheduled for collection at the same time point, assessments can be performed before or after the specified time point, within their permitted time windows, as required. Actual dates and times of all assessments will be recorded in the CRF.

6.2 SCREENING PHASE

The screening process will consist of the following stages: registration (if not already registered); pre-screening questionnaire (online or by phone); pre-screening visit for antibody tests; full screening visit. Screening will occur between days -90 to -3 prior to date of virus inoculation. Pre-screening and screening visits may be combined if the participant is joining a quarantine cohort that does not require pre-selection for antibodies. This will be decided in advance by the study team and communicated to the participant when booking the visit.

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Advertising

Advertising of the study will be done through a number of channels, such as:

- Website (e.g. study specific or sponsor's/site's website or webpages)
- Social media adverts and posts (e.g. Facebook, Twitter, LinkedIn and Instagram)
- GP invitation letters and GP text messaging services (local GP practices can be approached via the local CRN to contact potentially eligible participants registered with them and direct them to the study information on a website. The sponsor/site will not have access to their personal information)
- Direct mailouts and emails through any volunteer databases or mailing lists where members of these databases/lists have previously agreed/consented to be contacted about taking part in research. Examples include the NIHR Be Part of Research database, 1Day Sooner database and site specific research volunteer databases.
- Radio (including Spotify) or newspaper adverts
- Posters and leaflets
- Institute or Trust newsletters
- Referral
- Organic search (e.g. via Google or other search engines)

Registration and Pre-Screening Questionnaire

For volunteers interested in taking part at the London site, they will be asked to register their interest by completing an online pre-screening questionnaire and submitting their details to the study team. All advertising and media will direct volunteers to this web page. Volunteers already registered with an ethically-approved volunteer database may be contacted to determine their interest in participating in SARS-CoV-2 research and directed to the webpage with the pre-screening questionnaire. For volunteers unable to register on the study website, they may be registered by a member of the study team by telephone or in person.

For participants interested in taking part at the Oxford site, they will employ the same strategy as above and host their own webpage and online pre-screening questionnaire for this study on their own website. The same ethically approved website text including the pre-screening questions below will be used.

Pre-screening Questionnaire:

- Are you aged between 18 – 30 years?
- Are you registered with an NHS GP?
- For London site: This study involves several visits to the Hammersmith or St Mary's Hospital and a minimum 13-day quarantine stay at the Chelsea and Westminster Hospital, would you be able to commit to this?
  For Oxford site: This study involves several visits to either the Experimental Medicine Clinical Research Facility (EMCRF) and Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford and a minimum 13-day quarantine stay at the EMCRF, would you be able to commit to this?
- Have you ever had a positive test for COVID-19, either by PCR or Lateral Flow?
- Have you received a full course of COVID-19 vaccinations (at least 2 doses, or at least one dose of Johnson & Johnson)?
- Are any of the COVID-19 vaccines you have received not currently approved for use in the UK?

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- Are you currently pregnant, breastfeeding or planning to become pregnant in the next year, or have you been pregnant or breast-feeding within the last 6 months?
- Have you previously taken part in a COVID-19 Vaccine Trial or a COVID-19 Human Challenge Study?
- Do you have a Body Mass Index (BMI) between 18 kg/m2 and 30 kg/m2?
- Do you have close domestic contact (such as sharing a household with, or caring for), children under 2 years old, the elderly (>65 years), or any clinically vulnerable people?
- Have you taken part in any other human viral challenge studies in the last year (e.g. "flu camp")?
- Have you ever received chemotherapy, immunoglobulins or any other cytotoxic or immunosuppressive drugs?
- Have you had any first-degree relatives under 50yrs old who have had sudden cardiac or unexplained death?
- Do you have a history of or current alcohol addiction or excessive alcohol consumption which is defined as an average weekly intake in excess of 28 units alcohol; one unit being a half glass of beer, a small glass of wine or a measure of spirits)?
- Do you currently use recreational drugs or have you used recreational drugs in the last 3 months?
- Do you currently smoke or use e-cigarettes?
- Have you had any loss of taste or smell in the last 3 months?
- Have you had any significant nosebleeds in the last 3 months?
- Have you had nasal or sinus surgery within the last 6 months? (This includes rhinoplasty or "nose jobs")
- Do you have diabetes?
- Do you have an autoimmune disease, known immunodeficiencies of any cause or are immunosuppressed?
- Have you got a history of or currently have asthma, chronic obstructive pulmonary disease (COPD), pulmonary hypertension, reactive airway disease, or chronic lung condition? (This includes ever having severe wheeze or wheeze resulting in hospitalisation)
- Have you got a history or current cardiovascular, cerebrovascular or thromboembolic disease? (This includes strokes, aneurysms, heart attack/myocardial infarction, blood clots etc)

At the end of the pre-screening questionnaire, if they have made it through all the questions and are deemed eligible at this point, they will be able to read the Privacy Policy and then submit the following details:

- Full Name
- Gender
- Date of Birth
- Email
- Phone number
- Postcode
- How did you hear about the study?

These details and questionnaire responses will be downloaded by or sent by email to the research team at the site who may then contact them by phone to confirm the answers they provided on the pre-screening questionnaire and ask further questions pertaining to the full study inclusion/exclusion criteria. If inclusion/exclusion criteria are provisionally met based on answers to these questions, they will then be invited for a pre-screening visit and sent a

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confirmation email with the visit details and the Participant Information Sheet (PIS). They will also be encouraged to watch the information video on the website prior to their visit.

Pre-Screening visit

Pre-screening appointments for the London site will be conducted at the ICRF, Hammersmith Hospital; ICRRU, St Mary's Hospital, Imperial College Healthcare NHS Trust; Chelsea and Westminster Clinical Research Facility; or for the Oxford site, will be conducted at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) or the Experimental Medicine Clinical Research Facility (EMCRF). The participant information sheet (PIS) will be sent to the participant at least 24hours prior to the pre-screening visit and they will be encouraged to read this in advance. During the visit, the summary PIS and the pre-screening process will be discussed with the participant by the study doctor or nurse. When the participant 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 the relevant consent form in the presence of the study doctor or nurse who will also sign the consent form. Written consent for pre-screening will be obtained prior to any study procedures. A copy will be kept in the research file, a copy given to the participant and a copy put into their medical notes.

There may be optional statements on the pre-screening ICF. The participants will have these optional study procedures explained to them and these will be detailed in the PIS. The participant can then decide whether to agree to undergo these optional procedures and if they chose not to, this would not affect their ability to take part in the study.

Following informed consent, the following assessments will be completed at the pre-screening visit:

- Confirmation of name, age, gender, and contact details
- COVID-19 vaccination and infection history
- Venous blood sampling for Anti-S and Anti-N serology
- Optional: Dried blood spot (DBS) capillary sample (by finger prick)

Sero-suitability will be defined as positive anti-S AND negative anti-N antibody detection or positive anti-S AND positive anti-N antibody detection. Whilst we aim to recruit vaccinated participants with no previous SARS-CoV-2 infection, if very low numbers are found (i.e. anti-S positive, anti-N negative), enrolment of those with both anti-S and anti-N antibodies may occur, with prioritisation for low antibody titres, at the CI/PI's discretion. Those with an indeterminate anti-N antibody result and no history of laboratory-confirmed SARS-CoV-2 infection may be included or excluded at the CI/PI's discretion on a case-by-case basis.

If the volunteer is still deemed to be eligible for the study following the antibody test results, they will then be invited for a full screening visit. If they are not eligible, the research team will contact them by phone, email or letter to explain the outcome of their antibody tests results and thank them for their interest in taking part in the study.

Screening visit

Screening appointments for the London site will be conducted at the ICRF, Hammersmith Hospital; ICRRU, St Mary's Hospital, Imperial College Healthcare NHS Trust; Chelsea and

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Westminster Clinical Research Facility; or for the Oxford site, will be conducted at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) or the Experimental Medicine Clinical Research Facility (EMCRF). The participant information sheet (PIS) will be sent to the participant at least 24hours prior to the screening visit and they will be encouraged to read these in advance. During this visit, the full screening visit process as well as the PIS and ICF will be discussed with the participant by the study doctor or nurse. A multiple-choice quiz will then be conducted to check the volunteer's understanding of the study rationale, procedures and risks. If they do not pass the quiz, the study team member will check the incorrect quiz answers and re-review with the volunteer the relevant ICF sections to ensure understanding. When the participant 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 the relevant consent form in the presence of the study doctor or nurse who will also sign the consent form. Written consent for screening will be obtained prior to any study procedures. A copy will be kept in the research file, a copy given to the patient and a copy put into their medical notes.

There may be optional statements on the ICF and a separate ICF for genetic testing of samples which is also optional. The participants will have these optional study procedures explained to them and these will be detailed in the PIS. The participant can then decide whether to agree to undergo these optional procedures and if they chose not to, this would not affect their ability to take part in the study.

Following informed consent, the following assessments will be completed at the screening visit:

- Confirmation of name, demographics and contact details
- Full medical and medication history including questions about past and present health including clinically significant family history
- Quality of life questionnaires including the GAD-7 Anxiety Test questionnaire and PHQ-9 Depression Test questionnaire
- Questions about current weekly alcohol and/or smoking consumption and any history of use of drugs of abuse
- Check of any previous participation in clinical trials via The Over-Volunteering Prevention System (TOPS).
- Examination for signs of illness or disease (a medical examination).
- Height and weight measurements to calculate BMI
- Pulse rate, blood pressure, temperature, saturations and breathing rate checked (Vital Signs).
- Electrocardiogram (ECG)
- Urine samples for:
    - Evidence of infection or kidney/urinary tract conditions
    - Test for pregnancy (for WOCBP)
    - For drugs of abuse & cotinine
- Safety blood tests, including full blood count, renal and liver function tests.
- Blood samples for Hepatitis B and C and HIV (the virus that causes AIDS)
- Blood sample to save plasma and whole blood cells for HLA typing and other research assays if the participant passes screening
- Spirometry
- Chest X-Ray

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- Swabs taken from the nose and throat, nasosorption and nasal curettage/nasopharyngeal swab (tolerance testing)

The volunteer's medical history will either be reviewed during screening (after informed consent) using the hospital's electronic patient record system (such as Cerner) or using the ACCURX system as described in the PIS. If it is not possible to review their records using this system, or the record is not complete, the volunteer's medical history will be requested from their GP and reviewed to assess suitability. Participants may be invited for repeat assessment if required at the PI's discretion. If they are still eligible to take part, and when the participant has had enough time to consider their participation in this study, ask questions and if they are still willing to take part in the study, they will be invited to be admitted to the quarantine unit (Day -2/-1).

Pre-admission isolation and COVID-19 testing procedures may be required. These will align with whatever standard procedures for elective patient admissions are in place at Imperial College Healthcare NHS Trust, Chelsea and Westminster Hospital NHS Foundation Trust or Oxford University Hospitals NHS Trust at the time of admission. It is recognised that the Standard Operating Procedures for elective admissions may change over time, and as such the risk mitigating steps listed above may be adjusted to align with the procedures, as appropriate.

6.3 QUARANTINE PHASE

Participants will stay overnight for a period of at least 17 days (for those with evidence of infection) or 13 days (for those who remain uninfected), from 1-2 days before the viral challenge, to 10-14 days after viral challenge (see discharge criteria Section 6.3.1). This period of quarantine 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), without excessively long quarantine that might cause the participant mental or physical harm. During the quarantine period, all study procedures will take place in the site's designated quarantine unit for this study. Standard procedures for elective patient admissions at the quarantine site will be adhered to.

Before each cohort of participants at the London site, if there is a need to review clinical capacity at the Chelsea and Westminster Hospital, representatives of the bed management teams will meet with the CI, PI's and study teams to review daily capacity data, non-ITU and ITU bed state, plus local and national projections. The panel will provide an evidence-based opinion on whether they are happy for quarantine to go ahead. This decision will be recorded in the Trial Master File.

Before each cohort of participants at the Oxford site, there may be a need to review local hospital capacity and Oxford will follow their local SOPs and guidelines for quarantine admissions in relation to other potentially involved parties such as their local ITU teams.

Admission to quarantine

On Day -2/-1, the participant will be admitted to the quarantine unit. They will be assigned to their own room where they will remain for the duration of the quarantine phase.

Discharge from quarantine process

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Participants will remain in the quarantine unit until the following criteria are met:

- For participants with evidence of infection (defined as 2 consecutive viral detections by qPCR after day 2 post-inoculation):
    - A minimum of 14 days post-inoculation AND no viable virus in nose and throat swabs by overnight culture on two consecutive days AND a negative or overall decreasing viral load by quantitative PCR
    - Laboratory-performed lateral flow tests may be used in place of culture, if culture data are not available
    - A qualitative PCR may be used in place of quantitative PCR, if the latter is not available.
    - At the CI's discretion if protracted quarantine is deemed to be causing harm to the participant's mental or physical health and no viable virus is detected by culture or a lateral flow test is negative.

- For participants who remain uninfected:
    - A minimum of 10 days post-inoculation AND 2 consecutive swabs with undetectable virus by PCR only prior to discharge.

If the discharge criteria above are met then participants will be discharged from the confinement facility at the discretion of the PI/CI. Participants will be advised that they should stay in the quarantine unit they meet discharge criteria.

Self-discharge:

If a participant wishes to leave quarantine after virus inoculation but before discharge criteria are met, they will be counselled on the risk of transmission - particularly of transmitting a viral variant (Delta or pre-Alpha) that is not currently circulating in the community. They will be instructed on infection control measures and will need to self-isolate at home **for at least 14 days after inoculation AND have a negative lateral flow rapid antigen test**. Any household members will be asked to isolate for 14 days from the time they first contact the volunteer. The local health protection team (HPT) will be notified and may follow up as required. They will also be informed that "rescue" treatment may not possible in the community and therefore they may not receive it; and that they must remain in close contact with the study team to be monitored for any safety signals. If possible, a member of the study team will visit them at home and continue taking samples, including virology swabs to monitor whether they remain infectious.

In the event that a participant is suspected of becoming unwell or suffers a SAE deemed related to virus inoculation after discharge and requires further treatment, the participant will be provided with advice for immediate medical management followed by discussion with the PI/CI for possible referral for further assessment in accordance with "Criteria for Clinical Escalation of Participants" or elsewhere as appropriate (see Withdrawal criteria). Hospitalisation of any participant will lead to the immediate pausing of the trial and the DSMB will be convened to assess the clinical evidence in order to determine whether the study may proceed (see Data Safety Monitoring Board).

Post-discharge process

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Infected participants:

Infected participants who show persistent PCR quantifiable virus shedding may be requested to perform self-swabbing post discharge, at the discretion of the PI/CI.

- Weekly combined nose and throat swabs may be arranged for participants who show persistent PCR quantifiable virus shedding. If swab results raise any concern about infectiousness, e.g. PCR value <33.5, then a lateral flow test can be performed. If the lateral flow test is negative, isolation is not required.
- Additional swabs will not be required once a single swab has returned a "not detected" result.

Where low level virus can still be detected, participants will be reminded at each contact with the study team that they must follow government guidelines regarding infection control (including hand-washing and social distancing) and avoid contact with high-risk individuals.

Uninfected participants:

Uninfected participants who are eligible for discharge on Day 10 (as per Section 6.3.2) will be required to attend daily visits on Day 11, Day 12, Day 13 and Day 14 and will undergo procedures as specified in the SoA in Section 3. Procedures will be performed at maximum once daily. At the post-discharge visits, blood samples for safety (FBC, LFT, urea and electrolytes, CRP, clotting) and immunology (PAXgenes, cytokine profile, PBMCs, serum), vital signs and other clinical data will be taken. Nose/throat swabs, saliva, masks sampling may be taken as an optional sample, at the discretion of the PI/CI. Where it is not practical for volunteers to leave and reattend for Day 11-14 visits they may opt to stay in the quarantine unit until Day 14. In this case, they will remain in full quarantine on the unit until Day 14.

6.4 Follow Up Phase

Those who remain uninfected and are discharged on day 10 after inoculation will be required to attend daily visits on Day 11, Day 12, Day 13 and Day 14.

There will be a telephone call at Day 21 (+/- 2 days). During follow up phase, all participants will be required to attend 5 clinic visits on Day 28 (+/- 3 days), Day 90 (+/- 7 days), Day 180 (+/- 14 days), Day 270 (+/- 14 days) and Day 360 (+/- 14 days), as specified in the SoA in Section 3.

Testing of symptomatic volunteers during the follow up phase

At discharge from quarantine, all participants will be provided with lateral flow antigen test kits and RT-PCR swab kits with pre-paid postage and instructions. Participants will be reminded at each study visit to get in touch with the study team if they present with symptoms of COVID-19 (as detailed in NHS guidance (https://www.nhs.uk/conditions/coronavirus-covid-19/symptoms/main-symptoms/)), if they have tested positive for SARS-CoV-2 whilst asymptomatic (on lateral flow antigen test or RT-PCR) or if they are admitted to hospital for any reason.

Participants who become symptomatic during follow-up (up to Day 360) will be instructed to self-perform a lateral flow antigen test and call the study team who will then advise on how to

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proceed with further clinical testing as necessary. Participants without symptoms who test positive for SARS-CoV-2 in the community (on a lateral flow test or RT-PCR) will also be requested to contact the study team. For those who test positive for SARS-CoV-2, the study team may request the participant attends for Testing Visits at the study site, or they may arrange a Home Visit and if neither of those are possible, they will arrange a telephone assessment with the Study Doctor.

Testing Visit 1 should occur as soon as possible and within 7 days of symptom onset or within 7 days of a positive test if the person is asymptomatic. The participant may be asked to self-perform an RT-PCR swab before Testing Visit 1, at the CI/PI's discretion. This will be posted to the study team by the participant using the pre-paid postage label provided, or collected by courier from the participant's home, as arranged by the study team. Where possible, an onsite or home visit may be arranged for the study team to collect upper respiratory tract samples, collect blood samples for immunology (e.g. PAXgenes, cytokine profile, PBMCs, serum), measure vital signs, perform a physical exam and collect other clinical data if needed.

