CLINICAL STUDY PROTOCOL

AN EXPLORATORY STUDY TO ESTABLISH THE DOSE, SAFETY AND PATHOGENICITY OF A NEW INFLUENZA A H3N2 CHALLENGE STRAIN IN HEALTHY PARTICIPANTS 18 TO 55 YEARS OF AGE

TITLE PAGE

Brief Title	Dose, safety, and pathogenicity of a new influenza A H3N2 challenge strain
Protocol Version and Date	Final v4.0, 20 December 2024
Sponsor	hVIVO Services Limited 40 Bank Street, Canary Wharf, London E14 5NR, UK
hVIVO Study Number	HRD-vCS-006
Compound	Not applicable
IRAS ID	341061

Confidentiality and Protections Statement:

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Personal data included in the protocol is subject to the UK Data Protection Act considerations and protections.

Sponsor Statement

This protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the study intervention, and with the moral, ethical, and scientific principles governing clinical research as set out in the current Declaration of Helsinki and the principles of International Council for Harmonisation (ICH) Good Clinical Practice (GCP).

Sponsor Signatory

Name, title (typed or printed): Alex Mann, Senior Director, Clinical Science

Signature and Date:	

Investigator Agreement

I have read the protocol and agree to conduct the study in accordance with the approved protocol and any future amendments, the Declaration of Helsinki, the principles of ICH GCP, the current regulatory requirements as detailed in the Medicines for Human Use (Clinical Trial) Regulations (Statutory Instrument 2004/1031) and all subsequent amendments, the UK Data Protection Act 2018, any other applicable laws, and guidance.

I agree to conduct the procedures described in this protocol according to these guidelines and to appropriately direct and assist the study staff under my control.

Principal Investigator Signatory

Name (typed or printed): Dr. Alexandre Lima

Signature and Date:	

Note: In this protocol, the terms hVIVO and 'investigator' distinguish between the principal investigator's (PI's) responsibility, and actions required by the organization (hVIVO). The term 'investigator' includes appropriately qualified persons to whom the PI has formally delegated his/her investigator roles and responsibilities.

Study Staff Contact Information

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PROTOCOL AMENDMENT SUMMARY OF CHANGES

PROTOCOL HISTORY		
Document	**Date**	**Amendment Type**
Amendment 3 (Protocol v4.0)	20-December-2024	Substantial
Amendment 2 (Protocol v3.0)	31-October-2024	Non-Substantial
Amendment 1 (Protocol v2.0)	01-August-2024	Substantial
Original Protocol (Protocol v1.0)	29-February-2024	Not applicable

Amendment 3 (Protocol v4.0) – 20 December 2024

This amendment is substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Overall Rationale for the Amendment:

The primary reason for this protocol amendment was toinclude Throat swabs – virology: qRTPCR, viral culture assessments twice daily within 1.2, Schedule of Events.

TABLE OF CONTENTS

LIST OF TABLES

1. SYNOPSIS

Study Title	An exploratory study to establish the dose, safety and pathogenicity of a new influenza A H3N2 challenge strain in healthy participants 18 to 55 years of age
Brief Title	Dose, safety, and pathogenicity of a new influenza A H3N2 challenge strain
hVIVO Study Number	HRD-vCS-006
Sponsor	hVIVO Services Limited
IRAS ID	341061
Study Phase	Exploratory
Study Site(s)	hVIVO Services Limited 21 Plumbers Row, London E1 1EQ, UK hVIVO Services Limited 36 George Street, Manchester M1 4HA, UK hVIVO Services Limited 40 Bank Street, Canary Wharf, London E14 5NR, UK
Study Type	Interventional
Indication	Not applicable
Design	This is an exploratory study of an influenza A H3N2 challenge strain to determine the optimum infectious titer of challenge agent in healthy participants 18 to 55 years of age.

Objectives and Endpoints

Objectives	**Endpoints**
Primary	
Pathogenicity & Safety	
o To determine the safe and optimal titer of the influenza challenge agent.	To identify a safe and infectious dose of wild-type influenza virus in healthy participants, suitable for future intervention studies that: • Has an acceptable safety profile as measured by: o Occurrence of adverse events (AEs) related to the viral challenge from viral challenge (Day 0) to the Day 28 (±3 days) follow-up visit. o Occurrence of serious adverse events (SAEs) related to the viral challenge from viral challenge (Day 0) to the Day 28 (±3 days) follow-up visit. • Induces laboratory-confirmed infection in ≥40% of inoculated participants (ideally between 50% and 80%). Laboratory-confirmed infection is defined as: o Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)-confirmed influenza infection, defined as 2 quantifiable (≥ lower limit of quantification [LLOQ]) qRT-PCR measurements (reported over 4 planned consecutive assessments within 48 hours), from Day 1 pm to planned discharge from quarantine (Day 8 am).
Secondary	
Pathogenicity	
o To evaluate the incidence of viral culture-confirmed influenza infection.	o Viral culture-confirmed influenza infection, defined as a quantifiable viral culture measurement, from Day 1 pm to planned discharge from quarantine (Day 8 am).
o To evaluate the optimal titer of the influenza challenge agent, as measured by:	The following endpoints will be determined by both qRT--PCR and viral culture on nasal and throat samples: o Area under the viral load-time curve (VL-AUC) of the influenza challenge agent, from Day 1 pm to planned discharge from quarantine (Day 8 am).

o Viral load-related endpoints.	o Peak viral load (VLPEAK) of the influenza challenge agent, defined as the maximum viral load, from Day 1 pm to planned discharge from quarantine (Day 8 am).
o To explore the optimal titer of the influenza challenge agent, as measured by: o Clinical symptom-related endpoints.	o Area under the total clinical symptom score-time curve (TSS-AUC) as measured by graded symptom scoring system collected 3 times daily from Day 1 am to planned discharge from quarantine (Day 8 am). o Peak daily symptom score: individual maximum daily sum of symptom score from Day 1 (am) to planned discharge from quarantine (Day 8 am). o Peak symptom diary card score: peak total symptom score (TSS) as measured by graded symptom scoring system collected 3 times daily from Day 1 am to planned discharge from quarantine (Day 8 am).
o To explore the optimal titer of the influenza challenge agent, as measured by the incidence of influenza-like illness (ILI) and laboratory-confirmed symptomatic influenza infection.	o Laboratory-confirmed febrile influenza infection, defined as: o Laboratory-confirmed infection, AND o A febrile episode, defined as a temperature of ≥37.9°C, from Day 1 am to planned discharge from quarantine (Day 8 am). o qRT-PCR-confirmed ILI (Centers for Disease and Control [CDC]), defined as: o Laboratory-confirmed infection, AND o A febrile episode, defined as a temperature of ≥37.8ºC, from Day 1 am to planned discharge from quarantine (Day 8 am), AND o Cough and/or sore throat, from Day 1 am to planned discharge from quarantine (Day 8 am), in the absence of a known cause other than influenza. o qRT-PCR-confirmed ILI (World Health Organization [WHO]), defined as: o Laboratory-confirmed infection, AND o A febrile episode, defined as a temperature of ≥38.0ºC, from Day 1 am to planned discharge from quarantine (Day 8 am), AND

	○ Cough, from Day 1 am to planned discharge from quarantine (Day 8 am). ○ Laboratory-confirmed moderately severe symptomatic influenza infection, defined as: ○ Laboratory-confirmed infection, AND ○ Any symptoms from the symptom diary card of grade ≥2 at a single time point, from Day 1 am to planned discharge from quarantine (Day 8 am). ○ Laboratory-confirmed symptomatic influenza infection, defined as: ○ Laboratory-confirmed infection, AND ○ TSS ≥2 at a single time point, from Day 1 am to planned discharge from quarantine (Day 8 am). ○ Viral culture laboratory-confirmed moderately severe symptomatic influenza infection, defined as: ○ Viral culture laboratory-confirmed infection, AND ○ Any symptoms from the symptom diary card of grade ≥2 at a single time point, from Day 1 am to planned discharge from quarantine (Day 8 am). Further analyses may be performed on the above endpoints, using modified start or end times. Details will be provided in the statistical analysis plan (SAP). Further analyses may be performed on the above qRT-PCR-related endpoints where detection by qRT-PCR is reported above the lower limit of detection (LLOD) instead of the LLOQ. Details will be provided in the SAP.
Exploratory*	
○ To further explore the pathogenicity phenotype of wild-type influenza in healthy participants, as measured by: ○ Safety assessments.	○ safety laboratory tests, ○ lung function, ○ electrocardiograms [ECGs], ○ physical examinations, and concomitant medications.