Should it not be possible, or necessary for an in-person visit, a telephone call may be arranged with the study doctor. During the telephone call the participant will be instructed to perform an RT-PCR swab (if not already done). The study team will collect information on symptoms and concomitant medications. They will not be able to collect blood samples, take vital signs or perform physical exam if the visit has to be conducted over the phone.

If the visit is conducted at a participant's home, the study team will follow their SOP for home visits.

Testing Visit 2 should occur 28 days (+/-7 days) after Testing Visit 1. At Testing Visit 2, clinical data and additional blood samples for immunology may be taken.

If a Testing Visit falls within the window of a scheduled visit, the visits may be combined.

Symptomatic volunteers may be regularly reviewed over the phone or via video call using a smartphone or computer app if clinically appropriate.

End of Study Definition

A participant is considered to have completed the study if he/she has completed all phases including the last scheduled visit shown in the SoA Day 360 (± 14 days) or the last unscheduled visit as applicable. If a safety visit is required after the last scheduled visit, this will be at the PI's discretion as a duty of care, e.g. repeat spirometry or laboratory tests. These discretionary follow-up visits will not be considered part of the trial data unless they represent follow-up and closure on an AE or serious adverse event (SAE) identified during the trial period.

The end of the study is defined as the date of the last visit of the last participant in the study. See Section 7.4 for procedures in the event of discontinuation of study intervention or volunteer withdrawal.

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Participants will return to the care of their own GP following discharge from the quarantine unit. Should any abnormal assessment or finding be reported that is deemed clinically significant, the participant's GP will be informed.

6.5 STUDY POPULATION

This study will enrol healthy, fully-vaccinated individuals, who may or may not have also been infected with SARS CoV-2, aged between 18-30 years. The full inclusion and exclusion criteria can be found below. Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions are not permitted in this study.

Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

NO	INCLUSION CRITERIA
1	An informed consent form has been signed and dated by the participant and the Investigator.
2	Adults age between 18 and 30 years inclusive (at the time of consent)
3	Evidence of having had a complete COVID-19 vaccination course with the last vaccination at least 14 days before enrolment
4	Sero suitable as defined by: • positive anti-S AND negative anti-N antibody detection OR positive anti-S AND positive anti-N antibody detection (at the CI/PI's discretion). Those with an indeterminate anti-N antibody result and no history of laboratory-confirmed SARS-CoV-2 infection may be included or excluded at the CI/PI's discretion on a case-by-case basis.
5	Female participants must be willing and able to use contraception as described in the study protocol from 2 weeks before the scheduled date of viral challenge until 6 months after receipt of the final dose of study virus. Negative urine pregnancy tests will be required at screening and on day 0 prior to inoculation. On admission to the quarantine unit a Negative serum beta human chorionic gonadotropin (β-hCG) is required. **<u>Contraceptive requirements:</u>** Established use of hormonal methods of contraception described below (for 2 weeks prior to viral challenge). 1) combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: i. oral ii. intravaginal iii. transdermal 2). progestogen-only hormonal contraception associated with inhibition of ovulation: i. oral ii. injectable iii. implantable 3) Intrauterine device (IUD) 4). Intrauterine hormone-releasing system (IUS) 5) Barrier methods such as condoms 6). Bilateral tubal ligation 7). Male sterilisation where the vasectomised male is the sole partner for that woman.

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NO	INCLUSION CRITERIA
	8). True abstinence - sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the participant.
6	Male participants who are willing to use one of the contraception methods described in the study protocol, from the time of the date of viral challenge, for 6 months. **Contraceptive requirements:** a. Use a condom to prevent pregnancy in a female partner. b. Male sterilisation. This applies only to males participating in the study. d. True abstinence - sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. • In addition to the contraceptive requirements above, male participants must agree not to donate sperm following discharge from quarantine until 6 months after the date of study virus receipt.
7	In good health with no history of clinically significant medical conditions (as described in Exclusion criteria) that would interfere with subject safety, as defined by medical history, physical examination and routine laboratory tests, ECG, and Chest X-Ray and determined by the Investigator at an admission evaluation.
8	Participants will have a documented medical history either prior to entering the study and/or following medical history review with the study physician at screening.
9	Willing and able to commit to participation in the study.

Table 4. Inclusion Criteria.

Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

NO	STANDARD EXCLUSION CRITERIA
Any potential participant who meet any of the criteria below will be excluded from participating in this study.	
Clinical history	

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NO	STANDARD EXCLUSION CRITERIA
1.	History or evidence of any clinically significant or currently active cardiovascular, (including thromboembolic events), respiratory, dermatological, gastrointestinal, endocrine, haematological, hepatic, immunological, rheumatological, metabolic, urological, renal, neurological, psychiatric illness. Specifically: a) Participants with any history of physician diagnosed and/or objective test confirmed asthma, chronic obstructive pulmonary disease, pulmonary hypertension, reactive airway disease, or chronic lung condition of any aetiology or who have experienced: o Significant/severe wheeze in the past o Respiratory symptoms including wheeze which has ever resulted in hospitalisation o Known bronchial hyperreactivity to viruses b) History of thromboembolic, cardiovascular or cerebrovascular disease c) History or evidence of diabetes mellitus d) Any concurrent serious illness including history of malignancy that could interfere with the aims of the study or a participant completing the study. Basal cell carcinoma within 5 years of treatment or with evidence of recurrence is also an exclusion e) Migraine with associated neurological symptoms such as hemiplegia or vision loss. Cluster headache/migraine or prophylactic treatment for migraine f) History or evidence of autoimmune disease or known immunodeficiency of any cause. g) Other major disease that, in the opinion of the Investigator, could interfere with a participant completing the study and necessary investigations. h) Immunosuppression of any type
2.	Any significant abnormality altering the anatomy or function of the nose or nasopharynx in a substantial way (including loss of or alterations in smell or taste), a clinically significant history of epistaxis (large nosebleeds) within the last 3 months, nasal or sinus surgery within 6 months of inoculation.
3.	Clinically active rhinitis (including hay fever) or history of moderate to severe rhinitis, or history of seasonal allergic rhinitis likely to be active at the time of inclusion into the study and/or requiring regular nasal corticosteroids on an at least weekly basis, within 30 days of admission to quarantine.
4.	History of anaphylaxis and/or a history of severe allergic reaction or significant intolerance to any food or drug, as assessed by the PI.
5.	Significant history or presence of drug or alcohol misuse
6.	Current use of any drugs taken through the nasal or inhaled route including recreational drugs.
7.	Psychiatric illness including participants with a history of depression and/or anxiety with associated severe psychiatric comorbidities, for example psychosis. Consider exclusion in the following cases: (a) Participants with history of anxiety-related symptoms of any severity within the last 2 years if the Generalized Anxiety Disorder-7 score is ≥4; (b)Participants with a history of depression of any severity within the last 2 years if the Patient Health Questionnaire-9 score is ≥4. (c) severe claustrophobia

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NO	STANDARD EXCLUSION CRITERIA
8.	• Current active smokers, equivalent to ≥5 cigarettes per week, including use of tobacco in any form (e.g., smoking or chewing) or other nicotine-containing products in any form (e.g., gum, patch) or electronic cigarettes. Ex-smokers: • Participants who have smoked ≥5 pack years at any time [5 pack years is equivalent to one pack of 20 cigarettes a day for 5 years]). Ex-smokers that have smoked <5 pack years at any time must have not smoked in the last 3 months
9.	Family history of 1st degree relative aged 50 years or less with sudden cardiac or unexplained death
10.	Personal or Family History of unexpectedly severe COVID-19, adverse response to any other viral disease e.g. Guillain–Barré, or a family history (described as a 1st degree relative) with clotting disorders
Measurements and investigations	
11.	A total body weight of ≤ 50kg and a Body Mass Index (BMI) ≤18 kg/m2 and ≥28 kg/m2. The upper limit of BMI may be increased to ≤ 30kg/m2 at the PI's discretion, in the case of physically fit muscular individual
12.	Venous access deemed inadequate for the phlebotomy demands of the study.
13.	Any clinically significant abnormal finding on screening biochemistry, haematology and microbiology blood tests or urinalysis i.e. grade 1 lab abnormalities or above apart from minor deviations which are clinically acceptable and approved by the Principal Investigator a) Elevated random glucose and HbA1C b) Positive HIV, active/chronic hepatitis B or C test. c) Confirmed positive test for drugs of abuse on admission and urinary cotinine at quarantine.
14.	A forced expiratory volume in 1 second (FEV1) and a forced vital capacity (FVC) <80% of predicted value calculated using ATS/ERS guidance (refer to Section 8.4)
15.	Twelve-lead ECG recording with clinically relevant abnormalities as judged by the study physician/PI.
Recent respiratory infection	
16.	History of, or currently active symptoms suggestive of upper or lower respiratory tract infection (including reduced sense of taste and smell, raised body temperature and/or persistent cough) within 4 weeks prior to viral challenge.
17.	Presence of cold-like symptoms and/or fever (defined as participant presenting with a temperature reading of >37.9ºC) on Day -2, Day -1 and/or pre-challenge on Day 0.
18.	Evidence of any respiratory viruses (on Respiratory PCR from upper respiratory tract sample) prior to challenge virus inoculation on admission to the quarantine unit. These may include: VIRUSES: ● Adenovirus ● Coronavirus HKU1 ● Coronavirus NL63

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NO	STANDARD EXCLUSION CRITERIA
	● Coronavirus 229E ● Coronavirus OC43 ● Human Metapneumovirus ● Human Rhinovirus/Enterovirus ● Influenza A ● Influenza A/H1 ● Influenza A/H3 ● Influenza A/H1-2009 ● Influenza B ● Parainfluenza Virus 1 ● Parainfluenza Virus 2 ● Parainfluenza Virus 3 ● Parainfluenza Virus 4 ● Respiratory Syncytial Virus BACTERIA: ● Bordetella parapertussis ● Bordetella pertussis ● Chlamydia pneumoniae ● Mycoplasma pneumoniae
Receipt of medications and interventions	
19.	Evidence of a live vaccine within 60 days prior to the planned date of viral challenge, a non-live vaccine within 30 days prior to the planned date of viral challenge or intention to receive any vaccination(s) before the day 28 follow-up visit. (NB. No travel restrictions applied after the Day 28 Follow-up visit).
20.	Receipt of blood or blood products, or loss (including blood donations) of 550 mL or more of blood during the 3 months prior to the planned date of viral challenge or planned during the 3 months after the final visit.
21.	Medications a) Use of any medication or product (prescription or over-the-counter), for symptoms of hayfever, nasal congestion or respiratory tract infections or dermatitis/eczema including the use of regular nasal or medium-high potency dermal corticosteroids, antibiotics and First Defence™ (or generic equivalents) within 7 days prior to the planned date of viral challenge apart from those described in Table 7, Permitted Medication or agreed by the Principal Investigator b) Receipt of any investigational drug within 3 months prior to the planned date of viral challenge. c) Receipt of three or more investigational drugs within the previous 12 months prior to the planned date of viral challenge. d) Receipt of systemic (intravenous and/or oral) glucocorticoids or systemic antiviral drugs within 6 months prior to the planned date of viral challenge. e) Over the counter medications (e.g., paracetamol or ibuprofen) where the dose taken over the preceding 7 days prior to the planned date of viral challenge had exceeded the maximum permissible 24-hour dose (e.g., >4g per day of paracetamol over the preceding week).

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NO	STANDARD EXCLUSION CRITERIA
	f) Chronically used medications, including any medication known to be a moderate/potent inducer or inhibitor of cytochrome P450 enzymes, within 21 days prior to the planned date of viral challenge. g) Participants who have received any systemic chemotherapy agent, immunoglobulins, or other cytotoxic or immunosuppressive drugs at any time. h) Concurrent use of medications contraindicated for use with Paxlovid unless confirmed alternative rescue therapy will be available to the site at the time of enrolment
22.	Prior participation in another human viral challenge study in the preceding 6 months taken from the date of viral challenge in the previous study to the date of expected viral challenge in this study. The participant must also have completed the follow up visit requirements of the previous viral challenge study.
23.	Previous participation in a SARS-CoV-2 vaccine trial of a currently unapproved/unlicensed vaccine in the UK
24.	Any nasal sampling procedure in the month before date of expected viral challenge in this study (excluding study tolerance test or routine tests for COVID-19)
General	
25.	Participant is mentally or legally incapacitated in the opinion of the Investigator.
26.	Females who: a) Are breastfeeding within 6 months of study commencement, or b) Had been pregnant within 6 months prior to the study, or c) Had a positive pregnancy test at any point during screening or prior to inoculation with challenge virus
27.	Those in close domestic contact (i.e. sharing a household with, caring for, or daily face to face contact) with children under 2 years, the elderly (>65 years), immunosuppressed persons, or those with chronic respiratory disease
Other	
28.	Anyone who works on the study (e.g. a delegated study nurse) or in close proximity to the study at the sponsor organisation, participating trial sites or any contract research organisations involved in this study.
29.	Anyone who is first degree related to, or resides with, anyone who is a delegated member of the research team at a study site.
30.	Any other reason that the Investigator considered made the participant unsuitable to participate.
31.	Participants with no knowledge of their family history

Table 5. Exclusion criteria.

Lifestyle Considerations

3. Meals and dietary restrictions

No dietary restrictions are required before or after virus administration.

4. Caffeine, alcohol and tobacco

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Participants must not consume excessive amounts of alcohol for 72 hours prior to quarantine or any study visits. Participants cannot consume alcohol at all during quarantine. Participants must not smoke, use tobacco or any nicotine containing products for 3 months prior to and during quarantine. Participants will be allowed to drink caffeinated drinks during quarantine.

5. Activity

Participants will abstain from strenuous exercise that is unusual for them, for 48 hours prior and during quarantine and for 48 hours prior to each clinic visit (this is at the discretion of the Investigator).

Screen Failure

Screen failures are defined as participants who sign the Informed Consent Form (ICF) but who are not subsequently enrolled in the study. For individuals who do not meet the criteria for participation in this study (screen failure), the Investigator will decide whether the participant should be permanently excluded from the study or invited back for repeat assessments (i.e. repeat clinical laboratory test) if the initial screening assessments are still within the allowed screening windows, or rescreening for a later quarantine, as appropriate.

7 STUDY OUTCOME MEASURES

7.1 PRIMARY, SECONDARY AND TERTIARY ENDPOINTS

OBJECTIVES	ENDPOINTS
PRIMARY	
To identify a safe and infectious dose of Delta variant SARS-CoV-2 in healthy volunteers, suitable for future intervention studies	To evaluate the safety of Delta variant SARS-CoV-2 challenge in healthy participants by assessing: • Occurrence of unsolicited AEs within 30 days post-viral challenge (Day 0) up to Day 28 follow up. • Occurrence of SAEs related to the viral challenge from the viral challenge (Day 0) up to Day 28 follow up.
	To identify a Delta variant SARS-CoV-2 inoculum dose that safely induces laboratory confirmed infection in ≥50% of participants (ideally 70%, and not less than 50%). Laboratory confirmed infection is defined as: • Two quantifiable greater than lower limit of quantification (≥LLOQ) RT-PCR measurements from mid turbinate and/or throat samples, reported on 2 or more consecutive timepoints, starting from Day 2 post-inoculation and up to discharge from quarantine.
SECONDARY	
To further assess SARS-CoV-2 viral infection rates in upper respiratory samples in healthy volunteers	• To assess the incidence of laboratory confirmed infection rates using a) mid turbinate samples, b) throat swabs, and c) both mid turbinate and throat swabs.
To assess the incidence of symptomatic SARS-CoV-2 infection, in healthy volunteers	• To assess the incidence of lab-confirmed symptomatic SARS-CoV-2 infection using a) mid turbinate samples, b) throat swabs, and c) both mid turbinate and throat swabs.