o To further explore the pathogenicity phenotype of influenza challenge agent, as measured by: o Nasal discharge endpoints.	o Total weight of mucus produced from Day 1 to planned discharge from quarantine (Day 8 am). o Total number of tissues used by participant from Day 1 to planned discharge from quarantine (Day 8 am).
o To further explore the pathogenicity phenotype of the influenza challenge agent, as measured by: o Viral load-related endpoints.	The following endpoints may be determined by both qRT--PCR and viral culture on nasal and throat samples: o Duration of quantifiable influenza, from Day 1 pm to planned discharge from quarantine (Day 8 am). Duration is defined as the time (hours) from first quantifiable (≥LLOQ) until first confirmed unquantifiable (<LLOQ) assessment after their peak measure (after which no further virus is quantified). o Time to resolution from VLPEAK. Time to resolution is defined as the time (hours) from VLPEAK until first confirmed unquantifiable (<LLOQ) assessment (after which no further virus is quantified). o Time to resolution from first quantifiable (≥LLOQ) assessment. Time to resolution is defined as the time (hours) from first quantifiable (≥LLOQ) assessment until first confirmed unquantifiable (<LLOQ) assessment (after which no further virus is quantified). o Slope of the viral load over time (clearance rate), from VLPEAK to 4 days after VLPEAK.
o To further explore the pathogenicity phenotype of the influenza challenge agent, as measured by: o Clinical symptom-related endpoints.	o Duration (hours) of grade ≥2 clinical symptoms from Day 1 am to planned discharge from quarantine (Day 8 am). o Time to resolution of symptoms. Time to resolution is defined as the time (hours) from first occurrence of TSS above baseline until TSS returns to baseline or below (after which no further increase above baseline is observed).

	o Time to resolution from peak clinical symptoms as measured by graded symptom scoring system collected 3 times daily. Time to resolution is defined as the time (hours) from peak clinical symptoms until TSS returns to baseline or below (after which no further increase above baseline is observed). o Time to resolution of grade ≥2 symptoms. Time to resolution is defined as the time (hours) from first occurrence of any grade ≥2 symptom to occurrence of no grade ≥2 symptoms (after which no further grade ≥2 symptoms occur).
o To explore the incidence of symptomatic infection after influenza viral challenge.	o Clinical symptom-related endpoints may be further explored, as measured with either the full 13 symptoms within the graded symptom scoring system, a subset of these 13 symptoms, or including additional symptoms. o Patient perception questionnaire results (presence or absence of cold, change in severity of cold) may be compared to other virological and symptomatic endpoints in relation to respiratory viral disease including, but not limited to, construct validations.
o To explore immunity to influenza at baseline and follow-up.	o Baseline, and change from baseline of antibodies (hemagglutination inhibition [HAI], neutralization) after viral challenge. o Seroconversion, defined as follows: o HAI: If the pre-challenge serum is <10 (undetectable), the post-challenge serum must have a titer ≥40; if the pre-challenge serum is ≥10, at least a 4-fold titer increase is required. o Foci reduction neutralization assay (FRNA): at least a 4-fold titer increase is required. o Development of antibodies against the virus may be evaluated in serum samples
o To explore baseline immunology and response to	o Blood and respiratory samples may be used for exploratory assays related to respiratory viral infection, microbiology, genetics, and immunology.

infection with influenza.	
o To explore incidence of influenza virus particles in exhaled breath after virus challenge	o Air samples and sampling masks may be used for exploratory assays to for viral load assessments related to transmission potential of influenza virus

* Note that exploratory objectives and endpoints are optional and might be assessed only if needed; therefore, not all testing might be performed and reported.

Hypothesis	Experimental infection with an influenza A H3N2 challenge agent in healthy adults is safe and well tolerated. Experimental infection with an influenza A H3N2 challenge agent allows viral replication and signs and symptoms of disease to be measured and selection of a preferred dose for future interventional studies. There will be no inferential statistical analysis for the study, the analysis will be purely descriptive.
Investigational Medicinal Product (IMP)	Not applicable
Challenge Agent	Influenza A/England/7763/2022 H3N2 virus
Challenge Agent Route	Intranasal
Challenge Agent Titer	**Part A** o Dose Arm 1: Medium dose, expected to be approximately $10^{4.5}$ tissue culture infective dose 50% ($TCID_{50}$)/mL (titer may be adjusted based on stock titer) o Dose Arm 2: High dose, expected to be approximately $10^{5.5}$ $TCID_{50}$/mL (titer may be adjusted based on stock titer) **Part B** o Expansion Dose: Expansion of one of the Part A dose arms, to be determined (TBD), depending on outcome of Part A, AND/OR o Optional Dose Arm 3: TBD, depending on outcome of Part A

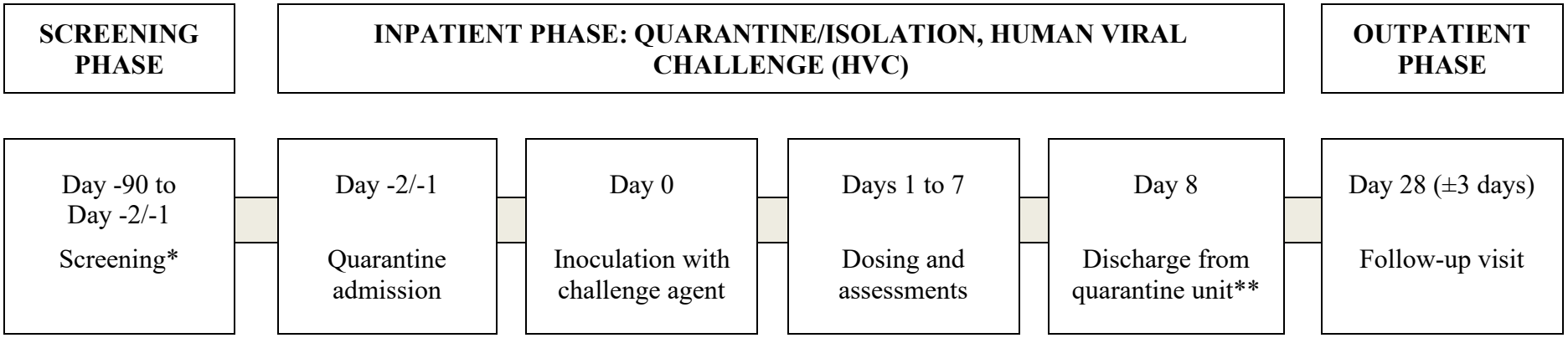
Study Population	Healthy adult male and female participants aged between 18 and 55 years, with a total body weight ≥50 kg and body mass index (BMI) ≥18 kg/m^2 and ≤35 kg/m^2, who have been screened to be serosuitable for infection with the influenza challenge agent.
Summary of Study Design	This is a single-center, randomized, single-blind, proof-of-concept study in healthy adult male and female participants 18 to 55 years of age, inclusive, utilizing an intranasally administered influenza A H3N2 challenge agent. The primary goal of this exploratory study is to establish the optimal dose of the influenza challenge agent that is safe and infectious in healthy adults for use in subsequent intervention studies. A total of up to 80 participants may be given the influenza A/England/7763/2022 H3N2 challenge agent. In Part A of the study, up to 40 participants will be randomized and enrolled (up to 20 participants in Dose Arm 1 [medium dose] and up to 20 participants in Dose Arm 2 [high dose]). Further to the outcome and analysis of inoculations in Part A, additional participants may be enrolled into an optional Part B to: • Expand the number of participants in one of the Part A dose arms, AND/OR • Inoculate different challenge agent titers in optional dose arms. The study is divided into the following study phases: • **Screening phase:** Screening will occur between Day -90 to Day -2/-1. Historical generic screening data collected through the hVIVO generic screening process may be transferred to this study after the study-specific informed consent form has been signed by the participant. • **Inpatient phase:** Participants will be resident in the quarantine unit for approximately 11 days (from Day -2/-1 to Day 8). o **Pre-human viral challenge (HVC)**: ▪ Quarantine admission on Day -2/-1. ▪ (Baseline) assessments, randomization (where applicable), and eligibility checks will be conducted as per Schedule of Events (SoE) up to Day 0, pre-challenge. o **HVC**: Influenza challenge agent inoculation on Day 0. o **Post-HVC**: ▪ Day 1 onwards and each day – study assessments will be conducted as per SoE.

	▪ Participants will be discharged from the quarantine unit on Day 8 (or may remain longer at the discretion of the principal investigator [PI]/investigator). o **Outpatient phase:** o Final follow-up visit: Day 28 (±3 days).
Randomization	Part A will be randomized in a ratio of 1:1. Part B: Expansion and/or additional dose arms to be confirmed. If more than one dose arm is enrolled in Part B, Part B will be randomized.
Participant Replacement Policy	[redacted]
Expected Duration of Participation per Participant	Approximately 4 months from screening to the participant's last scheduled visit.
Overall Duration of Clinical Phase	The length of the clinical phase is expected to be approximately 6 months from first participant's planned first screening visit to the last participant's last scheduled study visit.
End-of-Study Definition	The end of the study is defined as the date of the last visit of the last participant in the study.
Sample Size Determination	No formal sample size calculation has been performed for this exploratory phase study; the sample size is consistent with earlier influenza virus, respiratory syncytial virus, and human rhinovirus characterization studies conducted at hVIVO.
Statistics	The study will be summarized in terms of descriptive statistics; no formal statistical comparisons of groups are planned. Continuous variables will be summarized using number of available data, number of missing data, arithmetic mean (and/or

	geometric mean, where applicable), standard deviation, median, lower quartile, upper quartile, minimum, and maximum values. When relevant, confidence intervals (CIs) will be computed for the mean and/or the median. Categorical variables will be summarized using number of available data, number of missing values, frequency counts for each category and corresponding percentage. Percentages will be calculated using the number of available data as the denominator (i.e., not including missing values). When relevant, CIs will be computed. If not otherwise specified in the SAP, the Wilson Score Method will be used to compute CIs for proportions. o Participants will be grouped and presented against the titer of challenge agent they received. o The number of participants enrolled, randomized (where applicable), withdrawing (also split by reason for withdrawal) from and completing the study, and the number in each analysis population will be summarized for all participants and by group. o Any missing data will be accounted for, and the way their possible impact on the study analysis will be evaluated will be described within the SAP. Full details of the planned analysis will be presented in the SAP. Any deviations from the SAP will be documented in the clinical study report. **Analysis Sets:** The following analysis sets are defined for this study: o The safety analysis set is the same as the intent-to-treat (ITT) analysis set, defined as all participants that have been randomized (where applicable) and inoculated. o The per protocol (PP) population is defined as all participants having received the planned dose of challenge agent who have a valid result for at least 80% of the planned qRT-PCR nasal and throat sample analyses from Day 1 (pm) up to Day 8 (am), i.e., at least 11 out of 14 samples, and who present no important protocol deviations likely to impact the evaluation of the primary endpoint. o Intent-to-treat infected (ITT-I) analysis set is defined as all participants that have been inoculated with any dose of challenge agent and who are infected with challenge agent as per the definition of laboratory-confirmed infection for this protocol.

	For the pathogenicity analysis, the PP analysis set will be the primary analysis population. The ITT and the ITT-I analysis sets will be used for supportive analyses on all or part of the pathogenicity endpoints, as defined in the SAP. The safety evaluation will be performed on the safety analysis set.