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To assess the SARS-CoV-2 viral dynamics in upper respiratory samples (AUC, peak, duration, incubation period) in healthy volunteers	To assess the viral dynamics using a) mid turbinate samples, and b) throat swabs, as measured by: • Area under the viral load-time curve (VL-AUC) of SARS-CoV-2 as determined by qRT-PCR, starting from Day 1 post-inoculation and up to discharge from quarantine. • Peak viral load of SARS-CoV-2 as defined by the maximum viral load determined by quantifiable (≥LLOQ) qRT-PCR measurements, starting from Day 1 post-inoculation and up to discharge from quarantine • Duration of SARS-CoV-2 quantifiable (≥LLOQ) qRT-PCR measurements, starting from Day 1 post-inoculation and up to discharge from quarantine. Duration is defined as the time (hours) from the first quantifiable of the two viral quantifiable positives used to assess infection until first confirmed undetectable assessment after their peak measure (after which no further virus is detected). • Incubation period of SARS-CoV-2 qRT-PCR measurements. Incubation period is defined as the time (hours) from inoculation to the first quantifiable of the two viral quantifiable positives used to assess infection, and up to discharge from quarantine. The above endpoints will also be evaluated using quantitative viral culture.
To assess the SARS-CoV-2 induced symptoms, in healthy volunteers	• Sum total symptoms diary card score: sum total clinical symptoms (TSS) as measured by graded symptom scoring system, starting one day post-viral challenge (Day 1) up to discharge from quarantine • Area under the curve over time (TSS-AUC) of total clinical symptoms (TSS) as measured by graded symptom scoring system (categorical and visual analogue scales), starting one day post-viral challenge (Day 1) up to discharge from quarantine. • Peak symptoms diary card score: peak total clinical symptoms (TSS) as measured by graded symptom scoring system (categorical and visual analogue scales, starting one day post-viral challenge (Day 1) up to discharge from quarantine • Peak daily symptom score: Individual maximum daily sum of Symptom score starting one day post-viral challenge (Day 1) up to the end of quarantine. • Number (%) of participants with Grade 2 or higher symptoms
To assess the incidence of SARS-CoV-2 illness, in healthy volunteers	The incidence of: • Upper Respiratory Tract illness (URTI) • Lower Respiratory Tract illness (LRTI) • Systemic illness (SI) • Febrile illness (FI) • Proportion of participants with Grade 3 symptoms on any occasion at any time from the last assessment on Day 0 to quarantine discharge • Proportion of participants with Grade 2 or higher symptoms on any occasion at any time from the last assessment on Day 0 to quarantine discharge • Proportion of participants with Grade 2 or higher Symptoms on two separate occasions at any time from the last assessment on Day 0 to quarantine discharge

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	• Proportion of participants with any symptom (grade >=1) on any occasion at any time from the last assessment on Day 0 to quarantine discharge • Proportion of participants with any symptom (grade >=1) on two separate occasions at any time from the last assessment on Day 0 to quarantine discharge
TERTIARY Tertiary/exploratory endpoints include, but are not limited to;	
To explore the safety of Delta variant SARS-CoV-2 human challenge model in healthy adults	To explore safety related measures of SARS-CoV-2 challenge in healthy participants by assessing: • Changes in smell (anosmia/parosmia) and cognition through infection • Association of ABO blood group and susceptibility to infection • Occurrence of haematological and biochemical laboratory abnormalities during the quarantine period. • Use of concomitant medications within 30 days postviral challenge (Day 0 up to Day 28 follow up).
	•
To explore the SARS-CoV-2 viral infection rates in saliva in healthy volunteers	To measure the laboratory confirmed infection rates, as defined by: • Occurrence of at least two quantifiable (≥LLOQ) RT-PCR measurements, reported on 2 or more consecutive timepoints, starting from Day 2 post-inoculation and up to discharge from quarantine. • Occurrence of at least two detectable (≥LLOD) RT-PCR measurements, reported on 2 or more consecutive timepoints, starting from Day 2 post-inoculation and up to discharge from quarantine. • Occurrence of at least one quantifiable (≥LLOQ) SARS-CoV-2 viral cell culture measurement, starting from Day 2 post-inoculation and up to discharge from quarantine.
To explore the SARS-CoV-2 viral dynamics in saliva in healthy volunteers, by inoculum dose	To assess viral dynamics, as defined by: • Area under the viral load-time curve (VL-AUC) of SARS-CoV-2 as determined by qRT-PCR measurements in saliva, starting from Day 1 post-inoculation and up to discharge from quarantine. • Peak viral load of SARS-CoV-2 as defined by the maximum viral load determined by quantifiable qRT-PCR measurements in saliva, starting from Day 1 post-inoculation and up to discharge from quarantine. • Duration of SARS-CoV-2 quantifiable qRT-PCR measurements in saliva, starting from Day 1 post-inoculation and up to discharge from quarantine. Duration is defined as the time (hours) from first the quantifiable of the two viral quantifiable positives used to assess infection until first confirmed undetectable assessment after their peak measure (after which no further virus is detected). • Incubation period of SARS-CoV-2 qRT-PCR measurements in saliva. Incubation period is defined as the time (hours) from inoculation to the first quantifiable of the two viral quantifiable positives used to assess infection, starting from Day 2 post-inoculation and up to discharge from quarantine. • The above endpoints may also be evaluated using quantitative cell culture.
To explore SARS-CoV-2 viral infection in stool of healthy volunteers	To measure SARS-CoV-2 excretion in the stool by: • Virus detection and quantification using qRT-PCR

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To explore the host-pathogen relationship in the SARS-CoV-2 human challenge model in healthy adults	The primary, secondary, and tertiary endpoints may be explored in relation to immunological levels at baseline and after SARS-CoV-2 challenge. Assays performed on blood, stool and mucosal samples may include, but are not limited to: • Humoral immunity / systems serology SARS-CoV-2 (for example: SARS-CoV-2 neutralizing titres, ELISAs to IgG, IgM, IgA, sIgA, ADCC) • Proteomic levels and changes (for example, cytokine and chemokines) • Cellular cell quantification and quality of immunity (for example T and B cell frequencies, phenotypes and functionality assays, ELISPOTs, ICS, cytokine/chemokine responses) • Transcriptome levels and changes (for example, RNAseq, single cell RNAseq, microarray, PCR) • Human genomics in relation to SARS-CoV-2 susceptibility, infection (e.g. HLA typing, SNPs, GWAS) • Viral genomics to assess the possible emergence of mutations in SARS COV-2 over the duration of the study. • Microbiome analysis in relation to viral infection, disease and susceptibility (e.g. PCR, NGS, 16s rRNA)
To explore environmental contamination in the SARS-CoV-2 human challenge model in healthy adults	To explore the environmental contamination of SARS-CoV-2 as a result of infection in participants, as measured by: • Air sampling for virus detection and quantification • Exhaled breath sampling with the use of a face mask for virus detection and quantification • Aerosol quantification in exhaled breath using an optical particle sizer • Surface swabbing for virus detection and quantification
To explore changes in the vasculature during SARS-CoV-2 Delta infection in healthy adults	To measure changes in endothelial function during SARS-CoV-2 infection: • Using an EndoPAT™ device, to quantify flow mediated vascular dilatation • To assess levels of endothelial derived mediators (e.g. prostacyclin, endothelin-1, nitric oxide) in the plasma
To explore the performance of lateral flow tests (LFTs) during SARS-CoV-2 infection of healthy adults	To compare the performance of lateral flow tests performed: • By participants themselves following manufacturer's instructions (no additional training) • By trained laboratory personnel

Table 6. Primary, Secondary and Tertiary Endpoints.

7.2 WITHDRAWAL AND LOST TO FOLLOW UP CRITERIA

Participant Withdrawal (Early Discontinuation of Quarantine only)

A participant may elect to leave quarantine before discharge criteria are met without withdrawing their consent to continue participating in the study. Wherever possible, the tests and evaluations listed for the Early Withdrawal Visit (EWV) should be carried out prior to the participant leaving the quarantine unit, and the participant should attend planned follow-up visits. It is likely that some of the assessments required as part of the EWV will already have been performed for the study day as per the SoA, in this case the completed assessments will not be repeated on the same day unless clinically indicated and the participant agrees.

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Participants will be counselled that early withdrawal from the quarantine phase of the study is strongly discouraged, as it may pose a risk both to the participant and his/her contacts. In the event of a participant insisting on early withdrawal during the quarantine period, the participant will be advised of the potential risks of carrying SARS-CoV-2 infection into the community, including transmitting a viral variant that is not currently circulating locally, and the risks to vulnerable groups. However, if withdrawal occurs after virus inoculation, every effort will be made to manage their withdrawal and do the following:

- The participant will be counselled about the risk of onward transmission of SARS-CoV-2 and that they will need to remain in self-isolation until 14 days from the time of inoculation and have a negative lateral flow rapid antigen test before de-isolating.
- The participant will be counselled about any risks due to the withdrawal and specifically any risks due to missed safety monitoring that cannot be performed at home
- The participant will be reminded about infection control procedures and receive re-training if necessary, in handwashing and isolation rules
- The participant will be informed they will not receive rescue therapy and the potential consequences
- The participant will be transported home in private transport with appropriate PPE
- The local Health Protection Team may be notified and follow up as required
- Daily follow-up calls will be made to check on participant's health until the originally scheduled medical discharge, in line with their wishes. If possible, the study team will visit their home and perform tests including virology swabs to monitor infectiousness.
- Once the quarantine discharge criteria are met, the participant will be informed they can de-isolate. Where it is not possible to obtain further virology swabs following departure from the quarantine unit, de-isolation will be advised at 14 days after inoculation.
- Household members will also be advised to self isolate themselves for 14 days after first contacting the participant.

Participant Withdrawal (at any other time during the study)

A participant may withdraw their consent to participate in the study at any time and for any reason, without prejudice to his/her future medical care. Participants may decline to give a reason for their withdrawal. See Section 7.2.1 for when a participant withdraws from the study during the quarantine phase.

The PI may withdraw a participant if, in their clinical judgement, it is in the best interest of the participant or if the participant cannot comply with the protocol. Additionally, participants can be withdrawn from the study for the reasons listed below if the Investigator feels this is necessary:

- Non-compliance with the study requirements and restrictions.
- Clinically significant abnormal laboratory findings, which in the opinion of the Investigator(s) and/or Sponsor, precludes further participation in the study.
- Development of inter-current illness which, in the opinion of the Investigator would compromise the health of the participant or the study objectives.
- The Investigator's decision that withdrawal from further participation would be in the participant's best interest.

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- Termination of the study at the discretion of the Investigator(s) or Sponsor for safety, behavioural, or administrative reasons.
- Any intervention (Virus) related SAEs.
- The participant becomes pregnant.
- The wish of the participant.

The sponsor should be notified of all study withdrawals in a timely manner, and in cases where the withdrawal is due to a medical reason the participant would be referred to his/her GP. If the participant withdraws consent for future disclosure of information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent. If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records. In the event that the participant loses capacity during the course of the study, they will be withdrawn from the study.

7.3 Criteria for Clinical Escalation of Participants

Participants will be closely monitored throughout by the study medical team. Any illness recorded from the following list will lead to provision of immediate medical treatment, if necessary, followed by discussion with the CI/PI and possible referral for assessment by the local acute medicine and/or critical care team as agreed with the local site. In the event of a cardiac arrest, the hospital crash team will be summoned to respond.

- **Cardiovascular compromise**, such as -
  Sustained elevated heart rate >120bpm AND sustained low blood pressure SBP<100 for greater than 30 minutes
- **Respiratory compromise**, such as
  Sustained elevated respiratory rate >30/min AND sustained low blood oxygen SaO2<94% for greater than 30 minutes
  Fever (grade 2 or higher) for >48 hours
  Evidence of pneumonia on clinical examination
- **New ECG abnormalities** (compared to baseline), including
  Confirmed Fridericia-corrected QT (QTcF) >500 msec; confirmed increase of QTcF >60 msec above baseline value
- **Deranged and progressively worsening laboratory safety tests**
- **Any symptoms, signs or investigation results that are deemed to be of clinical concern by the CI / PI**

A complete medical history will be provided to the receiving physician including the possibility of SARS-CoV-2 infection. If following assessment by the receiving clinician the participant is transferred for further care in the emergency department or in-patient ward, infection control measures, transfer procedures and further medical or surgical management will be according to local NHS Trust SOPs. This may include COVID-19 treatments as per local SOPs. Hospitalisation of any participant for the above reasons will lead to immediate suspension of further inoculations.

The DSMB will be convened to assess the clinical evidence in order to determine whether the study may proceed (see Section 10.3.5, Data Safety Monitoring Board). Wherever possible, safety assessments and Follow Up Visits for participants who enter the NHS pathway, will continue, after their discharge from the NHS until completion of the study.

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7.4 LOST TO FOLLOW UP

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site, in accordance with the Sponsor's SOP. The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a follow-up letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

Participant Discontinuation of Study Intervention Therapy (Rescue therapy)

Participants will be discontinued from study intervention therapy for the reasons listed below and at the discretion of the Principal Investigator. Although Participants will not receive any further intervention therapy, they will continue to be followed for safety and monitoring as per the SoA, including any additional unscheduled assessments as required for safety reasons.

- Any intervention therapy related SAEs.
- Clinically significant abnormal laboratory findings, which in the opinion of the Investigator(s) and/or Sponsor, precludes further receipt of study intervention therapy.
- The Investigator's decision that withdrawal from further intervention receipt would be in the participant's best interest.
- Anaphylactic reaction following intervention therapy receipt.
- The wish of the participant.

Participants who are discontinued from the study intervention therapy will complete their Quarantine stay and be required to attend their Follow Up Visits, with assessments as detailed in the SoA.

Temporary Discontinuation/Temporary Delay in Enrolment

At the first study visit, if a participant is found to be ineligible due to transient circumstances (such as acute disease and/or fever), the inoculation will be postponed until transient circumstances have been resolved and the participant will be re-invited to a later quarantine group within the allowed time window.

Participant Replacement Strategy

Participants who withdraw or are discontinued from the study may be replaced in order to achieve the planned evaluable number of participants as follows, if deemed appropriate by the PI and with the approval of the CI/Sponsor. Withdrawals or early discontinuation prior to virus inoculation may be replaced.

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7.5 Stopping Rules

The CI, PI and the DSMB will perform safety reviews on available clinical and virology data during the quarantine period, as appropriate.

Any virus-related SAE or virus-related AEs of clinical concern that are reported following human viral challenge will trigger the following:

- If such a status occurs at any point during the study, then the CI, PI and the DSMB will review the data and make a decision.
- Further administration of the virus will be paused.
- The CI, PI and the DSMB will review the data and make a decision on whether it is appropriate to recommence inoculation (via a substantial amendment, if indicated) or terminate the study.

In any event, participant follow-up should continue until resolution or stabilisation of AEs and final follow-up on Day 360 (± 14 days).

8 Study Assessments and Procedures

Study procedures and their timing, including follow-up period (Day 28 – Day 360) are summarised in the SoA (Section 3). All screening evaluations must be completed and reviewed at D-2/-1 to confirm potential participants meet all eligibility criteria. A screening log will be maintained to record details of all participants screened and to document eligibility or record the reasons for screening failure, as applicable. For all study assessments, the value obtained nearest to inoculation will be used as the baseline measure for assessments. Where applicable, unless otherwise stated, normal ranges will be filed in the Trial Master File (TMF).

Protocol waivers or exemptions are not allowed. Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

8.1 Clinical Assessments

Medical and Medication History

Medical and medication histories including any allergies will be recorded at screening, including, but not limited to, detailed histories on allergies (e.g. rhinitis, dermatitis, food, aspirin/non-steroidal anti-inflammatory drugs [NSAIDs] and asthma). Medical history will either be reviewed during screening (after informed consent) using the hospital's electronic patient record system (such as Cerner) or using the ACCURX system as described in the PIS. If it is not possible to review their records using this system, or the record is not complete, the volunteer's medical history will be requested from their GP following screening and prior to challenge and reviewed for eligibility. Concomitant medications will be reviewed at each study visit.

Patient Health Questionnaire (PHQ-9) and Generalised Anxiety Disorder (GAD-7) Questionnaire

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PHQ-9 and GAD-7 questionnaire will be used at screening +/- admission to assess participants' eligibility in terms of ability to tolerate isolation in the quarantine unit.
Before discharge from quarantine they may undergo a repeat GAD-7 and PHQ-9 at the discretion of the Investigator.

Demographics

Demographic data will be recorded at screening. This will include:

- Age
- Sex/Gender
- Ethnicity/Race

Height, Weight and Body Mass Index (BMI)

Height and weight measurements will be recorded in compliance with local standard procedures.
BMI will be calculated as: $BMI\ (kg/m2) = \frac{Weight\ (kg)}{Height\ (m)2}$

Physical Examinations

A complete physical examination will include a full systemic assessment and will be conducted by an investigator or medically qualified designee.

Symptom-directed physical examinations will be conducted as deemed appropriate by the CI/PI.

Assessment and grading of any upper respiratory tract (URT) (nasal discharge, otitis, pharyngitis, sinus tenderness) and lower respiratory tract (LRT) symptoms (abnormal breath sounds externally [e.g. stridor] and on chest auscultation [wheezing or rhonchi, crepitations] will be performed. Physician-reported assessments of viral challenge related illness will be graded in accordance with their intensity and documented in the source data.

Following viral challenge, URT and LRT symptoms (as described above) will be expected and presumed to represent virus infection consequent to viral challenge and will not be additionally captured as AEs unless they meet the definition of an AE, and are deemed to be clinically significant (in the opinion of the Investigator) to be classed as AEs.

Following viral challenge, all unexpected (in the opinion of the Investigator) symptom-directed physical examination findings will be captured as AEs, along with all other occurrences that meet the criteria for an AE.

Vital Signs

During vital signs assessments, participants will be rested in a quiet setting without distractions (e.g., television, mobile phones, computers).

Vital signs assessments will be recorded as follows:

- Heart rate (HR) will be recorded in beats per minute.
- Respiratory rate (RR): respirations will be counted and recorded as breaths per minute.

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- BP: systolic BP and diastolic BP will be measured in millimetres of mercury (mmHg); measurements will be made supine. Where possible, the same arm will be used for all measurements.
- Peripheral oxygen saturation (SpO2%) will be assessed using pulse oximetry.

In the event of a participant having an unexpected abnormal or out of normal range result, the assessment may be repeated after at least 2 minutes to exclude a technical fault and confirm the original reading. The assessment may then be repeated at the PI's discretion and in accordance with local NHS SOPs.

Study specific normal ranges are provided in Appendix 3. If a result is out of the normal range and meets the criteria for an AE, the severity of the AE will be guided by the Division of Microbiology and Infectious Diseases (DMID) Adult Toxicity Table November 2007.

Deterioration in a vital sign (compared to baseline) should only be reported as an AE if the deterioration fulfils the criteria for an AE. If deterioration in a vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated vital sign will be considered as additional information.

Temperature

The study specific normal range for body temperature is detailed in Appendix 3. Temperature may be measured at tympanic, oral or axillary sites. The severity of out of normal range values will be assigned using the DMID toxicity scale as a guide. Temperature may be more frequently monitored in quarantine if deemed necessary by the Investigator.

Following viral challenge, pyrexia will be expected and presumed to represent virus infection consequent to viral challenge and will not be additionally captured as an AE unless it meets the definition of an AE and is deemed to be clinically significant (in the opinion of the Investigator) to be classed as an AE.

Following viral challenge all unexpected (in the opinion of the Investigator) pyrexia will be captured as an AE, along with all other occurrences that meet the criteria for an AE. Febrile illness (FI) is defined as any occurrence of temperature ≥ 37.8 ºC.

8.2 RADIOLOGY

Chest X-rays will be carried out in the Hammersmith, St Mary's, Chelsea and Westminster or Churchill Hospitals radiology department, according to local NHS SOPs. A chest X-ray will be performed at the screening visit to exclude clinically significant pulmonary abnormalities. Where possible, this should be acquired with the patient erect, taking a full inspiration and using a Posterior-Anterior (PA) projection. Where an admitted volunteer was unable to be inoculated in a quarantine and asked to return for a subsequent quarantine, a repeat X-ray may not be required for subsequent admissions, unless requested by the CI/PI.

8.3 ELECTROCARDIOGRAM

Twelve-lead ECGs will be obtained to evaluate the electrical activity of the heart, in accordance with local NHS SOPs. ECGs will be read on site by an appropriately qualified Investigator.

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Wherever possible the same Investigator will review subsequent ECGs from the same participant for the assessment of any change from baseline.