1.1. Study Schematic: On-study Participant Progression



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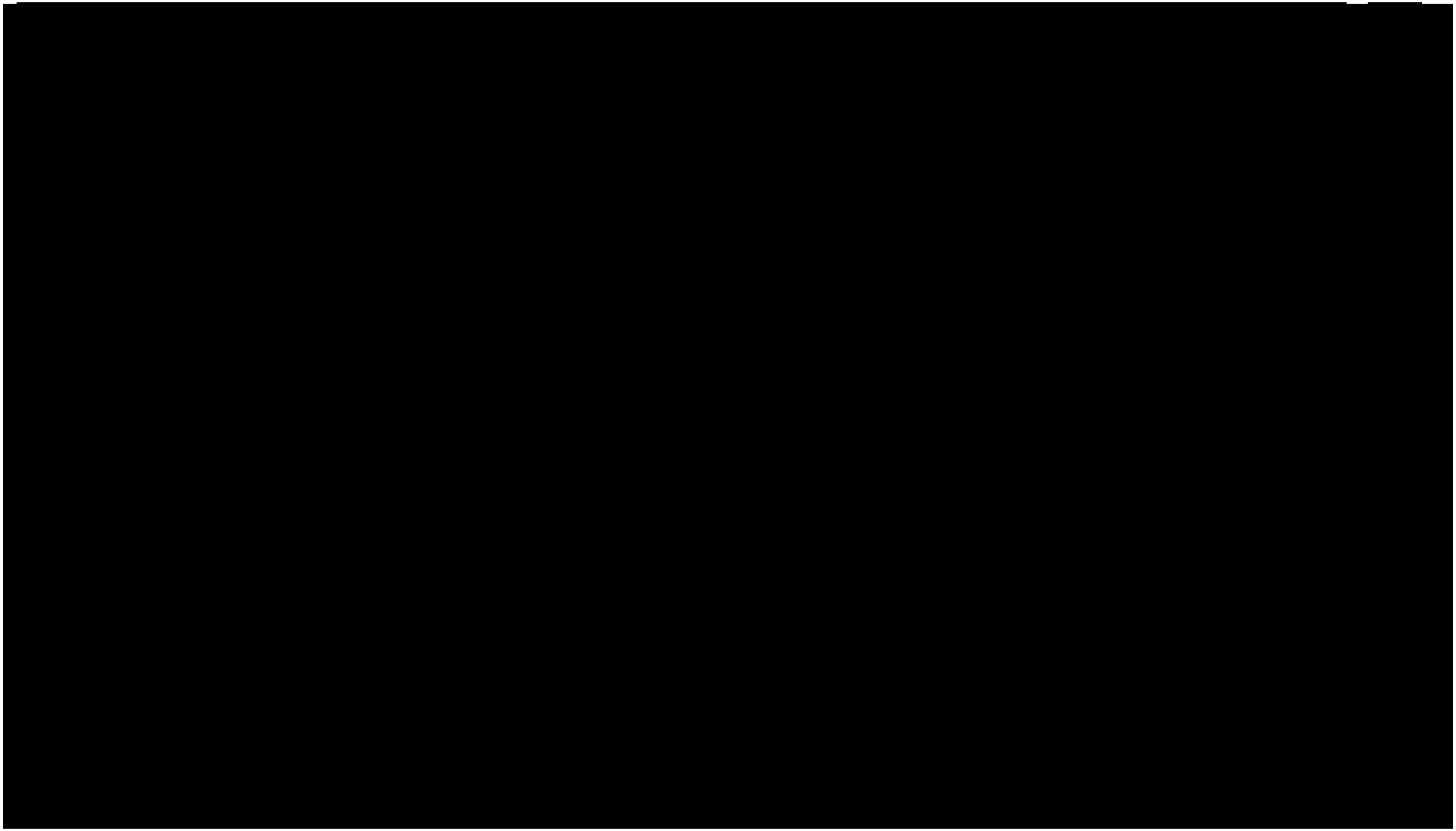
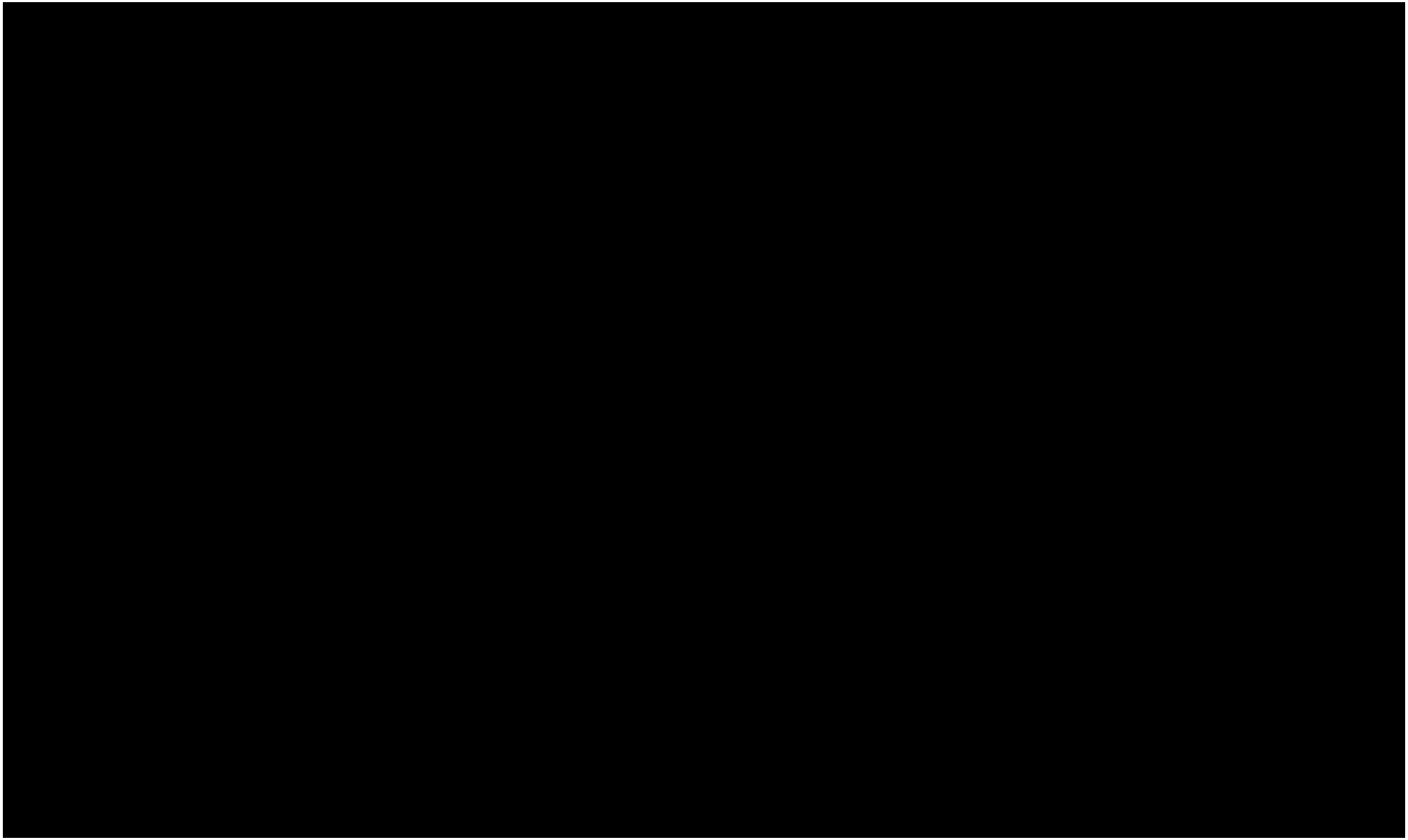
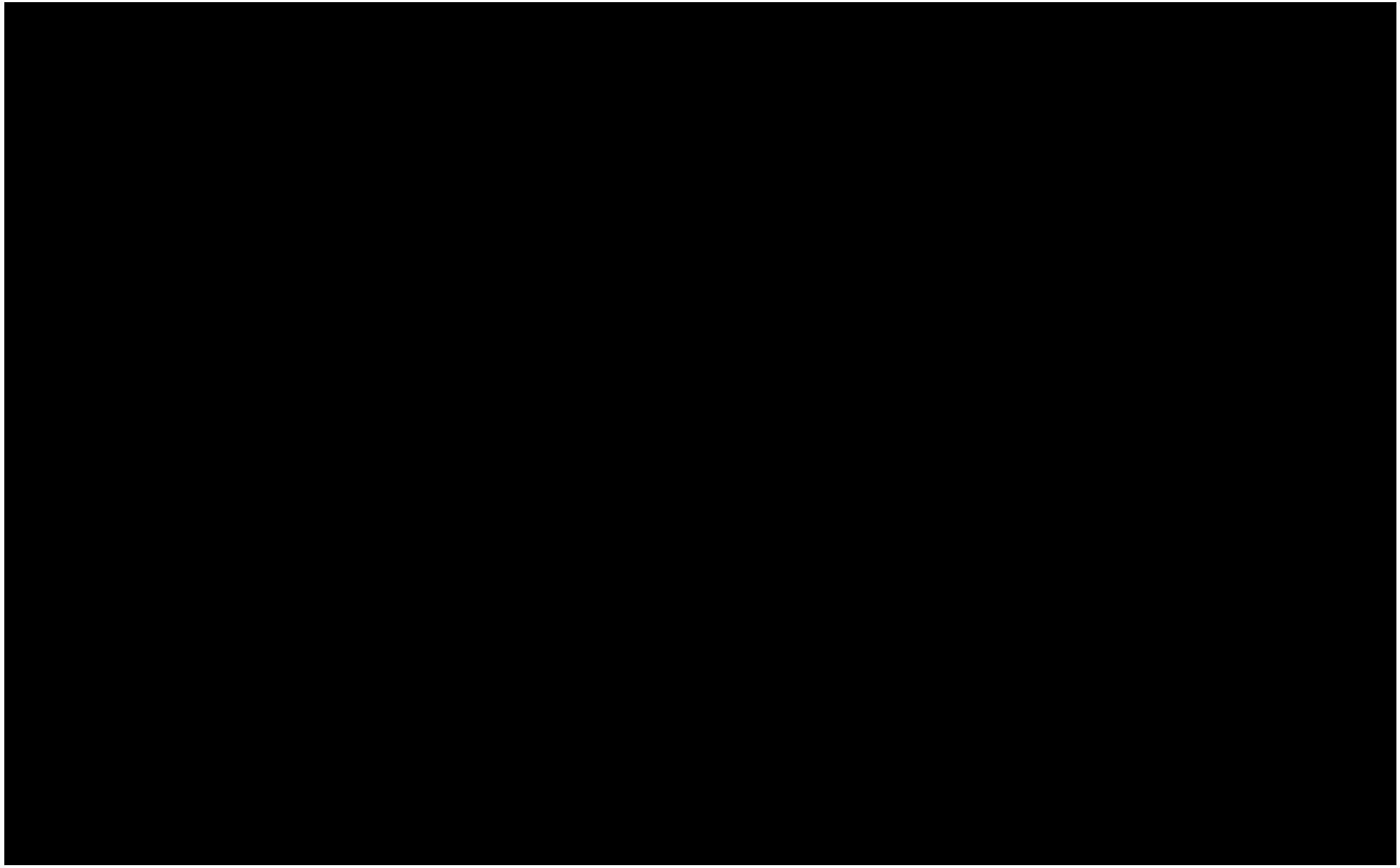
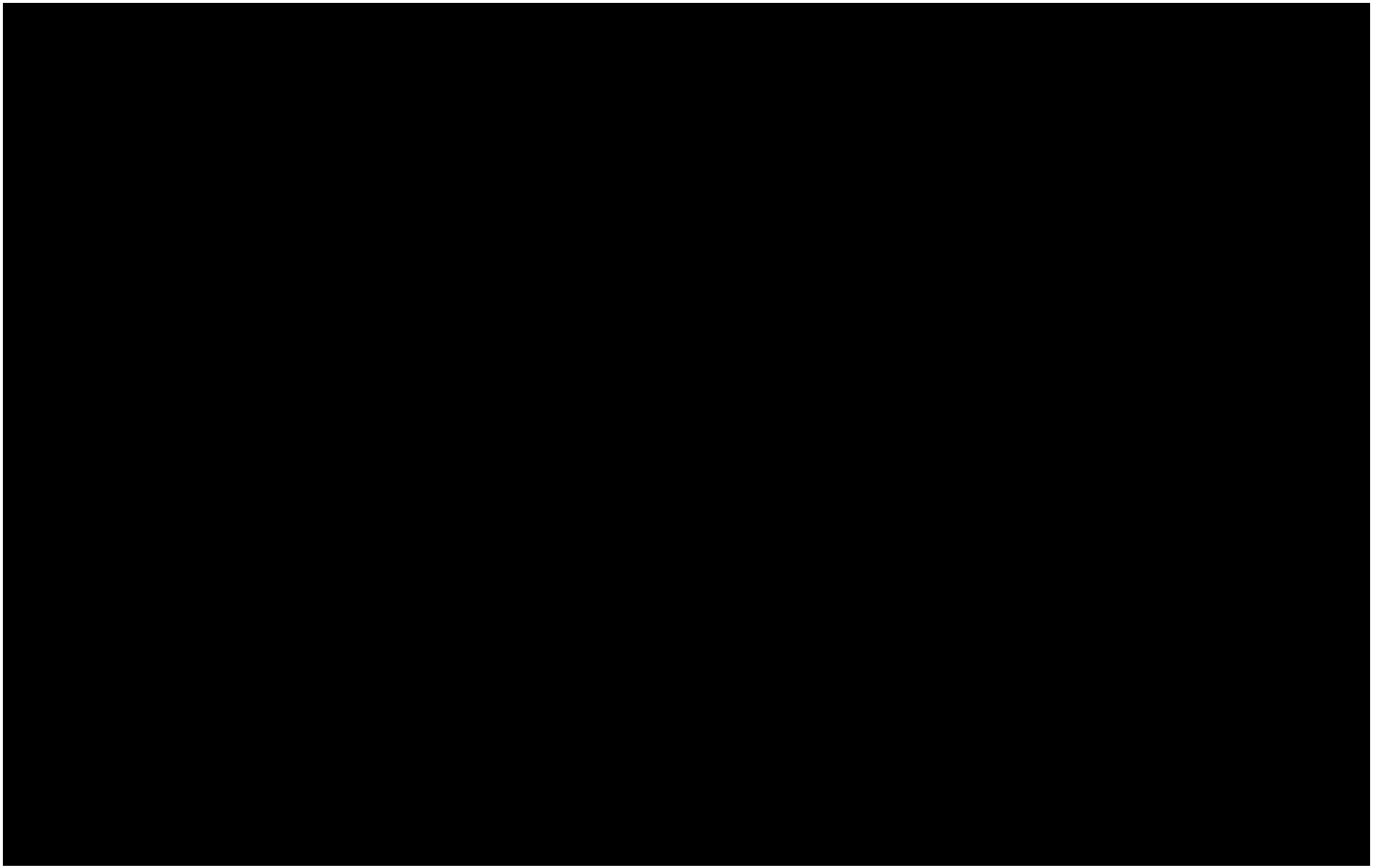
1.2. Schedule of Events









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KEY NOTES FOR SCHEDULE OF EVENTS

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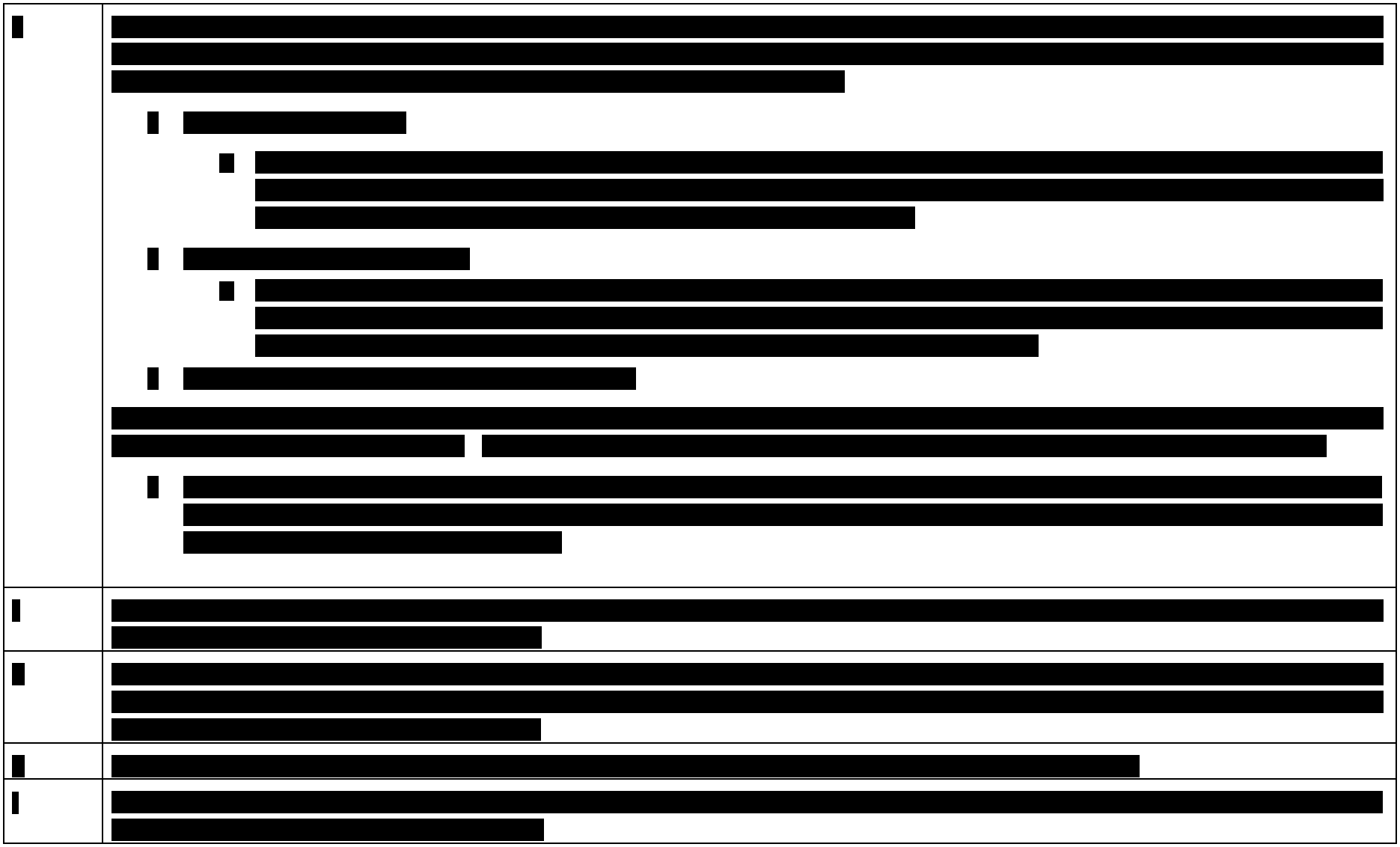

2. INTRODUCTION

2.1. Background

Influenza viruses are associated with significant human disease and cause annual epidemics during autumn and winter. Although most people recover within 1 to 2 weeks without requiring medical attention, seasonal influenza yearly results in approximately 3 to 5 million cases of severe illness and up to 500,000 deaths worldwide, particularly among the very young, elderly, and chronically ill. Influenza caused an estimated yearly average of 23,600 deaths between seasons 1976 and 2007 and more than 200,000 hospitalizations in the United States. Comparable mortality and morbidity rates have been reported for European countries. Currently, the influenza type A viruses H1N1 and H3N2 are circulating in humans, along with influenza type B viruses. H3N2 viruses have been predominant in most seasons and have caused a higher number of deaths and hospitalizations than H1N1 and influenza B viruses (Nunes et al, 2011; Thompson et al, 2004; Zucs et al, 2005).