Any changes from baseline during the study will be assessed for their clinical significance. Clinically significant changes will be reported as AEs. The PI or delegate will assess non-clinically significant changes to determine whether they should be recorded. Study specific normal ranges are provided in Appendix 3.

8.4 LUNG FUNCTION

Spirometry

Spirometry will be performed at screening, and at some timepoints during quarantine. It is optional at the investigator's discretion on other days during quarantine and at the follow up visits. Height at screening will be used as the baseline measurement for all spirometry assessments.

Predicted values will be calculated according to the formula of the European Coal and Steel Community (ECCS). Also incorporated are the recommendations of the British Thoracic Society and the Association of Respiratory Technicians and Physiologists.

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 participant should breathe in fully. A good tight seal by the lips round the mouthpiece is essential. The participant 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

At the screening visit, during quarantine, and at other visits as described in the SoA, measurements of FEV1 and FVC will be made as outlined above.

Spirometry may be repeated at any time in the event of respiratory signs or symptoms (repeated coughing, bradypnoea, tachypnoea, rales and rhonchi) or respiratory difficulties.

8.5 URINE TESTS

Urinalysis

Clinical urine safety analysis will be undertaken using commercially available urine test strips that provide an instant result and will be documented in the source data. Urinalysis will be performed to evaluate the parameters described in Appendix 1.

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If the dipstick yields abnormal results, a urine sample may be sent for microscopy, culture and sensitivity (MCS), at the Investigator's discretion. MCS will include but is not limited to RBC, WBC, epithelial cells, crystals, casts, and bacteria. Urine safety analysis values will be evaluated by the Investigator for clinical relevance. Those deemed to be clinically significant will be reported as AEs.

Urine Drugs of Abuse and Nicotine Test

Urinalysis will be performed for drugs of abuse and cotinine using commercially available kits that provide an instant result, which will be documented in the source data. Drugs of abuse screen will include (but is not limited to) amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines.

Pregnancy Test

Female participants of child-bearing potential are to have a urine pregnancy test at screening and at quarantine prior to inoculation (Day 0) and a serum pregnancy test on either day -2/-1. Participants will only be enrolled if the pregnancy tests are negative. Note: Pregnancy test must be performed even if the participant is menstruating at the time of the study visit.

8.6 PARTICIPANT SYMPTOM DIARY CARD – CLINICAL SCORES

Participants will report and assess the severity of any challenge virus-related signs and symptoms three times/day during quarantine, at a similar time each day. (using the Symptom Diary Card.
The following symptoms in the symptom questionnaire will be graded on a scale of 0-3

- grade 0: No symptoms;
- grade 1: Just noticeable;
- grade 2: Clearly bothersome from time to time but does not interfere with me doing my normal daily activities;
- grade 3: Quite bothersome most or all of the time, and it stops me participating in activities

Runny Nose	Cough	Chilliness/Feverishness
Stuffy Nose	Chest tightness	Dizziness
Sneezing	Shortness of Breath	Rashes
Sore Throat	Wheeze	Blisters
Hoarse voice	Malaise/Tiredness	Diarrhoea
Eye Soreness	Headache	
Earache	Muscle and/or Joint ache	

Table 7. List of symptoms recorded by participants

Participant cold perception questions

Two additional cold-related questions will also be answered by the participant each morning. The first question asks whether the participant's perception of whether they have a cold or not, the second asks the participant's perception of improvement/worsening of the cold.

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i. **Do you have a cold:** Yes/No

If the participant selects Yes to having a cold, then the second 7-point Likert scale "global change since yesterday" question is completed by the participant, as below.

ii. **Compared to yesterday**, I feel that my cold is:

- Very much better
- Somewhat better
- A little better
- The same
- A little worse
- Somewhat worse
- Very much worse

8.7 University of Pennsylvania Smell Identification Test (UPSIT)

UPSIT is a well-validated and reliable test (test-retest r = 0.94) that employs microencapsulated "scratch and sniff" odorants. It is provided as booklets containing a series of cards that the participants scratch and smell then asked to identify the odours, which have been previously validated in an English population. The test provides an index of absolute dysfunction (ie, anosmia, severe microsmia, moderate microsmia, mild microsmia, normosmia, factitious), as well as relative dysfunction based upon age and gender-adjusted normative percentile ranks. The total number of odorant stimuli out of 40 that is correctly identified serves as the test measure. Scores on this test correlate well with other types of olfactory tests, including threshold tests. The UPSIT is designed to be self-administered after explanation of the test by study staff and will be performed once before virus inoculation and then at least every third day starting from Day 1, though the test can be conducted more frequently at the discretion of the PI/study physician.

In addition, the participant will be asked each morning whether they have any smell disturbance:

i. **Do you have an altered sense of smell or taste:** Yes/No

If the participant selects Yes to have smell or taste disturbance, then they will be asked to specify:

ii. Do you have

a. Partly reduced sense of smell
b. Complete absence of sense of smell
c. Unusual or abnormal smells
d. Partly reduced sense of taste
e. Complete absence of sense of taste
f. Unusual or abnormal tastes

8.8 Cognitive Testing

To assess changes in cognition associated with infection, a computerised system for repeated assessment of cognitive function will be used. This has been developed by Dr Adam Hampshire

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(Imperial College London) and has been successfully used in healthy individuals and clinical populations inclusive of traumatic brain injury. Participants will be provided with a study tablet computer assigned to them for from the time of their admission to the quarantine unit until discharge that will contain a pre-loaded app (CogAsses) and factory-provided software only. This app will provide a brief battery of computerised tasks and questionnaires to track speed of information processing, memory, attention, executive function and sleep. The battery of tests will last for ~20-30 minutes. Participants will be asked to complete the cognitive assessment every day around the same time of day during their admission within the quarantine unit. In addition, a standardised questionnaire about the quality of sleep from the preceding night will be included in the app.

The cognitive tasks included within this battery of tests are:

1. Motor Control
2. Object memory-Immediate recall
3. Simple Reaction Time
4. Choice reaction time
5. 2D Manipulations
6. Allocentric towers
7. Spatial span
8. Target detection
9. Tower of London
10. Verbal analogies
11. Object memory-Delayed recall

Participants will also be invited to complete this assessment battery at each follow-up visit. All data from the cognitive assessments will be collected on a remote web server. The servers are behind a firewall in a secure cloud computing facility. These measures represent a high-level of data security that are the standard for any website. Remote storage of performance data will be in a pseudo-anonymised format and will be linked across tasks using anonymised user identifier codes. Identifiable information will be stored in an encrypted database 'key' that is separate from the test scores.

8.9 BLOOD SAMPLES

A maximum volume of 550 mL of blood may be taken from each participant in any 8-week period. If additional samples are required in excess of this amount, e.g., to monitor abnormalities, these will be collected at the discretion of the Investigator.

Blood will be taken for multiple purposes including:

- Eligibility, safety and pathogenicity assessments (haematology, biochemistry, and other eligibility safety monitoring tests, see Safety blood samples and Appendix 1.
- Immunology assays
- Exploratory research

Further instructions for the collection, handling, storage, shipment and analysis of samples are provided in the relevant SOPs.

Safety blood samples

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Appendix 1 describes the safety blood tests that will be performed including, but not limited to, haematology, biochemistry and cardiac enzymes. Additional safety assessments (e.g. coagulation) will be conducted at the discretion of the PI or delegated clinician as required.

Immunology and sero-suitability

Participants must be sero-suitable for inclusion in the study as defined by anti-S and anti-N antibody detection (positive anti-S and negative anti-N OR positive anti-S and positive anti-N). Whilst we aim to recruit vaccinated participants with no previous SARS-CoV-2 infection, if very low numbers are found (i.e. anti-S positive, anti-N negative), enrolment of those with both anti-S and anti-N antibodies will occur. Those with an indeterminate anti-N antibody result and no history of laboratory-confirmed SARS-CoV-2 infection may be included or excluded at the PI's discretion on a case-by-case basis. Serum levels of pre-existing SARS-CoV-2 specific antibodies to the Challenge Virus will be determined using the Roche quantitative anti-S and anti-N antibody test.

Additional exploratory immunological assays will be performed, as described in Exploratory Research**.**

Capillary blood sampling (fingerprick blood sample)

Two to four spots of blood will be collected onto an Ahlstrom card (or an alternative depending on availability) for serological analysis for Exploratory purposes.

Procedure:

- Lie the card on a flat surface and label with participant's details
- Using a lancet, prick the end of one finger
- Squeeze the finger from the base towards the fingertip
- Allow a large drop of blood to fall into each circle on the card. The blood should fill at least three quarters of the circle. The sample should soak through the filter paper and be visible on the back of the card.
- If the blood is not dripping freely, the card can be held to touch any blood droplets forming on the fingertip.
- Use gauze to clean the finger
- Allow card to dry for 3 hours untouched then place into sample sleeve

8.10 Respiratory and Oral Samples

The following exploratory respiratory sampling procedures will be performed during the study:

- Mid turbinate FLOQ swab
- Throat FLOQ swab
- Respiratory pathogen screen
- Participant performed swab for lateral flow rapid antigen test
- Saliva
- Nasosorption
- Nasal curettage (Rhinopro) for cells for RNA
- Nasopharyngeal swab for cells for RNA

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Where any nasal sampling time points occur together, the order of sampling will typically be (1) nasosorptions followed by (2) mid turbinate swab, (3) lateral flow test (4) nasopharyngeal swab and (5) nasal curettage.

Sample collection, handling, storage, shipment and analysis of samples from the respiratory tract will be performed in accordance with the relevant SOPs, or as detailed in the Analytical Plan, as appropriate. These samples will be collected for:

- Viral loads
- Respiratory Pathogen Screen
- Exploratory purposes e.g. immunology

FLOQ swabs for SARS-CoV-2 confirmation of infection endpoint analysis and viral loads

Infection will be determined by quantitative RT-PCR, quantitative viral culture and/or culture of mid-turbinate and throat swab samples taken daily. This is detailed in the objectives and endpoints, Section 7.1. Flocked swabs collected into viral transport medium (that allows for viable SARS-CoV-2 culture) will be used.

Mid turbinate FLOQ swab

Procedure:
Tilt patient's head back 70 degrees. Remove the swab from the container carefully to ensure the tip is not contaminated. While gently rotating the swab, insert it less than one inch (about 2 cm) into nostril parallel to the palate until resistance is met at turbinates. Rotate the swab 10 times against nasal wall. . Place swab, tip first, into the transport tube provided. Once the tip is near the bottom, break the swab handle at the swab breakpoint by bending back and forth or cut it off with sterile scissors. The swab should fit in the tube comfortably so that the cap can be screwed on tightly to prevent leakage and contamination.

Throat FLOQ swab

Procedure:
A tongue depressor can be used 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 participants may experience a strong gag reflex. Rotate the swab ten times along the desired area then remove, avoiding contact with the teeth. Place swab, tip first, into the transport tube provided. Once the tip is near the bottom, break the swab handle at the swab breakpoint by bending back and forth or cut it off with sterile scissors. The swab should fit in the tube comfortably so that the cap can be screwed on tightly to prevent leakage and contamination.

Respiratory pathogen screen

On entry to quarantine, an upper respiratory tract sample (e.g. nasopharyngeal swab or nasal wash, per local SOP) will be collected and tested to detect the presence of a set of respiratory pathogens, including Covid-19, that could potentially contraindicate a participant's

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participation in the study. The methodology to be used to conduct the respiratory virus screen will be documented in the Analytical Plan (AP). Additional test may be conducted if the results from the first test were invalid to support study eligibility prior to virus inoculation, or if a community acquired infection is suspected during quarantine. Any additional screening tests will be conducted at the discretion of the CI/PI.

Participant performed swab for lateral flow antigen tests

The participant will perform daily lateral flow antigen tests on themselves, according to manufacturer's instructions. The aim is to assess the performance of self-performed lateral flow tests with the same test performed on viral transport media by a trained laboratory staff member.

Procedure: The volunteer will be provided with a lateral flow test and asked to read the instructions and perform the test themselves (without training).

A typical lateral flow test involves the following steps:

- Open the kit and remove components
- Perform an anterior nares or throat-and-nose swab as indicated on packaging
- Insert swab into buffer solution and rotate to mix, as per instructions
- Place 2-4 drops of buffer solution on the lateral flow cartridge

The lateral flow test will be taken from the participant before the result appears. A study team member will read and document the result, if the result is unclear, the study team member will request verification from a second team member.

Saliva collection

Saliva will be collected in a universal container. The participant will be asked to produce saliva directly into the container, aiming to produce 1-2mL in total.

Nasosorption

Up to two strips of SAM will be used and will always be the first nasal sample collected in that sampling session. . The participant is asked to not blow or clean their nose for 30 minutes prior to sample collection.
The SAM is placed into the nose for 2 minutes to obtain repeated samples of neat nasal ELF. The participant is required to use a finger to close the nostril and keep the SAM in place, alternatively a nose clip will be provided.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, immediately placed on wet ice, then onto dry ice for cross site transport (as required) and frozen at -80°C. Further details are given in the Clinical Sample Collection SOP and Sample Management Plan,

Nasal curettage using Rhinopro

Rhinopro® curettes will be used to obtain a sample of nasal epithelial cells from each nostril, one side at each time point, alternating nostril at each successive time point. This is a painless procedure and will not require local anaesthetic. The following technique is used:

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Procedure:

- The participant 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 2 times.
- Note that this area has limited sensitivity and the participant 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 90% heat inactivated fetal bovine serum and 10% of dimethyl sulfoxide (DMSO) or RNA Cell Protect (Qiagen) or Trizol. The tube will be placed on dry ice for cross site transport (as required) as soon as reasonably possible (FBS/DMSO only) and transported to be frozen at -80°C for storage prior to analysis. Further details are given in the SOP Sample Management Plan.

Nasopharyngeal swab for cells for RNA

A nasopharyngeal (NP) FLOQ (flocked) swab will be used. The patient is asked to clear any mucus from their nasal passages and close their eyes to minimise discomfort. The patient's head is tilted back slightly, and the swab inserted along the nasal septum, above the floor of the nasal passage to the nasopharynx until slight resistance is felt. The swab is then rotated in this position in both directions, a total of 6 rotations and slowly removed whilst rotating.

The swab is cut, using sterile scissors, ~2cm up from the swab tip and placed into a pre-cooled sterile cryogenic vial containing 90% heat inactivated fetal bovine serum and 10% of dimethyl sulfoxide (DMSO) and placed on ice. The cryovial will be moved onto dry ice for cross site transport (as required) then to -80C storage as soon as reasonably possible.

Where a nasopharyngeal swab for cells for RNA is taken at the same time as curettage, this will be from the opposite nostril.

8.11 EXHALED BREATH SAMPLING

Facemask Sampling

Volunteers will be asked to wear one or two single use face masks fitted with a polyvinyl alcohol (PVA, Orbi-Tech, Leichlingen, Germany) sampling matrix insert, capable of capturing virus. They will do this on study Day -1 and then daily during quarantine from Study Day 1 plus, at the discretion of the PI/CI, on day 28, for up to 60 minutes per mask. A second mask sample may be taken during which volunteers may be asked to sing or speak a defined phrase

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at certain time points during sample collection, and they may be asked to audio record this on the study tablet device that will be provided to them.

No hazards have been identified by the manufacturer of the PVA material and these face masks have been validated for the detection of M. tuberculosis in infected participants[70]. They have been successfully demonstrated to detect SARS-CoV-2 in exhaled breath[71] and were used successfully to collect virus in the COVHIC001 study. Up to five PVA strips will be harvested from each exposed mask and analysed for virus and virus-related signals.

Breath aerosol sampling

Volunteers will be asked to breathe into a mouthpiece or via a facemask, attached via tubing to an optical particle sizer (TSI OPS 3330), which enables quantification and sizing of exhaled aerosols. They will do this on study Day -1 and then as detailed in the SoA, up to 15 minutes on each occasion. At certain time points volunteers may be asked to vocalise, cough or perform a forced exhale.

If equipment is unable to be supplied to the Oxford site, only participants enrolled at the London site will undergo this sampling. If equipment allows, this will be performed on participants at both sites.

8.12 Swabs for Microbial Analysis

Throat swab

A sterile dry cotton-headed swab is used to obtain daily samples from the pharynx for bacterial 16S gene analysis. This is performed with the participant sitting and with adequate lighting to view the sample site, a tongue depressor will be used 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. Rotate the swab ten times along the desired area then remove, avoiding contact with the teeth. Some participants may experience a strong gag reflex. Place swab imeediately into a dry container ont wet ice and then later freeze at -80°C.

Stool swab

Sterile dry cotton-headed swabs will be used to obtain stool samples for analysis of bacteria and viruses. These will be collected by the participant from the toilet paper at any time after opening their bowels, up to once a day during the quarantine period.

Procedure:

- Remove the swab from the collection tube by holding it firmly by the cap. Do not touch the cotton part with your bare hands.
- Collect a small amount of faecal 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 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.

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If a participant does not open their bowels on a given sampling day, the stool swab will be missed but not counted as a protocol deviation.

8.13 NON-INVASIVE VASCULAR MEASUREMENTS USING ENDOPAT™

Clinical data clearly identifies a significant impact of COVID-19 on the vasculature. However, the mechanisms that lead to endothelial dysfunction and thrombosis, and how they might cause the cardiovascular pathology seen in COVID-19, are incompletely understood. To assess endothelial function during SARS-CoV-2 infection, peripheral arterial tonometry (PAT) is measured using an EndoPAT™ device (Itamar Medical), which quantifies flow mediated dilatation non-invasively. PAT allows beat-to-beat recording of the finger arterial pulse amplitude with pneumatic probes[72]. These clinical measurements will be combined with measurement of vasoactive mediators on plasma samples to assess endothelial function.

A trained researcher will perform clinical measurements using the non-invasive EndoPAT™ 2000 device. The measurements take around 20 minutes and are performed as detailed in the SoA. The right arm will be the active arm and left arm control in all participants at all study visits.