The neuraminidase inhibitors zanamivir and oseltamivir are currently recommended for the treatment and/or (post-exposure) prophylaxis of influenza A and B virus infections (Fiore et al, 2011; Weinstock and Zuccotti, 2009). However, new drugs are now required as there have been reports of the emergence of resistance after oseltamivir treatment (Dharan et al, 2009; Poland et al, 2009; Stephenson et al, 2009) and the increasing use of zanamivir monotherapy may lead to the development of zanamivir resistance. Xofluza (baloxavir marboxil) was subsequently developed and is available in several countries for treatment and post-exposure prophylaxis for influenza A and B. However, there remains a need to develop better vaccines (demonstrating high efficacy, with longer or broader protection, or with faster manufacturing) as well as additional antivirals and immunomodulators to manage infection, transmission, and disease.

The influenza human challenge model was established to not only aid understanding of influenza disease and transmission, but to also assess the efficacy of antivirals, immunomodulators, and vaccines (as reviewed by Bueno de Mesquita et al, 2021; Lambkin-Williams et al, 2018; Nguyen-Van-Tam et al, 2020; Ramos-Sevillano et al, 2019; Yogaratnam et al, 2019). hVIVO has given different influenza strains to over 2,000 volunteers over the last 20 years. The various influenza challenge strains have been well tolerated with no challenge agent-related serious adverse events (SAEs) occurring in any of the participants inoculated to date. Furthermore, the influenza challenge viruses have been shown to induce measurable disease profiles with clear distinction from non-infected participants and study participants have approximately 60% to 75% chance of becoming infected following the administration of the viruses. Typical influenza illness is characterized by an abrupt onset of rhinitis, nasal stuffiness, fever, malaise, myalgia (muscle aches), and sore throat. In healthy adults the illness usually resolves without any treatment, with relief of symptoms occurring naturally within 3 to 5 days. The disease profiles of the challenge agents are consistent with the mild to moderate disease profiles expected with wild-type challenge viruses in healthy adult participants (Fragaszy et al, 2017).

Both influenza A and B types circulate seasonally, and both types contribute to the morbidity and mortality seen each year. This study is specifically intended to characterize a new, more recent influenza A H3N2 strain for further usage in a vaccine efficacy study. However, prior to the study, hVIVO will know the virus stock titer and based on this, will select the doses to be given to the participants. The doses planned in this study are based on hVIVO's experience with other H1N1 and H3N2 influenza challenge viruses and may be adjusted based on the final virus stock titer.

The known risks to participants in this study have been addressed in the study eligibility criteria, and qualified study site staff in the quarantine unit will manage any symptoms that develop during the study.

2.2. Study Rationale

The purpose of this exploratory study is to establish the dose, safety, and pathogenicity of a new influenza challenge strain in healthy participants 18 to 55 years of age.

2.3. Benefit/Risk Assessment

Healthy participants will not directly benefit from this clinical study. Some limited protection from related circulating influenza A strains may be imparted on those who seroconvert following viral challenge. Results from this clinical study may be useful in developing new approaches to controlling influenza A H3N2 virus-related infections and disease.

2.3.1. Risk Assessment

The known risks to participants are detailed below. However, there may also be risks that are unforeseen and unanticipated (e.g., unknown allergies). Every effort will be made to monitor the health of the participants to ensure that such risks are minimized. Trained medical staff and appropriate facilities will be available to provide medical emergency care.

Potential Risk of Clinical Significance	Description of Risk	Mitigation Strategy
Study Intervention		
Study Procedures		
Blood sampling	Pain or bruising at the site where blood is drawn.	Blood samples will be obtained by a trained professional.
	Syncope (fainting) can occur following or even before any blood draw as a psychogenic response to the needle insertion.	Blood samples will be obtained by a trained professional and procedures will be put in place to avoid injury from fainting.
	There is a possibility that, in the process of collecting blood, a nerve may be injured.	Procedure to be performed by qualified personnel.
	Blood tests performed to address the health of the participants at screening and during the study may indicate that a participant has an infection that they were not previously aware of (such as human immunodeficiency virus [HIV] or hepatitis) or an unexpected illness.	If the participant agrees, the hVIVO doctor will provide the participant's general practitioner (GP) or other appropriate doctor with a referral letter.
Nasal and throat sampling	Collection of respiratory (nasal) and throat samples may cause e.g., discomfort, sneezing, watery eyes, irritated nose, or nose bleeding.	Sample collection will be performed by appropriately qualified and trained study staff to minimize the discomfort.

Potential Risk of Clinical Significance	Description of Risk	Mitigation Strategy
Influenza A/England/7763/2022 H3N2 Infection from Inoculation		
Influenza A/England/7763/2022 H3N2 infection & severe complications	There is a chance of becoming infected with influenza A/England/7763/2022 H3N2 virus. One of the objectives of this study is to establish this infectivity rate. Typical influenza illness: abrupt onset of rhinitis, nasal stuffiness, fever, malaise, myalgia (muscle aches), and sore throat. Severe complications are not expected as these tend to occur almost exclusively in infants, the elderly, and persons of any age with chronic comorbidities and significant immune compromise and not in young, healthy cohorts with no comorbidities or coinfections. Influenza virus, like many viruses, can cause more substantial health issues such as myocarditis (inflammation or damage to the heart muscle). However, the chance of this resulting in serious or permanent changes is rare, as most cases are minor and resolve without any lasting changes.	Characterizing the safety profile of the influenza A/England/7763/2022 H3N2 virus is an objective of this study. However, influenza infection in healthy adults is usually mild and resolves without treatment within around 7 days (Woods et al, 2013; Wilkinson et al, 2012; Zaas et al, 2009; Florman et al, 1946). Strict inclusion and exclusion criteria will apply to ensure only healthy adults are enrolled in this study. There will be daily medical monitoring in a quarantine unit for at least 8 days post viral challenge. Qualified medical and nursing staff in the quarantine unit will monitor for and manage any symptoms.
Transmission of influenza A/England/7763/2022 H3N2 to participants' close contacts	The presence in nasal secretions can cause infection in close contacts.	Virus is usually absent from the nose by the time participants are discharged from quarantine. This will be confirmed by testing a nasal swab sample by using a qualitative virus antigen test (influenza discharge test [e.g., rapid viral antigen test {RVAT}]) or PCR to determine participants' suitability for discharge.

Potential Risk of Clinical Significance	Description of Risk	Mitigation Strategy
		In addition, participants will be instructed to avoid close contact with vulnerable individuals as described in Section 2.3.1.1, Vulnerable Persons, for 2 weeks after they leave the quarantine unit.
Risk of reactivation of herpes infection	If a participant ever had a herpes infection (e.g., cold sores, genital herpes, or shingles) there is a low possibility that this infection could return after viral challenge.	Participants will be instructed to inform the study staff if they currently have an active herpes infection or have had one during the 30 days before enrollment. If a participant develops any cold sore, herpes, or shingles they may be treated symptomatically while at the quarantine unit. If it continues, they will be followed up until resolved or, if necessary and dependent on medical history, will be referred to their GP or any specific department at hospital, as required.

2.3.1.1. Vulnerable Persons

For the purposes of possible contact after leaving the quarantine unit, the participant should avoid close contact with vulnerable individuals for 2 weeks after they leave the quarantine unit. A vulnerable individual is a person including but not limited to:

- Persons ≥65 years of age.
- Children ≤2 years of age.
- Residents of nursing homes.
- Women who are pregnant or who are trying to become pregnant.
- Persons of any age with significant chronic medical conditions such as:

[redacted]

2.3.1.2. Risk Associated With Coronavirus Disease 2019 Pandemic

hVIVO has implemented enhanced infection control measures during the pandemic to minimize risks of COVID-19 infection.

Risk of Increased Severity of COVID-19 Infection if Contracted After Challenge Agent Inoculation:

[redacted]

2.3.2. Benefit Assessment

Healthy participants will not receive direct benefit from treatment during their participation in this clinical study.

Participants may develop some immunity to influenza A/England/7763/2022 H3N2 challenge agent and benefit from a general health check at screening. Benefit may also be derived from the medical evaluations and assessments associated with study procedures. In addition,

participants are contributing to the process of developing new therapies in an area of unmet medical need.

2.3.3. Overall Benefit/Risk Conclusion

Considering the measures taken to minimize risk to participants in this study, the potential risks identified in association with influenza A/England/7763/2022 H3N2 virus infection are justified by the anticipated benefits linked to characterization of a new influenza challenge strain for future evaluation of the efficacy of novel vaccines and therapies in an influenza challenge model.

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
Pathogenicity & Safety	
o To determine the safe and optimal titer of the influenza challenge agent.	To identify a safe and infectious dose of wild-type influenza virus in healthy participants, suitable for future intervention studies that: o Has an acceptable safety profile as measured by: o Occurrence of adverse events (AEs) related to the viral challenge from viral challenge (Day 0) to the Day 28 (±3 days) follow-up visit. o Occurrence of SAEs related to the viral challenge from viral challenge (Day 0) to the Day 28 (±3 days) follow-up visit. o Induces laboratory-confirmed infection in ≥40% of inoculated participants (ideally between 50% and 80%). Laboratory-confirmed infection is defined as: o Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)-confirmed influenza infection, defined as 2 quantifiable (≥ lower limit of quantification [LLOQ]) qRT-PCR measurements (reported over 4 planned consecutive assessments within 48 hours), from Day 1 pm to planned discharge from quarantine (Day 8 am).
Secondary	
Pathogenicity	
o To evaluate the incidence of viral culture-confirmed influenza infection.	o Viral culture-confirmed influenza infection, defined as a quantifiable viral culture measurement, from Day 1 pm to planned discharge from quarantine (Day 8 am).
o To evaluate the optimal titer of the influenza challenge agent, as measured by:	The following endpoints will be determined by both qRT-PCR and viral culture on nasal and throat samples: o Area under the viral load-time curve (VL-AUC) of the influenza challenge agent,

Objectives	Endpoints
o Viral load-related endpoints.	from Day 1 pm to planned discharge from quarantine (Day 8 am). o Peak viral load (VLPEAK) of the influenza challenge agent, defined as the maximum viral load, from Day 1 pm to planned discharge from quarantine (Day 8 am).
o To explore the optimal titer of the influenza challenge agent, as measured by: o Clinical symptom-related endpoints.	o Area under the total clinical symptom score-time curve (TSS-AUC) as measured by graded symptom scoring system collected 3 times daily from Day 1 am to planned discharge from quarantine (Day 8 am). o Peak daily symptom score: individual maximum daily sum of symptom score from Day 1 (am) to planned discharge from quarantine (Day 8 am). o Peak symptom diary card score: peak total symptom score (TSS) as measured by graded symptom scoring system collected 3 times daily from Day 1 am to planned discharge from quarantine (Day 8 am).
o To explore the optimal titer of the influenza challenge agent, as measured by the incidence of influenza-like illness (ILI) and laboratory-confirmed symptomatic influenza infection.	o Laboratory-confirmed febrile influenza infection, defined as: o Laboratory-confirmed infection, AND o A febrile episode, defined as a temperature of ≥37.9°C, from Day 1 am to planned discharge from quarantine (Day 8 am). o qRT-PCR-confirmed ILI (Centers for Disease and Control [CDC]), defined as: o Laboratory-confirmed infection, AND o A febrile episode, defined as a temperature of ≥37.8ºC, from Day 1 am to planned discharge from quarantine (Day 8 am), AND o Cough and/or sore throat (symptoms and signs), from Day 1 am to planned discharge from quarantine (Day 8 am), in the absence of a known cause other than influenza. o qRT-PCR-confirmed ILI (World Health Organization [WHO]), defined as: o Laboratory-confirmed infection, AND

Objectives	Endpoints
	o A febrile episode, defined as a temperature of ≥38.0ºC, from Day 1 am to planned discharge from quarantine (Day 8 am), AND o Cough, from Day 1 am to planned discharge from quarantine (Day 8 am). o Laboratory-confirmed moderately severe symptomatic influenza infection, defined as: o Laboratory-confirmed infection, AND o Any symptoms from the symptom diary card of grade ≥2 at a single time point, from Day 1 am to planned discharge from quarantine (Day 8 am). o Laboratory-confirmed symptomatic influenza infection, defined as: o Laboratory-confirmed infection, AND o TSS ≥2 at a single time point, from Day 1 am to planned discharge from quarantine (Day 8 am). o Viral culture laboratory-confirmed moderately severe symptomatic influenza infection, defined as: o Viral culture laboratory-confirmed infection, AND o Any symptoms from the symptom diary card of grade ≥2 at a single time point, from Day 1 am to planned discharge from quarantine (Day 8 am). Further analyses may be performed on the above endpoints, using modified start or end times. Details will be provided in the statistical analysis plan (SAP). Further analyses may be performed on the above qRT-PCR-related endpoints where detection by qRT-PCR is reported above the lower limit of detection (LLOD) instead of the LLOQ. Details will be provided in the SAP.
Exploratory*	
o To further explore the pathogenicity phenotype of wild-type influenza in healthy participants, as measured by:	o Safety laboratory tests, o lung function, o electrocardiograms [ECGs], o physical examinations, and concomitant medications.

Objectives	Endpoints
o Safety assessments.	
o To further explore the pathogenicity phenotype of influenza challenge agent, as measured by: o Nasal discharge endpoints.	o Total weight of mucus produced from Day 1 to planned discharge from quarantine (Day 8 am). o Total number of tissues used by participant from Day 1 to planned discharge from quarantine (Day 8 am).
o To further explore the pathogenicity phenotype of the influenza challenge agent, as measured by: o Viral load-related endpoints.	The following endpoints may be determined by both qRT-PCR and viral culture on nasal and throat samples: o Duration of quantifiable influenza, from Day 1 pm to planned discharge from quarantine (Day 8 am). Duration is defined as the time (hours) from first quantifiable (≥LLOQ) until first confirmed unquantifiable (<LLOQ) assessment after their peak measure (after which no further virus is quantified). o Time to resolution from VLPEAK. Time to resolution is defined as the time (hours) from VLPEAK until first confirmed unquantifiable (<LLOQ) assessment (after which no further virus is quantified). o Time to resolution from first quantifiable (≥LLOQ) assessment. Time to resolution is defined as the time (hours) from first quantifiable (≥LLOQ) assessment until first confirmed unquantifiable (<LLOQ) assessment (after which no further virus is quantified). o Slope of the viral load over time (clearance rate), from VLPEAK to 4 days after VLPEAK.
o To further explore the pathogenicity phenotype of the influenza challenge agent, as measured by:	o Duration (hours) of grade ≥2 clinical symptoms from Day 1 am to planned discharge from quarantine (Day 8 am). o Time to resolution of symptoms. Time to resolution is defined as the time (hours) from first occurrence of TSS above baseline until TSS returns to baseline or

Objectives	Endpoints
o Clinical symptom-related endpoints.	below (after which no further increase above baseline is observed). o Time to resolution from peak clinical symptoms as measured by graded symptom scoring system collected 3 times daily. Time to resolution is defined as the time (hours) from peak clinical symptoms until TSS returns to baseline or below (after which no further increase above baseline is observed). o Time to resolution of grade ≥2 symptoms. Time to resolution is defined as the time (hours) from first occurrence of any grade ≥2 symptom to occurrence of no grade ≥2 symptoms (after which no further grade ≥2 symptoms occur).
o To explore the incidence of symptomatic infection after influenza viral challenge.	o Clinical symptom-related endpoints may be further explored, as measured with either the full 13 symptoms within the graded symptom scoring system, a subset of these 13 symptoms, or including additional symptoms. o Patient perception questionnaire results (presence or absence of cold, change in severity of cold) may be compared to other virological and symptomatic endpoints in relation to respiratory viral disease including, but not limited to, construct validations.
o To explore immunity to influenza at baseline and follow-up.	o Baseline, and change from baseline of antibodies (hemagglutination inhibition [HAI], neutralization) after viral challenge. o Seroconversion, defined as follows: o HAI: If the pre-challenge serum is <10 (undetectable), the post-challenge serum must have a titer ≥40; if the pre-challenge serum is ≥10, at least a 4-fold titer increase is required. o Foci reduction neutralization assay (FRNA): at least a 4-fold titer increase is required. o Development of antibodies against the virus may be evaluated in serum samples
o To explore baseline	o Blood and respiratory samples may be used for exploratory assays related to respiratory

Objectives	Endpoints
immunology and response to infection with influenza.	viral infection, microbiology, genetics, and immunology.
o To explore the incidence and magnitude of influenza virus particles from exhaled breath after virus challenge	o Air samplers and sampling masks to collect exhaled breath samples to be used for exploratory assays to for viral load assessments related to transmission potential of influenza virus

* Note that exploratory objectives and endpoints are optional and might be assessed only if needed; therefore, not all testing might be performed and reported.