Procedure:
1) Place the apparatus on a stable platform together with the computer in close proximity to the volunteer's chair. Any restrictive clothing that could interfere with blood flow to the arms should be removed.
2) Ask volunteer to lie down on their back on the bed and to close their eyes and remain silent, avoiding movement during the procedure.
3) Place blood pressure cuff on the lower arm of the designated test arm and place volunteer's fingers into the PAT™ probes.
4) Once the volunteer is comfortable perform a pre-recording signal quality reading for 1-2 minutes
5) If there are no issues with signal quality or probe leaks the study will begin. There will be a 5-minute period of baseline measurement.
6) Inflate cuff to at least 60mmHg above the systolic blood pressure (minimum 200, max 300mmHg) for 5 minutes.
7) Rapidly deflate cuff and continue a further 5 minutes of recording.
8) At the end of the procedure the probes will be removed. Data is automatically stored on the EndoPAT™ software and formal analysis to generate EndoPAT™ score will be undertaken away from the patient.

The procedure is non-invasive though it is acknowledged there could be some level of discomfort from inflating the pressure cuff. This will be explained to the volunteer and is minimised by the participant lying down with their arms resting comfortably on purpose made arm rests.

If equipment is unable to be supplied to the Oxford site, only participants enrolled at the London site will undergo these measurements. If equipment allows, this will be performed on participants at both sites.

8.14 ENVIRONMENTAL SAMPLING

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Exploratory environmental sampling may be conducted one day before (Day -1) and daily during quarantine following intranasal inoculation of volunteers with SARS-CoV-2 (from Day 1).

Daily sampling will entail:

- Swabs collected from environmental surfaces such as bedside furniture, bathroom surfaces, door handles, computer keyboards, mobile phone and ventilation outlets in the volunteer quarters. Details will be provided in the Sampling SOP. Each day volunteer rooms will undergo a surface clean to avoid contamination from the previous day's virus deposition.
- Swabs collected from volunteers' hands.
- Air sampling to detect and quantify airborne virus will be performed using a variety of devices. At certain time points volunteers may be asked to sing or speak loudly and collect samples before and after. Other than generating some noise, the samplers do not have any other impact on study participants.

Following collection, samples will be kept cold and returned to the laboratory for downstream processing. This may involve different viral assays such as:

- Quantitation of extracted viral RNA using RT-PCRs
- Quantitation of infectious material by immunofluorescence or viral culture

Room temperature, humidity and carbon dioxide levels will be recorded. Baseline room ventilation rates (air changes per hour) will be measured.

If equipment and staffing cannot to be supplied to the Oxford site, only participants enrolled at the London site will undergo this sampling. If equipment and staffing allow, this will be performed on participants at both sites.

8.15 EXPLORATORY RESEARCH

The primary, secondary, and tertiary endpoints may be explored in relation to immunological levels at baseline and after SARS-CoV-2 challenge. Assays performed on blood, stool and respiratory samples may include, but are not limited to:

- Humoral immunity / systems serology SARS-CoV-2 (for example: SARS-CoV-2 neutralizing titres, ELISAs to IgG, IgM, IgA, sIgA, ADCC)
- Proteomic levels and changes (for example, cytokine and chemokines)
- Cellular cell quantification and quality of immunity (for example T and B cell frequencies, phenotypes and functionality assays, ELISPOTs, ICS, cytokine/chemokine responses)
- Transcriptome levels and changes (for example, RNAseq, single cell RNAseq, microarray, PCR)
- Human genomics in relation to SARS-CoV-2 susceptibility, infection (e.g. HLA typing, SNPs, GWAS)
- Viral genomics in relation to SARS-CoV-2 population changes through infection

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- Microbiome analysis in relation to viral infection, disease and susceptibility (e.g. PCR, NGS, 16s rRNA)
- Measurement of plasma mediators to investigate endothelial dysfunction (e.g. prostacyclin, endothelin-1, nitric oxide)

Results of these analyses may be reported separately to the final study report.

9 Study Intervention(s)

Study interventions administered to participants are described in Table 9.

9.1 Summary of Study Intervention(s) Potentially Administered

Intervention Name	SARS-CoV-2 Delta variant	Paxlovid (nirmatrelvir with ritonavir)	Legevrio (Molnupiravir)	Veklury® (Remdesivir)
Type	Virus	Drug (Rescue treatment)	Drug (Rescue treatment)	Drug (Rescue treatment)
Dose Formulation	Ampoule, Liquid	PF-07321332 – film-coated tablet. Pink, oval, with a dimension of approximately 17.6mm in length and 8.6mm in width debossed with 'PFE' on one side and '3CL' on the other side Ritonavir – film-coated tablet. White to off white, capsule shaped tablets with a dimension of approximatel 17.1mm in length and 9.1mm in width, debossed with H on one side and R9 on other side	Lagevrio (molnupiravir) is a Swedish Orange size 0 dry filled capsule with the corporate logo printed in white ink on one half and "82" pprinted in white ink on the other half. Each capsule has an overall close length of approximately 21.7mm and maximum external diameter of approximately 7.64mm.	VEKLURY™ (Remdesivir) for injection, 100 mg, available as a sterile, preservative-free, white to off-white to yellow lyophilized powder

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Unit Dose Strength(s)	The titre of the undiluted Challenge Virus stock (Master Virus Bank) is determined in an infectivity assay and is reported in Tissue Culture Infective Dose units per mL (TCID50/mL). The inoculum virus vials are produced by dilution of the master virus bank to achieve targeted challenge doses, as follows: • Dose level 1 ~ 10^2 $TCID_{50}$ (~100 $TCID_{50}$ dose) • Dose level 2 ~ 1,000 $TCID_{50}$ (~10^3 $TCID_{50}$ dose) • Optional additional Dose levels (e.g. ~10 $TCID_{50}$, ~10,000 $TCID_{50,}$ ~ 100,000 $TCID_{50,}$	Each pink film-coated tablet contains 150mg of PR-07321332 Each white film-coated tablet contains 100mg of ritonavir	Each capsule contains 200mg of molnupiravir active substance.	Each 100 mg single-dose vial contains a sterile, preservative-free lyophilized powder that is to be reconstituted with 19 mL of Sterile Water for Injection and diluted into 0.9% sodium chloride prior to administration by intravenous infusion. Following reconstitution, each vial contains 100 mg/20 mL (5 mg/mL) of VEKLURY (Remdesivir) concentrated solution.
Dosage Level(s)	Different doses of virus will be evaluated. Dose volume delivery method is provided in the Section 9.2.4.	PF-07321332 must be co-administered with ritonavir. The dose is 300mg (two 150mg tablets of PF-0321332) and 100mg (one tablet) of ritonavir taken orally every 12 hours for 5 days.	The dosage is 800mg (administered as four 200mg capsules) taken orally every 12 hours for 5 days.	The recommended dosage for adults weighing at least 40 kg is a single loading dose of VEKLURY (Remdesivir) 200 mg on Day 1 followed by once-daily maintenance doses of VEKLURY (Remdesivir) 100 mg from Day 2. The treatment duration is 3 days.
Route of Administration	Intranasal	Oral	Oral	IV infusion
Use	Infectious challenge agent	Rescue treatment	Rescue treatment	Rescue treatment
IMP and NIMP	N/A	NIMP	NIMP	NIMP
Sourcing	Provided by hVIVO	Provided centrally by the Sponsor.	NHS hospital pharmacy stock	NHS hospital pharmacy stock

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Packaging and Labelling	Challenge Inoculum will be provided in single-dose vials. The details of the virus challenge agent will be provided in the Analytical Plan	Packaging and Labelling of the study Intervention is described in the Summary of Product Characteristics..	Packaging and Labelling of the study Intervention is described in the Summary of Product Characteristics.	Packaging and Labelling of the study Intervention is described in the Summary of Product Characteristics.
Current/ Former Name(s) or Alias(es)	N/A	PAXLOVID (PF-07321332 (nirmatrelvir) with ritonavir)	Lagevrio (molnupiravir)	Remdesivir ™, Veklury

Table 8. Study Interventions.

Refer to the Summary of Product Characteristics (SmPC) for details regarding administration of rescue treatments. All rescue treatments are UK licensed products used within indication. Refer to Virus Administration SOP for further details of the SARS-CoV-2 Challenge Virus.

Where necessary, participants being given a rescue treatment will also be provided further information about the rescue treatment, including any known side effects, contra-indicated medications and effects on contraceptives.

9.2 PROVENANCE OF THE SARS-COV-2 CHALLENGE VIRUSES

The SARS-CoV-2 Delta variant challenge virus strain was originally obtained in mid-2021 from a nose-throat swab from an otherwise healthy young adult with mild COVID-19 in the community. A SARS-CoV-2 wild-type challenge virus strain was originally obtained in mid-2020 from a nose/throat swab taken from a patient in the UK who developed respiratory symptoms consistent with COVID-19. The wild-type challenge virus was used in a previous challenge study, as described in Killingley et al[73]. The Delta challenge virus has not been used in previous human virus challenge studies.

The virus was isolated by inoculation with the clinical sample of a qualified cGMP Vero Cell line. Seed Virus Stocks for each virus were then generated by a further passage on the same cGMP Vero Cell line. The Zayed Centre for Research (ZCR) GMP manufacturing facility of Great Ormond Street Hospital (GOSH) subsequently used the Seed Virus Stocks to manufacture the Delta variant Challenge Virus in accordance with cGMP and produce a Challenge Virus Master Virus Bank. The ZCR GMP manufacturing facility of GOSH was also used to generate the challenge agent in the first SARS-CoV-2 human challenge study (COVHIC001).

Individual person inoculum vials were then produced in accordance with cGMP by GOSH by dilution of the cGMP MVB with cGMP sucrose diluent. Inoculum vials have been produced at a range of viral concentrations for each virus. The challenge viruses have undergone extensive quality testing performed as part of the GMP manufacturing release processes according to pre-determined specifications (including identity, infectivity and contaminant / adventitious agent tests). The challenge viruses will be stored in a secure –80°C freezer (normal temperature range –60°C to –90°C).

The procedure for isolation, storage, preparation and administration of Challenge Virus in this study (COVHIC002) is the same as used in the first SARS-CoV-2 human challenge study

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(COVHIC001) at the Imperial site and the same as used in the COV-CHIM01 (NCT04864548) SARS-CoV-2 human challenge study at the Oxford site.

Supply and accountability of challenge virus

The challenge virus will be transferred under appropriate containment to the study site on Day 0 for each quarantine cohort. The study site will establish a system for control of challenge virus in accordance with site SOPs and as detailed in the Analytical Plan. The PI or appropriate PI delegated research staff will maintain accurate records of receipt and condition of all challenge virus inoculum stock used for challenge in accordance with the site SOPs, including details and dates of the batch and vial numbers, quantities dispensed and used in the study. Any departures from the protocol-dispensing regimen will be fully documented. Accountability records will be maintained as per the relevant SOPs.

Storage of challenge virus

Stocks of challenge virus for use at the London site will be maintained at Cryostore London and delivered to the quarantine site before quarantine cohorts.
Stocks of the challenge virus for use at the Oxford site will be maintained on-site at the Centre for Clincial Vaccinology and Tropical Medicine and transferred to the EMCRF on D0 of each quarantine cohort.
Vials will be thawed in the quarantine unit just before inoculum preparation. Once thawed, vials of stock virus will not to be re-frozen for later use in human challenge studies. The residual of the inoculum in the vials used for challenging the participants with the challenge virus will be frozen at the study site (on dry ice or into a -80C freezer) , and delivered to the Section of Virology, Department of Infectious Disease, Imperial College London for viral titration or disposed of in accordance with SOPs.. All storage records will be maintained.

Preparation and administration of challenge virus

All participants will be administered GMP-compliant SARS-CoV-2 at one of dose levels by intranasal drops on Day 0, starting at 100 $TCID_{50}$ dose. Higher doses of Delta variant SARS-CoV-2 may be given via nasal administration to healthy volunteers, if the targeted attack rate is not achieved (see Section 4.6.1). Up to three viral dose levels are anticipated to be needed, however higher doses may be required, as appropriate:

- Dose level 1 ~ 100 $TCID_{50}$ (~10^2 $TCID_{50}$ dose)
- Dose level 2 ~ 1000 $TCID_{50}$ (~10^3 $TCID_{50}$ dose)
- Optional additional Dose levels that may be evaluated
    - > 1000 $TCID_{50}$ (i.e. ~10,000 $TCID_{50}$, ~ 100,000 $TCID_{50}$), 10 $TCID_{50}$

On the day of inoculation (Day 0), aliquots of challenge virus will be removed from storage in the -80 °C freezer and transferred from Cryostore London/ CCVTM (dependent on site) to the quarantine unit on dry ice in accordance with the SOPs.

A clinically trained staff member will inoculate each volunteer in the high containment room in which the volunteer will subsequently remain quarantined, with another staff member acting as scribe-/timekeeper/assistant. A sign will be placed on the door of the room to indicate that inoculation is taking place and to prevent accidental entry of others. Staff will wear full PPE (FFP3 mask or respirator, long sleeved fluid resistant gown, eye protection, single use plastic

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gloves; and shoe covers) when in the participant's room during and at all times after inoculation. For the inoculation, the participant will wear an apron and eye protection.

The inoculum dose will be rapidly defrosted by warming in the hand and then placed on ice (if necessary). Participants will then be inoculated with intra-nasal drops (two 50 µl drops per naris) with inoculum at a given dose divided equally between the two nostrils (if higher doses of SARS-CoV-2 are used the volume of each drop may be under or over 50µL and additional drops may be required). Inoculations using intranasal drops will be done using a pipette with participants' supine (face and torso facing up and remaining so for at least 10 minutes post inoculation). This will be done slowly with at least 30s intervals between each nostril (inoculation) to ensure maximum contact time between with the nasal and pharyngeal mucosa. Participants will be asked not to swallow during the procedure to ensure maximal pharyngeal contact and not to shower or blow their nose for at least two hours. Following inoculation, advice regarding infection control, emergency call and hand hygiene will be given. Further details are provided in the Virus Inoculation SOP.

To quantify the amount of SARS-CoV-2 inoculated into each participant within a cohort, a quantitative culture (infectivity assay) may be performed on residual challenge inoculum after participant administration, which will be stored on ice and transported to the virology laboratory. Volunteer inoculations may also be back titrated using qRT-PCR. Where doses given that may not be quantifiable by cell culture given (e.g. 10^1 $TCID_{50}$) due to the limitations of the assay sensitivity, the genome copies may be used to confirm that the intended dose was given.

9.3 EARLY "RESCUE" THERAPY

Participants administered SARS-CoV-2 may receive a "rescue" therapy to limit the risk of progression to more severe COVID-19. Early "rescue" therapy, when backed by compelling evidence of efficacy and when available, is indicated for challenge model infections that demonstrate warning features beyond mild signs and symptoms that are confined to the upper respiratory tract. The oral antiviral agent Paxlovid will be preferentially used and a dedicated stock has been supplied by the manufacturer (Pfizer) for the purpose of the study. In the event that Paxlovid is not available or not appropriate, molnupiravir or remdesivir may also be used. Indications to consider rescue therapy in those participants confirmed to be infected include:

- Persistent tachypnoea – Respiratory Rate ≥ 21 for ≥ 8 hours
- Persistent Fever – Fever (≥ 37.9) from a time point 5 days post symptom onset and present for at least once each day for ≥ 72 hours
- Severe and persistent cough – Grade 3 (defined as "quite bothersome most or all of the time, and it stops me from participating in activities") reports of coughing via symptom diary cards that is largely persistent over 48 hours
- Any event of confirmed hypoxia (≤ 94%, usually confirmed over a 1-hour period) should this occur without the above 'warnings'

Importantly CI/PI discretion will be used at all times in the decision to start Rescue Therapy. Biochemical markers (e.g. elevated CRP/D-dimer) will not be used in isolation, though a combination of factors outside of the triggers above could still lead to a decision to start therapy.

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Once a decision to commence rescue therapy has been made by the CI/PI, the NHS acute medicine team at the Chelsea and Westminster Hospital / OUHFT will be made aware. The participant will remain within the quarantine unit to receive rescue therapy unless protocol stated criteria for transfer to NHS care are met.

6. Dose Preparation

Rescue therapy doses for each individual participant will be prepared by a pharmacist or designee (e.g. nurse) in the quarantine unit at the Chelsea and Westminster Hospital or the Oxford EMCRF. Dose preparation process will be described in the Summary of Product Characteristics (SmPC).

7. Handling, Storage, and Accountability

Paxlovid will be shipped to the research pharmacy at Chelsea and Westminster Hospital and to the hospital pharmacy at the Churchill Hospital. A trained and delegated pharmacist will take receipt of the shipment. They will then maintain accountability logs for the Paxlovid. At the Chelsea and Westminster hospital, some doses of Paxlovid will remain in the research pharmacy and some doses will be transferred to a specially designated, restricted access locked cupboard on the quarantine unit to be available to the study participants out of hours. At the Oxford site, all doses of Paxlovid will be transferred to the EMCRF pharmacy. Trained and delegated members of the study team will also maintain an accountability for the doses stored on the quarantine unit.

All study interventions:

1. Only participants enrolled in the study may receive study intervention and only authorised site staff may supply or administer study intervention. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the Investigator and authorised site staff.
2. The Principal Investigator, is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).
3. Disposal of used and unused Virus inoculum vials will be done in accordance with the Virus Administration SOP.

9.4 RANDOMISATION AND BLINDING

Not applicable.

9.5 STUDY INTERVENTION COMPLIANCE

Interruptions from the protocol-specified intervention plan require consultation between the investigator and the Sponsor and written documentation of the collaborative decision on participant management.
Any non-compliance or problems with the administration of the study intervention will be recorded in the participant's source notes and documented as a protocol deviation if appropriate.

9.6 CONCOMITANT AND PRIOR THERAPY

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Permitted Medication

Participants who were taking, wanted to take, or were required to take regular medication (whether prescribed or not) during their participation are excluded from this study with the exception of those therapies listed below or unless agreed with the PI. Use of all permitted therapies has to be documented and agreed at the screening visit.

The use of concomitant medications other than those listed in Table 10 is prohibited unless approved by the PI, including over-the-counter or prescribed medications. Where there is uncertainty, the PI is encouraged to discuss the use of any concomitant medications with the CI/Chief Investigator before initiating therapy.

The Investigator is to be informed as soon as possible about any medication taken by a participant from the time of screening until the completion of the follow-up visit on Day 28. Agreed concomitant medications taken during the quarantine phase will be stored, prescribed and administered in line with their label-specific requirements and full accountability will be maintained. Use of all concomitant medications will be recorded in the case report form (CRF). Concomitant medications include all prescription drugs, herbal preparations, over-the-counter medications, vitamins and minerals.

Permitted medication	**Restrictions**
Oral, injected or implanted contraceptives or hormone replacement therapies	Recommended dosing
Paracetamol	Maximum daily dose of 4 g from 7 days before Day -1 and throughout the duration of the study
Mild potency topical steroids	Recommended dosing
Over-the-counter creams and topical treatments	Recommended dosing
Prescription and non-prescription medications, including vitamins or herbal and dietary supplements, not listed in prohibited medications are subject to approval by the PI.	

Table 9. Permitted medications and restrictions.

Prohibited Medication

All medications, other than those noted above are to be stopped before the planned date of viral challenge unless in the opinion of the PI/CI, the medication will not interfere with the study procedures or compromise participant safety. Certain medications requiring a specific washout period before a participant is eligible to enter the study; details are provided in . These medications are prohibited until day 28 post-challenge.

Prohibited medication	**Washout required**
Systemic corticosteroid (oral and parenteral) therapy	6 months before the planned date of viral challenge
Systemic (oral and parenteral) antiviral drugs	6 months before the planned date of viral challenge
Vaccinations	30 days (non-live vaccine) or 60 days (live vaccine) before the planned date of viral challenge

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Short and long-acting anti-histamines	7 days before the planned date of viral challenge
Any medication or product (prescription or over-the-counter), for symptoms of nasal congestion or respiratory tract infections including nasal steroids	7 days before the planned date of viral challenge
Herbal supplements	7 days before the planned date of viral challenge
Chronically used medications, vitamins or dietary supplements, including any medication known to be an inducer or inhibitor of cytochrome P450 enzymes	21 days before the planned date of viral challenge

Table 10. Prohibited medications.

10 ADVERSE EVENTS

10.1 DEFINITIONS

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

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

- **Results in death**
- **Is life-threatening** – *refers to an event in which the participant 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, or prolongation of existing inpatients' 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 participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Risks and expected adverse events

8. 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, including fingerprick blood sample (for DBS): risks include discomfort as the needle goes through the skin and/or bruising. Infection, excess bleeding, clotting, or fainting are also possible, although unlikely.

SAM strips (a soft strip placed into the nose for two minutes), nasal curettage (scrape) and swabs (nose/throat/nasopharyngeal) may tickle, make the eyes water or be slightly

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uncomfortable. They should not be painful. The throat, nose and oral swabs are not painful. EndoPAT™ measurements, using a blood pressure cuff, may be slightly uncomfortable when the cuff is inflated, but not painful.

9. Potential adverse events related to SARS-CoV-2 infection

Participants are expected to experience typical symptoms of a common cold or flu-like illness (including, but not limited to: fever, headache, malaise, rhinorrhoea, nasal congestion, sneezing, sore throat, smell and/or taste disturbance and cough). These would not be deemed adverse events, unless in the opinion of the study doctor. However, symptoms requiring rescue therapy (see Section 7.3) or withdrawal from the study due to intolerable symptoms in more than two participants will lead to a suspension of the study. The DSMB will be convened to determine any systematic cause for unexpectedly severe symptoms.

10. Potential adverse effects of chest x-ray

At screening, participants will have a single chest X-ray to exclude those with clinically significant lung abnormalities. The estimated dose will be 0.014mSv (table 11, HPA-CRCE-012 2010 dose review) which is approximately equivalent to 2 days natural background radiation and carries risk of inducing a cancer of approximately 1:1,428,000 based on risk factors for a healthy adult. This is classified as a negligible risk level (HPA-CRCE-028).

10.2 REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. AEs will be fully recorded in the source documents as they are reported, whether spontaneously volunteered by a participant or in response to questioning about wellbeing at telephone or face-to-face study visits. Enquiries about AEs should cover the period between the previous and current visit. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non serious AEs

All such events, whether expected or not, should be recorded - it should be specified if only some non-serious AEs will be recorded, any reporting should be consistent with the purpose of the trial end points.

Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours of the Investigator becoming aware of the event. However, hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

All SAE reports should be directed to the CI within 24hours of the Investigator or any site personnel's knowledge of an SAE. An update SAE report form should be forwarded to the Sponsor within 24 hours of receipt of the new/updated information as relevant. Information relating to the participant's subsequent medical progress must be submitted to the Sponsor as

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available, until the SAE has resolved or, in the case of permanent impairment, until it stabilises, and the overall clinical outcome has been ascertained.

The Investigator will also provide additional information, including a copy of the following documents (where applicable):

- Copies of test results, as available
- Concomitant medications
- Hospital discharge summary (as soon as it is available to the PI)
- Autopsy report (as soon as it is available to the PI)

The investigator at the site is responsible for ensuring that a member of the Sponsor study team is made aware of any SAE reports that have been transmitted.

Contact details for reporting SAEs
mailto:**Please send SAE forms to: RGIT@imperial.ac.uk and the CI, c.chiu@imperial.ac.uk**

In addition, any AE resulting in permanent study discontinuation for a participant, even if not serious and regardless of expectedness or causality, must be reported by email to the CI and Sponsor within 7 calendar days of the investigator or any other site personnel's knowledge of the event.

Recording of Adverse Events and Serious Adverse Events

The PI is responsible for ensuring that all AEs, SAEs and pregnancies are identified, evaluated, recorded and reported in a timely manner as per the Sponsor's SOPs, and also for ensuring that the medical management (including follow up) of AEs, SAEs and, where appropriate, pregnancy symptoms/complications is provided by site staff.

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting AE, SAE, and other reportable safety event reports can be found in 2.

Time Period and Frequency for Collecting AE and SAE Information

All AEs/SAEs will be collected from the signing of the ICF until the last Follow-up Visit at the time points specified in the SoA (Section 3)

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the sponsor.

Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in 2.

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Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. Additional Information/clarification may be required to ensure accurate completion of safety reports. The follow up reports should include a detailed Investigator's clinical assessment. All AEs/SAEs, will be followed until resolution, stabilisation, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is provided in 2.

Regulatory reporting requirements of SAEs

Prompt notification by the Investigator (PI) to the CI and Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

All SAEs should be reported to the 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 within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

SAEs will be documented and reported in accordance with the Sponsor's SOPs:

PI will send SAE/pregnancy forms to the sponsor and CI within 24 hours of becoming aware of the event.

RGIT@imperial.ac.uk
c.chiu@imperial.ac.uk

The sponsor ensures compliance with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and Investigators. Annual safety/progress reports and final Study reports will be generated and submitted to the relevant ethics committee.

An Investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and will notify the IRB/IEC, if appropriate according to local requirements.

Further information on regulatory reporting requirements is provided in Section 12.

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Reporting of events related to rescue therapy

Any SAEs deemed to be related to a rescue therapy will be reported within 24 hours of investigator awareness following the Sponsor's safety reporting SOP.

Relatedness will be assessed by study doctors, PIs and CI, taking into consideration the nature of the SAE, the timing of onset in relation to rescue therapy commencement, similarity to reported AEs/SAEs in relation to the rescue therapy, and similarity to symptoms or AEs known to be related to COVID-19.

Contraceptive requirements with Paxlovid:

There are currently no data on the use of Paxlovid in pregnancy and so it is not recommended during pregnancy.

Participants will be counselled to use effective barrier contraception (for example a condom, female condom, diaphragm or cap) for the duration of Paxlovid treatment and until one full menstrual cycle is completed after the last dose of Paxlovid. Paxlovid can stop the combined oral contraceptive pill from working properly, so for those taking the combined oral contraceptive pill, a barrier method of contraception in addition to the oral contraceptive pill for the duration of treatment with Paxlovid and for 30 days after (or until one full menstrual cycle is completed) will be strongly recommended.

Any pregnancy while taking Paxlovid or within 30 days will be reported to the UK COVID-19 Antivirals in Pregnancy Registry in line with NHS guidelines.

Pregnancy

Details of all pregnancies in female participants and, if indicated, female partners of male participants will be collected from study specific informed consent and until the last study assessment as outlined in the SoA. If a pregnancy is reported, the Investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in 2.

Abnormal pregnancy outcomes that occur while the participant is in the study (e.g., spontaneous abortion, foetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

Disease-related events and/or disease related outcomes not qualifying as AEs or SAEs

Not applicable.

Treatment of overdose

For this study, any dose of any drug administered as part of the study greater than the dose prescribed by the protocol will be considered an overdose.

In the event of an overdose, the Investigator should:

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1. Contact the Medical Monitor (Professor Chris Chiu, Chief Investigator) immediately.
2. Closely monitor the participant for any AE/SAE and laboratory abnormalities associated with overdose and participants will be clinically followed up until the AE has resolved.
3. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

The Sponsor is responsible for notifying the REC of the potential serious breach within 7 days of becoming aware of it.

10.3 Safety Oversight Procedures

Procedures to be followed in the event of abnormal findings

Eligibility for enrolment in the trial in terms of laboratory findings will be assessed by clinically qualified staff. Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. Laboratory AEs will be assessed using specific toxicity grading scales adapted from the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. For the Imperial and Chelsea study sites, the Toxicity Grading Scales can be found in the "Toxicity Grading for Lab AEs SOP" and for the Oxford study site, these will be found in their "COVHIC002 CSP". If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate and with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

Ongoing and interim safety reviews

The safety profile will be assessed on an on-going basis by the study Investigators. The CI/PI and relevant Investigators (as per the trial delegation log) will be reviewing safety issues and SAEs as they arise. The Chief Investigator will be informed of any safety concerns.
Safety reviews are planned as follows:

- After the first 6-10 volunteers at each dose level (Sentinels), available safety data will be reviewed by the PI, CI and the chair of Data Safety Monitoring Board (DSMB) (or full DSMB at the discretion of the DSMB chair), before inoculating further participants
- After each cohort (6-12 participants) has been challenged, a review will be performed by the chair of Data Safety Monitoring Board (DSMB) (or full DSMB at the discretion of the DSMB chair) before proceeding with challenge of further volunteers.

The DSMB will review safety data accumulated after each dose group and evaluate frequency of events, safety and infection rate / symptom data. The DSMB will make recommendations to the investigators concerning the conduct, continuation or modification of the study.

Safety holding rules

Safety holding rules have been developed considering the fact that this is the first study in which SARS-CoV-2 virus has been administered experimentally.

Group holding rules

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Group holding rules below will apply:

- **Adverse events:**
  - If 2 or more individuals suffer the same Grade 3 adverse event persisting at Grade 3 for >72 hrs following virus challenge
- **Laboratory adverse event:**
  - If 2 or more individuals suffer the same Grade 3 laboratory adverse event persisting at Grade 3 for >72 hrs following virus challenge
- **A serious adverse event considered possibly, probably or definitely related to inoculation occurs**
- **Withdrawal from the study of 2 or more individuals due to subjectively intolerable symptoms considered possibly, probably or definitely related to SARS-CoV-2 inoculation**

If a holding rule is activated, then further virus inoculations will not occur until a safety review by the DSMB, study Sponsor, CI and PI has been conducted and it is deemed appropriate to restart challenges. Follow-up visits and procedures, including safety assessments for all study participants already challenged with the virus will continue. Intervention treatment will be given as planned unless the adverse events in question are deemed possibly, probably or definitely related to such treatment. The safety review will consider:

- The relationship of the AE or SAE to the virus inoculation, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms and alterations to the current Participant Information Sheet (PIS)
- New, relevant safety information from ongoing research programs on COVID-19.

The ethics committee will also be notified if a holding rule is activated or released.

Data Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) will be appointed for this study to periodically review and evaluate the accumulated study data for participant safety, study conduct, progress, and efficacy and make recommendations concerning the continuation, modification, or termination of the trial. Specifically, the DSMB will meet before study start and (at the discretion of the DSMB chair) after every 6-12 challenged participants.

The DSMB will operate in accordance with the study-specific charter, which will be established before recruitment starts.

The chair of the DSMB may be contacted for advice and independent review by the Investigator or Sponsor in the following situations:

- Following any SAE deemed to be possibly, probably or definitely related to a study intervention.
- Any other situation where the Investigator or trial Sponsor feels independent advice or review is important.

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The DSMB will review SAEs deemed possibly, probably or definitely related to study interventions. The DSMB will be notified within 24 hours of the Investigators' being aware of their occurrence. The DSMB has the power to place the study on hold if deemed necessary following a study intervention-related SAE. Additionally, the DSMB will review safety data after each dosing cohort to assess the safety of progressing to the next cohort at the same dose, higher dose or lower dose. The DSMB is expected to meet approximately every 4 weeks during the period in which quarantines are taking place or as required.

Trial Steering Committee

A Trial Steering Committee (TSC) will be convened to provide overall guidance for the project on behalf of the Project Sponsor (Imperial College London) and to ensure the project is conducted to the rigorous standards set out in the Department of Health's Research Governance Framework for Health and Social Care and the Guidelines for Good Clinical Practice. It should be noted that the day-to-day management of the trial is the responsibility of the Chief Investigator. The Chief Investigator in conjunction with the Sponsor's Project Manager is responsible for overseeing Trial management and progress. The TSC will comprise at least 3 independent members (including the Chair). The TSC will operate in accordance with the study-specific charter, which will be established before recruitment starts.

The study will be terminated if this is recommended by the DSMB and/or TSC following any safety review. Additionally, the study will be terminated if data becomes available that raises concern about the safety of the study so that continuation would have posed significant new risks to the participants. If the Investigators, DSMB, or TSC become aware of such data, they will call an extraordinary meeting to discuss the implications and assess the risk to study participants in light of the new data, after which the DSMB and TSC will provide a recommendation as to whether the study can continue.

Pharmacokinetics

Pharmacokinetic parameters are not evaluated in this study.

Pharmacodynamics

Pharmacodynamic parameters are not evaluated in this study.

11 STATISTICS AND DATA ANALYSIS

This section describes the statistical analyses that will be performed in this study.

11.1 STUDY ANALYSIS SETS

The following analysis sets are defined for this study:

- **Full Analysis Set (FAS)** is defined as all participants that are inoculated with each SARS-CoV-2 challenge virus. The FAS will be considered as the primary analysis set for all primary, secondary and exploratory endpoints.
- **Safety Analysis Set** is defined as all participants that are inoculated with SARS-CoV-2 challenge virus. The Safety Analysis Set is identical to FAS and will be used for all safety endpoints.

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- **Per Protocol (PP) Analysis Set** is defined as all FAS participants that are sero-suitable, who have no major protocol deviations and who complete the quarantine period up to the final day of quarantine. The PP Analysis Set will be considered as the secondary analysis set for pre-specified primary, secondary and exploratory endpoints.

Membership of participants in each analysis set will be determined prior to any analysis and database lock.

11.2 SUBGROUP ANALYSIS

A 'Laboratory confirmed infected' subgroup will be identified and will be presented for certain pre-specified analyses (as described in this protocol). The 'subgroup is defined as those subjects that fulfil the following criteria:

- Two quantifiable (≥LLOQ) RT-PCR measurements from mid turbinate or throat samples, reported on 2 or more consecutive timepoints, starting from Day 2 post-inoculation and up to discharge from quarantine.

The participants not part of the 'Laboratory confirmed infected' (i.e. those that are uninfected) will also be presented for certain pre-specified analyses. Some pre-specified endpoints will additionally be explored in relation to subsets of symptoms from the symptom diary card. Additional subgroup analysis may be performed as detailed in the AP.

11.3 SAMPLE SIZE AND POWER

No formal sample size calculation has been performed for this dose-finding study, but a sample size of 30 participants per dose level/condition (made up of cohorts of between 6-12 participants) is felt sufficient to meet the primary objective of escalating/expanding in a safe manner whilst providing information on the attack rate.

To support future studies, a target attack rate of 70% has been deemed desirable and should not be below 50%. A two-sided 95% confidence interval (CI) approach will be used for assessing the precision of the point estimate for the attack rate. This approach is consistent with previous human infection dose escalation studies. Given that sample sizes are relatively small, an exact (Clopper-Pearson) confidence interval will be used. Assessment of the early (first and second) cohorts of the dose escalation scheme will be based primarily on safety together with the fixed attack rate criteria. Participant numbers will then be expanded with a third cohort at the selected dose level and a 95% CI calculated to obtain a level of precision for the observed attack rate. For the level of precision, if an observed 21 out of 30 (70%) participants are infected a 95% CI of (51%, 85%) would provide the necessary CI width to have the lower bound above the required 50%.

In comparing Delta and pre-Alpha groups, we calculate that a sample size of 29 participants in each arm will be sufficient to find a difference of at least 30% (e.g. reduction from 50% to 20%) between Delta and pre-Alpha infection rates using the same optimised conditions, given a 90% confidence interval and 80% power, using the formula:

$n = (Z_{\alpha/2}+Z_{\beta})^2 * (p_1(1-p_1)+p_2(1-p_2)) / (p_1-p_2)^2$,
where $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$, Z_{β} is the critical value of the Normal distribution at β and p_1 and p_2 are the expected sample proportions of the two groups[74].

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Further details on exploratory comparisons between pre-Alpha and Delta groups will be provided in the AP.

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.

11.4 Cohort and Dose Escalation

An overview of the cohort and dose escalation scheme is presented in Figure 6 in Section 6. The following describes the statistical aspects to support the cohort and dose escalation scheme in relation to the first, second and third cohorts each of an expected 5-12 participants at each condition/dose level.

To support future studies, a target attack rate of 70% has been deemed desirable and should not be below 50%. A two-sided 95% confidence interval (CI) approach will be used for assessing the precision of the point estimate for the attack rate. This approach is consistent with previous human infection dose escalation studies[75]. Given that sample sizes are relatively small, an exact (Clopper-Pearson) confidence interval will be used. The assessment of the early (first and second) cohorts of the dose escalation scheme will be based primarily on safety together with clinically relevant fixed attack rate criteria. Participant numbers will then be expanded with a third cohort at the selected dose level and a 95% CI calculated to obtain a level of precision for the observed attack rate. If a pre-specified attack rate (as shown in Figure 6 in Section 6) after the third cohort hasn't been achieved then further dose levels or participant populations will be explored.

11.5 Interim Statistical Analysis

No formal interim statistical analysis will be performed. However, two analyses are planned to be conducted. The primary analysis of study data will be conducted when all data through the Day 28 post-inoculation are available, monitored, and locked for all participants. This will be followed by a final analysis which will include all study data through to the extended (Day 365) follow-up.

11.6 Statistical Analysis Plan

Data will be analysed and reported using Graphpad Prism version 9.3.1.

No statistical comparison of dose groups is planned. Primary, secondary and exploratory endpoints will be analysed descriptively. Continuous variables will be summarised using number of observations, mean (and/or geometric mean, where applicable), standard deviation, standard error, median, lower quartile, upper quartile, minimum and maximum values. Categorical variables will be summarised using proportions (counts and percentages). A 95% confidence interval (CI) may be presented for certain pre-specified endpoints.

An AP will be developed and approved prior to any lock of the study database. The AP will give a more detailed description of the report presentations to be produced for the study, expanding on the protocol specified analysis. Any deviation(s) from the original statistical plan will be described and justified in an amendment to the protocol and/or AP as appropriate and also referenced in the end of study report. The AP will describe and account for the occurrence of and extent of missing data, and its possible impact on the study analysis. Any required sensitivity analyses will be specified in the AP.

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Further post-hoc evaluations of any exploratory endpoints may be conducted and reported separately.

Protocol deviations

Participant data will be reviewed for major protocol deviations prior to database lock and decisions will be documented within the meeting minutes. At this meeting, participants will be reviewed for their inclusion/exclusion from the analysis sets. Protocol deviations will be listed.

Demographic and baseline characteristics

Descriptive statistics of demographics (age, sex, height, weight, BMI, and ethnicity) will be presented by dose group and across all participants. Medical history information will be listed. Other baseline characteristics will be defined in the AP.

Primary Endpoint Analysis

The primary objective is to identify a safe and infectious dose of Delta SARS-CoV-2 in healthy volunteers, suitable for future intervention studies.

The following primary endpoints will be analysed as follows:

- To evaluate the safety of Delta SARS-CoV-2 challenge in healthy participants the following endpoints will be summarised by dose group, as well as the subgroups of infected and uninfected, and for all participants:
  - Occurrence of unsolicited AEs within 30 days post-viral challenge (Day 0) up to Day 28 follow up.
  - Occurrence of SAEs related to the viral challenge from the viral challenge (Day 0) up to Day 28 follow up.

  Additional safety data presentations are also described in Section 10.
- To evaluate the infection rate, the laboratory confirmed infection rate will be summarised by dose group.
  The laboratory confirmed infection is defined as: Two quantifiable greater than lower limit of quantification (≥LLOQ) RT-PCR measurements from mid turbinate and/or, throat samples, reported on 2 or more consecutive timepoints, starting from Day 2 post-inoculation and up to discharge from quarantine.
  In addition, the laboratory confirmed infection will be assessed using at least two detectable (≥LLOD) in place of quantifiable (≥LLOQ) RT-PCR measurements and will be summarised by dose group.

Secondary Endpoint Analysis

Secondary endpoints as described in Section 7.1 will be summarised by dose group as appropriate. In addition, subgroup analyses may be performed as per Section 11.2. Further details will be described in the AP.

Exploratory Endpoints

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Exploratory endpoints as described in Section 7.1 may be summarised by dose group as appropriate. In addition, subgroup analyses may be performed as per Section 11.2. Further details will be described in the AP.

Safety Analysis

Safety data will be summarised descriptively.

All Adverse Events (AEs) will be coded using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA). Virus Challenge emergent adverse events will be summarised by MedDRA system organ class (SOC), preferred term and dose group, for those participants infected and uninfected and for all participants.

An AE will be defined as Virus Challenge emergent if the onset date is on or after the date of inoculation. Any AE with an onset date earlier than the date of inoculation will be considered as a pre-Virus Challenge AE. If a participant experiences more than one AE with the same preferred term, that preferred term will be counted only once within summary presentations.

It will be assigned the highest severity and the strongest relationship to Virus Challenge among those events for the summaries in which those characteristics are considered. Pre-Virus Challenge AEs will be identified in a listing.

Summary presentations will be performed for the number and percentage of participants reporting Virus Challenge emergent: AEs, severity of AEs and AEs related to Virus Challenge. In addition, SAEs and AEs directly resulting in withdrawal from the study will be listed.

Other safety endpoints that will be presented by dose group include laboratory evaluations (biochemistry, haematology, coagulation, cardiac enzymes and urine analysis), vital signs assessments, 12-lead ECG. Additionally, physical examinations will be listed.

Concomitant medications will be recorded. A medication will be assigned as being prior to Virus Challenge or concomitant with Virus Challenge, based on the start and stop dates of the medication and the date of inoculation. If the medication stop date is before the date of inoculation, the medication will be assigned as being prior to Virus Challenge. In all other situations, the medication will be assigned as being concomitant with Virus Challenge. Prior medications will be identified in a listing. If a participant has separate periods of taking specific medications, then that medication is only counted once within the specific period of observation (i.e. prior or concomitant) where it is taken.

Further details of the safety analyses will be documented in the AP.

12 REGULATORY ISSUES

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines

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- Applicable laws and regulations

The protocol, protocol amendments, PIS, ICF and other relevant documents must be submitted to an IRB/REC by the Chief Investigator and reviewed and approved y the IRB/REC before the study is initiated.
Any amendments to the protocol or other study documents will require IRB/REC approval before implementation of changes made to study design, except for changes necessary to eliminate an immediate hazard to study participants. These will be reviewed by the Sponsor prior to submission.

The Sponsor will be responsible for the following:

- Notifying the IRB/REC of SAEs or other significant safety findings as required by the IRB/REC procedures
- Providing written summaries of the status of the study to the IRB/REC annually or more frequently in accordance with the requirements, policies and procedures established by the IRB/REC.
- Provide the relevant manufacturer with necessary information to facilitate safety reporting related to the "Rescue" therapy
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/REC, European regulation 536/2014 for clinical studies (if applicable) and all other applicable local regulations

12.1 Ethics Approval

The Study Coordination Centre will obtain approval from a Research Ethics Committee (REC) and the Health Research Authority (HRA) before beginning this study. 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.

12.2 Informed Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information sheet offered, and time allowed for consideration. Signed participant consent should be obtained. 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.

Informed consent will be obtained at the pre-screening visit by a study nurse or doctor, and at the screening visit by a s study doctor, prior to any study specific procedures as described in Section 6.2.3 and Section 6.2.4. If the participant information sheet or informed consent form is updated at any point during the study, the participant's will be provided with the new version of the PIS and asked to reconsent to the latest version at their next scheduled study visit.

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All participants will be required to have a good understanding of English and the Investigator will be responsible for ensuring that the participant understands the information contained in the ICFs. Once they have confirmed that the participant has understood the study, including the benefits and risks of participation, the participant and the Investigator can sign and date the ICF.

The consent process will be documented in line with the Sponsor's SOPs, which require that documented and signed evidence must be available to confirm staff obtaining consent are trained and passed as competent, prior to independently obtaining consent. The Investigator will document the consent process in the participant notes and progress notes as applicable. This documentation will include details of capacity assessment, discussion with participant, and when/how consent has been obtained.

Participants will be assured that they can withdraw from the study at any time and for any reason without prejudice to their future medical care, and that they will be informed in a timely manner if new information becomes available that may affect their willingness to continue their participation in the study. This information will be included within the ICF.

The ICF will contain a separate section that addresses the use of samples for future research. The investigator or authorised designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason.

12.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

Data will be pseudonymised

Data will be transferred to research collaborators under appropriate collaboration agreements.

12.4 INDEMNITY

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

12.5 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to Imperial College Healthcare NHS Trust, Chelsea and Westminster Hospital NHS Trust and the University of Oxford taking part in this study.

12.6 FUNDING

This study is funded by the Wellcome Trust. They are acting as sole funders and the contract and agreement are in place. The investigators will not receive any additional payment above their normal salaries.

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Participants will be reimbursed £4,470 to compensate for the time and inconvenience of taking part in the study (including the quarantine stay). This was calculated using the NIHR formula and national living wage. It is similar to the market rate for in-patient human infection challenge studies. These expenses will be paid in line with standard procedures. If participants require additional visits or if they fail to attend scheduled visits, they will be reimbursed on a pro-rata basis. This will be detailed fully in the PIS.

12.7 AUDIT

The study may be subject to audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

12.8 SAMPLE AND DATA STORAGE AND USAGE

Samples of tissue, cells and fluids will be stored according to the Sponsor and local SOPs as appropriate. Samples will be anonymised with participant identification numbers only and the anonymisation key kept in a separate locked location accessible only to the clinical study team. Samples 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 committees and subject to a valid Materials Transfer Agreement. Data and samples sent outside the UK will be labelled with the identification number only and no patient identifiable data transferred.

There are some exceptions where participant identifiable data may be used:
- For safety reasons, radiological investigations will have the participant's identifying details as per NHS trust policy.
- In addition, during the inpatient quarantine period, participant identifiers will be recorded as part of standard of care as per NHS trust protocols. e.g. blood testsData and all appropriate documentation will be stored for a minimum of 10 years and maximum of 25 years after the completion of the study, including the follow-up period according to Imperial College London policy.

At the end of the study any remaining samples will be either destroyed or transferred to the sponsor or subject to consent, transfer to and maintained under ICHNT tissue bank sub-collections HTA licenses.

12.9 DISSEMINATION OF CLINICAL STUDY DATA

The key design elements of this Protocol will be posted on publicly accessible registers, such as ISRCTN. Where required, protocol summaries will also be posted on national or regional clinical trial registers or databases in compliance with the applicable regulations.

It is the Sponsor's (or Sponsor delegate) responsibility to send the Clinical Trial Summary Report to the REC (if required) within 1 year of the end of the trial. In addition, the Sponsor or Sponsor delegate is responsible for entering appropriate data into the ISRCTN database within 1 year of the end of the trial.

The CI/PI/Investigator shall provide assurance to participants that their confidentiality will be maintained.

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12.10 DATA PROTECTION

Participants will be assigned a unique identifier by the study team when they attend their first screening visit. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law and UK GDPR. The level of disclosure including the participant's rights under the applicable data protection laws must also be explained to the participant in the Informed Consent Form.

The participant must be informed that his/her medical records may be examined by third parties such as Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate REC members, and by inspectors from regulatory authorities.

12.11 DATA QUALITY ASSURANCE

Participant data will be collected at site using paper source casebooks which will then be data entered into the electronic case report form (eCRF) database unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents. Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (remote or on-site monitoring) are provided in the Monitoring Plan.

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator during the retention period as agreed with the sponsor and as required by local regulations or institutional policies. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

12.12 SOURCE DOCUMENTS

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

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Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Definition of what constitutes source data can be found in the Source Data Agreement which is in the Data Management Plan.

12.13 STUDY DISCONTINUATION

The Sponsor reserves the right to temporarily suspend or discontinue the study for any reason at any time. In addition, the study may be stopped at any time if, in the opinion of the CI, the safety data suggest that the medical safety of participants is being compromised.

If the study is suspended or terminated for safety reason(s), the Sponsor will promptly inform the PI, and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action.

The CI is responsible for promptly informing the REC and providing the reason(s) for the suspension or termination of the study.

If the study is prematurely terminated, all study data must be returned to the Sponsor. In addition, the site must conduct final disposition of all unused intervention treatment in accordance with the Sponsor's procedures for the study.

Termination of the clinical trial may also be initiated by the REC.

13 STUDY MANAGEMENT AND GOVERNANCE

The study will be registered on ISRCTN.

By signing the study protocol, the CI/PI agrees that the results of this study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals by the Sponsor and collaboration partners.

In order to allow the use of the information derived from this clinical study, the CI/PI understands that he has an obligation to provide complete test results and all data developed during this study to the Sponsor.

If the study is to be published, the Sponsor and collaboration partners may jointly prepare and co-author manuscript(s) that could result from the clinical trial. In the case the Sponsor acts as fully responsible for the publication, the Sponsor agrees to allow the partners time to review all manuscripts and abstracts prior to submission for publication. All proposed publications that discuss or disclose any part of the Study Data will be submitted to the Human Challenge Steering Committee (HCSC) and will be subject to the prior approval of the Human Challenge Steering Committee, or its successor The Sponsor also reserves the right to delete any confidential information from any proposed manuscripts prior to submission for publication. Confirmation of study specific arrangements can be found in the partnership collaboration agreement.

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The expectation is that after analysis aggregated anonymised 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 will work with public engagement and involvement groups and the media department at Imperial College and the collaboration partners to publicise research that is of public interest. No personal data from the participants will be published.

14 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.

15 Appendices: Supporting Documentation and Operational Considerations

15.1 APPENDIX 1: CLINICAL LABORATORY TESTS

- The tests detailed in Table 12 will be performed for the London site by North West London Pathology (NWLP) and for the Oxford site by Oxford University Hospitals NHS Foundation Trust laboratories
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 6.5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Laboratory Assessments	Parameters
Haematology	Platelet Count. White blood cell (WBC) count (absolute) WBC differential: Neutrophils Lymphocyte Monocytes Eosinophils Basophils Red blood cell (RBC) count Haemoglobin Haematocrit Mean corpuscular volume (MCV) Mean corpuscular haemoglobin (MCH) MCH concentration (MCHC).
Coagulation	Prothrombin Time (PT) Activated Partial Thromboplastin Time (APTT) D-dimer
Biochemistry	Sodium Potassium Albumin Chloride Bicarbonate Calcium

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Laboratory Assessments	Parameters
	Total protein Creatinine eGFR Total bilirubin Inorganic phosphate C-reactive protein (CRP) Gamma glutamyl transferase Alkaline phosphatase (ALP) Alanine transaminase (ALT) Aspartate transaminase (AST) Urea.
Cardiac enzymes	Creatine Kinase (CK) Troponin (T)
Routine urinalysis	Colour Specific gravity Appearance pH Presence of blood, glucose, leukocytes, ketones, nitrites, proteins, urobilinogen, bilirubin by dipstick Microscopy, culture and sensitivity examination (If the dipstick yields abnormal results)
Other screening/eligibility tests	Glucose (random) HbA1c Total cholesterol (and full lipid profile at Investigator's discretion) Thyroid function test [thyroid stimulating hormone (TSH), free thyroxine (T4)] Antibodies against HIV-1 and HIV-2 Hepatitis B surface antigen (HBsAg) Hepatitis C antibodies (HepC)

Table 11. Protocol-Required Safety Laboratory Assessments

Investigators must document their review of each laboratory safety report.

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15.2 APPENDIX 2: ADVERSE EVENTS: PROCEDURES FOR RECORDING, EVALUATING, FOLLOW-UP, AND REPORTING

Recording, assessment and follow-up of AE and/or SAE

11. AE and SAE recording

All AEs and SAEs will be collected from the time of written informed consent until study completion/final study contact or until the resolution of the AE. AEs will be fully recorded in the source documents as they are reported, whether spontaneously volunteered by a participant or in response to questioning about wellbeing at telephone or face-to-face study visits. Enquiries about AEs should cover the period between the previous and current visit.

The following are examples of open ended, non-leading questions that may be used to obtain this information:

- How are you feeling?
- Have you had any medical problems since your last visit/assessment?
- Have you taken any new medicines, other than those given to you in this study, since your last visit/assessment?

Following the reporting of AEs and concomitant medication, the Investigator should assess the participant's eligibility to continue in the study.

The PI or delegated study clinician will record all relevant information regarding an AE/SAE in the source documents and evaluate AEs using the following guidelines:

- Description of events (if the event consists of a cluster of signs and symptoms, a diagnosis should be recorded)
- Seriousness
- Severity (or grade)
- Onset date and time
- Frequency
- Date and time of resolution (or 'continuing' if unresolved)
- Action taken
- Concomitant medication
- Clinical outcome
- Relationship or causality (Intervention treatment/ Challenge Virus/ study procedures/ concomitant medication/other).

Any clinically significant abnormal laboratory result, vital sign or other measure will be followed until it returns to normal or baseline values, stabilises, or is judged by the Investigator to be no longer clinically significant.

If an AE is not resolved at the end of the study, the AE should be followed until it has resolved or (in the case of pregnancy) the pregnancy has been terminated (including spontaneous abortion), resulted in a birth, or a decision has been made by the Sponsor that no further follow-up is required.

Even if the AE or SAE is assessed by the PI as not reasonably attributable to the challenge virus, its occurrence must be fully documented in the source notes.

12. Assessment

<u>Description</u>

If the event consists of a cluster of signs and symptoms, a diagnosis should be recorded (e.g. gastroenteritis) rather than each sign and symptom.

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Onset and end

The dates and times of the onset and end of the event should be recorded.

Assessment
Challenge Virus Symptoms The Investigator will assess, and review Challenge Virus related symptoms recorded in participants' Symptom Diary Cards. Symptoms greater than Grade 0 will be expected and presumed to represent virus infection consequent to Viral Challenge, and will not be additionally captured as AEs unless they meet the definition of an AE, and are deemed to be clinically significant (in the opinion of the Investigator) to be classed as AEs. Following Viral Challenge all unexpected (in the opinion of the Investigator) symptoms post inoculation will be captured as AEs, along with all other occurrences that meet the criteria for an AE.
Physical Examination Any clinically significant change in complete physical examination findings during the study will be documented as an AE.
Direct Physical Examination Following Viral Challenge, upper and lower respiratory symptoms (nasal discharge, otitis, pharyngitis, sinus tenderness, new wheezes, rales and rhonchi) will be expected and presumed to represent virus infection consequent to Viral Challenge, and will not be additionally captured as AEs unless they meet the definition of an AE, and are deemed to be clinically significant (in the opinion of the Investigator) to be classed as AEs.
Vital Signs Deterioration in a vital sign (compared to baseline) should only be reported as an AE if the deterioration fulfils the criteria for an AE. If deterioration in a vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated vital sign will be considered as additional information.
Temperature Following Viral Challenge, pyrexia will be expected and presumed to represent virus infection consequent to Viral Challenge, and will not be additionally captured as an AE unless it meets the definition of an AE, and is deemed to be clinically significant (in the opinion of the Investigator) to be classed as an AE. Following Viral Challenge all unexpected (in the opinion of the Investigator) pyrexia post inoculation will be captured as an AE, along with all other occurrences that meet the criteria for an AE.
Spirometry A 15% drop in a spirometry value (compared to baseline, and confirmed by a repeat on the same day) may be judged a Grade 1 (mild) AE. However, due to variability in participants' ability to perform these tests with adequate technique, the Investigator will use his/her clinical judgement to assess whether abnormal spirometry readings are consistent with a true drop and whether an AE should be raised. The PI/Investigator will use his/her clinical judgement to assign severity grades above Grade 1, based on evaluation of clinical signs and symptoms. If a spirometry reading on repeat assessment has returned to normal an AE will not be raised.
Laboratory Values Deterioration in a laboratory value (compared to baseline) should only be reported as an AE if the deterioration fulfils the criteria for an AE. If deterioration in a laboratory result is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result will be considered as additional information. The Investigator will judge whether abnormal laboratory values are clinically significant or not clinically significant, and record this in the source document. This entry should be signed and dated by the relevant Investigator. Laboratory abnormalities detected at screening will be considered as part of the medical history and will not be reported as AEs Challenge Virus associated laboratory abnormalities (e.g.: elevated ALT, AST or GGT; decreased neutrophils) may be recorded as AEs (at the discretion of the Investigator).

13. Assessment of intensity

The term 'severe' is often used to describe the intensity (severity) of a specific event. This is not the same as 'serious' which is based on participant/event outcome or action criteria.

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The PI and delegated study clinicians will use the grading scale for AEs as a reference when collecting, reporting and clarifying database queries of AEs and SAEs.
The severity of an AE that does not appear in the grading scale for AEs should be determined according to the definitions in Table 12. Classification of Adverse Event Severity.

Grade	Definition
Grade 0	**Absent**
Grade 1	**Mild** level of discomfort, and does not interfere with regular activities
Grade 2	**Moderate** level of discomfort that intermittently interferes with regular activities
Grade 3	**Severe:** Significant level of discomfort and prevents regular activities
Grade 4	**Potentially life threatening**

Table 12. Classification of Adverse Event Severity

An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe. It is important to distinguish between serious and severe AEs. An AE of severe intensity needs not necessarily be considered serious. For example, a migraine headache that incapacitates a participant for many hours may be severe, whereas a stroke that results in a limited degree of disability may be considered mild but should be reported as a SAE.

Vital Signs	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Grade 4 Potentially Life threatening
Fever	**37.9°C - 38.4°C**	**38.5°C – 38.9°C**	**39.0°C - 40°C**	> 40°C
Tachycardia (bpm)*	**101 – 115**	**116 – 130**	**>130**	A&E visit or hospitalisation for arrhythmia
Bradycardia (bpm)**	**45-49**	**40-44**	**<40**	A&E visit or hospitalisation for arrhythmia
Systolic hypertension (mmHg)	**141 – 150**	**151 – 155**	**≥155**	A&E visit or hospitalization for malignant hypertension
Diastolic hypertension (mmHg)	**91 – 95**	**96 – 100**	**>100**	A&E visit or hospitalization for malignant hypertension
Systolic hypotension (mmHg)***	**85 – 89**	**80 – 84**	**<80**	A&E visit or hospitalization for hypotensive shock
Respiratory Rate –breaths per minute	**17 – 20**	**21-25**	**>25**	Intubation

Table 13. Severity grading criteria for physical observations

*Taken after 5 minutes at rest **When resting heart rate is between 50 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy participant populations, for example, conditioned athletes. ***Only if symptomatic (e.g. dizzy/ light-headed)

14. Frequency

The frequency of the AE should be categorised as one of the following:

- Single
- Intermittent
- Continuous

For every AE, an assessment of the relationship of the event to the administration of the challenge virus will be undertaken by the PI. An interpretation of the causal relationship of the challenge virus to the AE in question will be made, based on the type of event; the relationship

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of the event to the time of challenge virus administration; and the known biology of the challenge virus. Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to challenge virus will be considered and investigated. Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding or stopping rule is activated) and at the final safety analysis, except for SAEs, which should be assigned by the reporting investigator immediately.

For every AE, an assessment of the relationship of the event to the administration of the challenge virus will be undertaken by the PI. An interpretation of the causal relationship of the challenge virus to the AE in question will be made, based on the type of event; the relationship of the event to the time of challenge virus administration; and the known biology of the challenge virus (Table 16). Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to challenge virus will be considered and investigated. Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding or stopping rule is activated) and at the final safety analysis, except for SAEs, which should be assigned by the reporting investigator immediately.

- The Investigator is obligated to assess the relationship between challenge virus and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the sponsor and CI. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data.
- The Investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements – AE/SAE related to the challenge virus will be reported to the REC.
- The relationship of an AE to the challenge virus will be categorised as shown in Table 16

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Classification	Definition
Not related	The AE is related to an aetiology other than the challenge virus (the alternative aetiology must be documented in the participant's medical record).
Unlikely to be related	The AE is unlikely to be related to the challenge virus and likely to be related to factors other than challenge virus
Possibly related	There is an association between the AE and the administration of the challenge virus, and there is a plausible mechanism for the AE to be related to the challenge virus, but there may also be alternative aetiology, such as characteristics of the participant's clinical status or underlying disease.
Probably related	A reasonable temporal sequence of the AE and the challenge virus administration exists and, based upon the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the Investigator's clinical experience, the association of the AE with the challenge virus seems likely.
Definitely related	A definite causal relationship exists between the AE and the administration of the challenge virus, and other conditions do not appear to explain the AE.

Table 14 Classification of Adverse Event Relationship

Unless an AE is 'definitely related' to the challenge virus, a causal relationship to one of the following should be considered, and full details provided on the AE reporting form as appropriate.

- Study procedures
- Concomitant medication
- Other

15. Action taken

The Investigator should ensure that adequate medical care is provided to participants for any AEs, including clinically significant laboratory values related to the study intervention. In addition, the Investigator will describe whether any treatment was given for the AE.

The Investigator will classify the action taken with regard to the AE. The action taken should be classified according to the following categories and full details provided as appropriate:

- None
- Non-drug therapy given
- Concomitant medication taken
- Study intervention dose not changed
- Study intervention dose adjusted
- Study intervention administration temporarily interrupted
- Study intervention administration permanently discontinued
- N/A Study intervention not administered
- Participant withdrawn
- Participant hospitalised
- Other

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16. Outcome

An AE should be followed until the Investigator has determined and recorded the outcome or an alternative explanation. The outcome should be classified according to the categories shown in Table 17.

Classification	Definition
Resolved	Resolution of the AE with **no** residual signs or symptoms
Resolved with sequelae	Resolution of the AE with residual signs or symptoms
Ongoing	Either incomplete improvement or no improvement of the AE, such that it remains on-going
Fatal	Outcome of the AE was death. 'Fatal' should be used when death was at least possibly related to the AE.
Unknown (e.g. Lost to follow-up)	Outcome of the AE is not known (e.g. the participant is lost to follow-up).

Table 15 Classification of Adverse Event Outcome

17. Follow up

All AEs and SAEs must be followed-up by the Investigator, or where appropriate, be referred to the participant's GP or other healthcare professional for follow-up until they are:

- Resolved (return to normal or baseline values), or
- Stabilised, or
- Judged by the PI/Investigator to be no longer clinically significant, or
- An alternative explanation has been provided.

Additional measurements and/or evaluations may be necessary to investigate the nature and/or causality of an AE or SAE. This may include additional laboratory tests, diagnostic procedures, or consultation with other healthcare professionals. If the participant dies, any post-mortem findings (including histopathology) will be provided to the Sponsor if possible.

Reporting of SAEs

Prompt notification and assessment of SAEs to ethics committee(s) where necessary for this study will be the responsibility of the sponsor. Contact details are detailed in Table 18.

Annual safety/progress reports and final Study report will be generated and submitted to relevant ethics committee(s). This will be the responsibility of the study team as described in Section 10.

Notification should be made:

- In a detailed written SAE form report within 24 hours of the Investigator becoming aware of the event.

All reports should be directed to the CI and the sponsor mailbox. The Investigator at the site is responsible for ensuring that a member of the Sponsor study team is made aware of any SAE reports that have been transmitted.

Contact	Details

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Sponsor SAE reporting email address:	RGIT @imperial.ac.uk
Chief Investigator email address:	c.chiu@imperial.ac.uk

Table 16 Contact Details for Reporting SAEs

In addition, any AE resulting in permanent study discontinuation for a participant, even if not serious and regardless of expectedness or causality, must be reported by telephone, email or fax to the CI and Sponsor within 7 calendar days of the PI or any other site personnel's knowledge of the event.
The SAE form, AE record and relevant concomitant medication record should be faxed/emailed to the Sponsor within 24 hours of the Investigator or any site personnel's knowledge of a SAE. An updated SAE report form should be forwarded to the Sponsor within 24 hours of receipt of the new/updated information as relevant.
Information relating to the participant's subsequent medical progress must be submitted to the Sponsor as available, until the SAE has subsided or, in the case of permanent impairment, until it stabilises and the overall clinical outcome has been ascertained.
The Investigator will also provide additional information, including a copy of the following documents (where applicable):

- Copies of test results, as available
- Hospital discharge summary (as soon as it is available to the PI)
- Autopsy report (as soon as it is available to the PI).

The Investigator must report SAEs to the relevant REC in accordance with applicable regulatory requirements and within the relevant timelines.
The REC will be sent annual safety updates in order to facilitate their continuing review of the study.

Adverse reactions to non-IMPS

Any AEs and SAEs which are related to/caused by a concomitant medication or Challenge agent, should not be classed as ARs, SARs, or SUSARs (ARs, SARs, SUSARs relate only to IMP by definition). However, an SAE caused by a non-IMP would need to be reported to the REC for the appropriate action to be taken.

Post-study AEs and SAEs

All SAEs that occur during the study must be reported by the Investigator to the Sponsor as soon as possible, in accordance with the Sponsor's SOPs, and at the latest within 24 hours of becoming aware of the event.

Pregnancy

If a female participant or partner of a male participant becomes pregnant after being discharged from the quarantine unit at Day 14 until their final scheduled study visit at Day 360, this must be reported by the Investigator to the Chief Investigator and Study Monitor by telephone as soon as possible, in accordance with the Sponsor's SOPs, and at the latest within 24 hours of becoming aware of the event.
Following the telephone notification, the Investigator must fully and accurately complete the appropriate pregnancy reporting form, which must be e-mailed to the pharmacovigilance department and the Study Monitor at the latest within 24 hours of becoming aware of the pregnancy.
Participants will be advised to contact their GP or a specialist, as appropriate.

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Provided that the appropriate consent is in place, information related to the pregnancy will be collected as per the Sponsor's SOPs and the Sponsor's requirements. The completed reporting form(s) will be sent to the Sponsor for review and assessment, and subsequent reporting as required.

- A complete evaluation will be documented in the source data to permit transfer to the clinical database.
- The study site team will maintain contact with the participant for a protracted period of time, but certainly until after the birth, in order to assess for outcomes that may be reportable as related AEs, and for reporting to the Sponsor as appropriate.
- The study site team in consultation with the participant will keep the participant's GP informed.
- All cases of foetal drug exposure via the parent as a study participant will be reported to the Sponsor and the REC.

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15.3 APPENDIX 3: NORMAL RANGES

Vital Signs

Vital sign Parameters	Lower limit	Higher limit	Units
Temperature (above 37.8 classed as pyrexia)	35.5	37.8	°C
Oxygen saturation	Normal is ≥ 95		%
Respiratory rate	10	17	breaths per minute
Heart rate	50	100	beats per minute
Systolic BP	90	140	mmHg
Diastolic BP	50	90	mmHg

ECG

ECG Parameters	Lower limit	Higher limit	Units
HR	**50**	**100**	**bpm**
QRS	**60**	**120**	**ms**
PR interval	**120**	**220**	**ms**
QT	**320**	**450**	**ms**
QTc	Normal for females is < 470		ms
	Normal for males is < 450		
QTcF	320	450	ms
QTcB	320	450	ms

Spirometry

Spirometry parameters	Lower limit	Higher limit	Units
FEV1	Normal if ≥ 80% of the predicted value		litres
FEV1/FVC	Normal if ≥70% (≥ 0.7) of the base value		litres

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15.4 APPENDIX 4: ABBREVIATIONS

ADCC	Antibody-Dependent Cellular Cytotoxicity
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALRI	Acute Lower Respiratory Infection
ALT	Alanine Aminotransferase
AP	Analytical Plan
APTT	Activated Partial Thromboplastin Time
AR	Adverse Reaction
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
A&E	Accident and Emergency
BD	Twice Daily
BEIS	Department of Business, Energy and Industrial Strategy
BP	Blood Pressure
BPM	Beats Per Minute
BMI	Body Mass Index
cGMP	Current Good Manufacturing Practices
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CK	Creatine Kinase
CHIM	Controlled human infection model
CI	Chief Investigator
CIOMS	Council for International Organizations of Medical Sciences
CMI	Cell Mediated Immunity
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRP	C-reactive Protein
CT	Computed Tomography
CTL	Cytotoxic T cell
COVID-19	Coronavirus Disease 19
CYP450	Cytochrome 450
DBP	Diastolic blood pressure
DBS	Dried blood spot
DHSC	Department for Health and Social Care
DLCO	Diffusing Capacity for Carbon Monoxide
DNA	Deoxyribonucleic acid
DMID	Division of Microbiology and Infectious Disease
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ELISA	Enzyme-linked Immunosorbent Assay
EMCRF	Experimental Medicine Clinical Research Facility
EUA	Emergency Use Authorisation
EWV	Early withdrawal visit
FAS	Full Analysis Set
FBC	Full Blood Count
FDA	Food and Drug Administration
FEV	Forced Expiratory Volume

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FEV1	Forced Expiratory Volume in One Second
FFA	Focus forming assay
FI	Febrile illness
FSH	Follicle Stimulating Hormone
FVC	Forced Vital Capacity
GAD	Generalised Anxiety Disorder
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GGT	Gamma-glutamyl transferase
GMP	Good Manufacturing Practice
GOSH	Great Ormond Street Hospital
GP	General Practitioner
GWAS	Genome-wide association studies
HAV	Hepatitis A
HbA1c	Haemoglobin A1c
HBV	Hepatitis B
HCSC	Human Challenge Steering Committee
HCV	Hepatitis C
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigen
HR	Heart Rate
HRA	Health Research Authority
HTA	Human Tissue Act
HVC	Human Viral Challenge
IB	Investigator Brochure
ICF	Inform Consent Form
ICH	International Council for Harmonisation
ICRF	Imperial Clinical Research Facility
ICRRU	Imperial Clinical Respiratory Research Unit
ICS	Intracellular staining
ICU	Intensive care unit
IEC	Independent Ethics Committees
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Intramuscular
IMP	Investigational Medicinal Product
IRB	Institutional Review Boards
IUD	Intrauterine Device
IUS	Intrauterine hormone-releasing system
IV	Intravenous
LDH	Lactate dehydrogenase
LFA	Lateral flow antigen
LFT	Liver function tests
LLOD	Lower limit of detection
LLOQ	Lower limit of quantification
LRT	Lower Respiratory Tract
LRTI	Lower Respiratory Tract Infection

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mAb	Monoclonal antibody
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCID	Minimal Clinically Important Difference
MCS	Microscopy, culture and sensitivity
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
MOI	Monoamine Oxidase Inhibitors
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA (ribonucleic acid)
MVB	Master Virus Bank
NERVTAG	New and Emerging Respiratory Virus Threats Advisory Group
NGS	Next Generation Sequencing
NHC	n-hydroxycytidine
NHS	National Health Service
NIHR	National Institute for Health Research
NIMP	Non-Investigational Medicinal Product
NPS	Nasopharyngeal Swab
NRES	National Research Ethics Service
NSAIDs	Non-steroidal anti-inflammatory drugs
OAS	Original antigenic sin
ONS	Office for National Statistics
PBMC	Peripheral Blood Mononuclear Cell
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PEF	Peak Expiratory Flow
PFM	Peak flow meter
PFU	Plaque Forming Unit
PHQ	Patient Health Questionnaire
PI	Principal Investigator*
PIS	Participant Information Sheet
PK	Pharmacokinetic
PP	Per Protocol
PPE	Person Protective Equipment
PT	Prothrombin Time
PVA	Polyvinyl alcohol
QDS	Four Times Daily
qPCR	Quantitative Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcriptase-Polymerase Chain Reaction
RBC	Red Blood Cell
REC	Research Ethics Committee
RNA	Ribonucleic acid
RSI	Reference Safety Information
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SAE	Serious Adverse Event
SAGE	Scientific Advisory Group for Emergencies

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SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SBP	Systolic blood pressure
SI	Systemic illness
SNP	Single nucleotide polymorphisms
SoA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
SpO2	Peripheral arterial oxygen saturation
SUSAR	Suspected Unexpected Serious Adverse Reaction
T	Troponin
TDS	Three Times Daily
TMF	Trial Master File
TOPS	The over-volunteering prevention system
TSC	Trial Steering Committee
TSH	Thyroid Stimulating Hormone
TSS	Total Symptoms Score
UK	United Kingdom
UKHSA	United Kingdom Health Security Agency
UPSIT	University of Pennsylvania smell identification test
URT	Upper Respiratory Tract
UTRI	Upper Respiratory Tract Infection
US	United States
VE	Vaccine effectiveness
VL	Viral load
VOC	Variant of Concern
VTF	Vaccines Taskforce
WBC	White Blood Cell
WHO	Word Health Organisation
WOCBP	Woman of Childbearing Potential
β-HCG	β-human chorionic gonadotrophin

* Delegation Log will list all investigators / study physicians delegated by PI to perform study activities.

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