4. STUDY DESIGN

4.1. Overview of Study Design

This is a single-center, randomized, single-blind, proof-of-concept study in healthy adult male and female participants 18 to 55 years of age, inclusive, utilizing:

- Intranasally administered influenza A H3N2 challenge agent.

A total of up to 80 participants may be given the influenza A/England/7763/2022 H3N2 challenge agent.

In Part A of the study, up to 40 participants will be randomized and enrolled (up to 20 participants in Dose Arm 1 [medium dose] and up to 20 participants in Dose Arm 2 [high dose]. Further to the outcome and analysis of inoculations in Part A, additional participants may be enrolled into an optional Part B to:

- Expand the number of participants in one of the Part A dose arms, AND/OR
- Inoculate different challenge agent titers in optional dose arms.

Both parts of the study will be single-blinded; participants will not know the challenge agent dose level (or relative dose level [i.e., higher or lower than other participants]) that they receive.

The primary goal of this exploratory study is to establish the optimal dose of the influenza challenge agent that is safe and infectious in healthy adults for use in subsequent intervention studies.

The expected duration of study participation for a participant is 4 months, with the following sequence and duration of study phases:

- **Screening phase:** From Day -90 to Day -2/-1.
  Historical generic screening data collected through the hVIVO generic screening process may be transferred to this study after the study-specific informed consent form (ICF) has been signed by the participant.
    - **Inpatient phase:** Participants will be resident in the quarantine unit for approximately 11 days (from Day -2/-1 to Day 8). Procedures will include:
  - **Pre-human viral challenge (HVC)**:
    - Quarantine admission on Day -2/-1.
    - (Baseline) assessments, randomization (where applicable), and eligibility checks will be conducted as per Schedule of Events (SoE) up to Day 0, pre-challenge.
  - **HVC**:
    - Influenza challenge agent inoculation on Day 0

      Following challenge agent inoculation, participants will be closely observed specifically for potential allergic reactions and any AEs for the following 24 hours. Post inoculation, participants will remain under observation for 30 minutes. Participants will continue to be monitored throughout the clinical phase of the study.
  - **Post-HVC**:
    - Day 1 onwards and each day – study assessments will be conducted as per SoE.
    - Participants will be discharged from the quarantine unit on Day 8 [redacted]

- **Outpatient phase**:
- Final follow-up visit: Day 28 (±3 days).

The Study Schematic, showing participant progression through the study, is presented in Section 1.1, Study Schematic. The SoE is presented in Section 1.2, Schedule of Events.

4.2. Scientific Rationale for Study Design

The study will be conducted by hVIVO Services Limited, which has extensive experience with influenza challenge studies. Numerous studies have been performed using experimental influenza infection in human participants. To date, in hVIVO's studies, over 2,000 participants have been successfully and safely inoculated with influenza. These studies demonstrated that adults could be infected by nasal inoculation and that experimental infection was safe. Influenza challenge strains have been shown to cause symptoms and virus shedding that closely match natural infection. In this study a new influenza challenge strain will be used; influenza A/England/7763/2022 H3N2 virus.

Challenge with influenza will take place in hVIVO's specialized quarantine unit. Standard study procedures (including collection of blood, urine, and nasopharyngeal secretions for assessment of safety and pathogenicity) have been employed in previous studies conducted by hVIVO.

Randomization and Blinding

Randomization and participant blinding will be applied in Part A (and in Part B, if more than one dose arm is enrolled) of the study.

Randomization will be used to prevent bias in the assignment of participants to dose arms, to increase the likelihood that known and unknown participant characteristics (e.g., demographic and baseline characteristics) are equally balanced across dose arms, and to enhance the validity of statistical comparisons across dose arms.

Blinding will prevent the occurrence of conscious and unconscious bias in the conduct and interpretation of the study.

Together, the randomized, and blinded features of the study will allow a causal interpretation of the study results.

4.3. Justification for Dose

An inoculum titer of approximately $10^{4.5}$ tissue culture infective dose 50% ($TCID_{50}$)/mL to $10^{5.5}$ $TCID_{50}$/mL of the influenza A/England/7763/2022 H3N2 challenge agent is expected to cause disease profiles that are consistent with the mild to moderate disease profiles expected with wild-type challenge viruses in healthy adult participants. The objective of this study is to confirm the optimal dose that is both safe and induces suitable infection rates in healthy adults.

4.4. End-of-Study Definition

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

The end of the study is defined as the date of the last visit of the last participant in the study.

5. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1. Inclusion Criteria

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

No.	Inclusion Criteria
1.	Written study-specific informed consent signed and dated by the participant and the PI/investigator obtained before any study-specific assessment is performed.
2.	Aged between 18 and 55 years old, inclusive, on the day prior to signing the consent form.
3.	A total body weight ≥50 kg and body mass index (BMI) ≥18 kg/m^2 and ≤35kg/m^2.
4.	In good health with no history, or current evidence, of clinically significant medical conditions, [redacted]
5.	Participants will have a documented medical history either prior to entering the study or following medical history review with the study physician at screening.
6.	The following criteria are applicable to female participants participating in the study: a) Females of childbearing potential must have a negative pregnancy test prior to enrollment. b) Females of non-childbearing potential: a. Postmenopausal females [redacted] b. Documented status as being permanently sterile [redacted]

7.	<u>The following criteria apply to female participants:</u> Female participants of **childbearing potential** must use **one form** of highly effective contraception (birth control). [redacted] <u>The following criteria applies to male participants:</u> Male participants must agree to use at least one of the contraceptive requirements [redacted]

No.	Inclusion Criteria
	[redacted] d) In addition to the contraceptive requirements above, male participants must agree not to donate sperm [redacted]
8.	Serosuitable for the challenge agent: • The serology result obtained from the influenza A H3N2 virus antibody assay suggests that the participant is sensitive to influenza infection (i.e., they are likely to be infected following inoculation with the challenge agent).

5.2. Exclusion Criteria

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

No.	Exclusion Criteria
Medical History	
1.	History of, or currently active, symptoms or signs suggestive of upper or lower respiratory tract infection within 4 weeks prior to the first study visit (i.e., quarantine admission).
2.	Any history or evidence of any clinically significant or currently active cardiovascular, respiratory, dermatological, gastrointestinal, endocrinological, hematological, hepatic, immunological (including immunosuppression), metabolic, urological, renal, neurological, or psychiatric disease and/or other major disease that, in the opinion of the PI/investigator, may interfere with a participant completing the study and necessary investigations. [redacted] [redacted] [redacted]

No.	Exclusion Criteria
	[redacted]
3.	Any participants who have smoked ≥10 pack years at any time (10 pack years is equivalent to one pack of 20 cigarettes a day for 10 years), including any calculable or self-reported significant vaping history.
4.	Females who: a) Are breastfeeding, or b) Have been pregnant [redacted] or c) Have a positive pregnancy test at any point during screening or prior to viral challenge.
5.	Lifetime history of anaphylaxis and/or a lifetime history of severe allergic reaction. [redacted]
6.	Venous access deemed inadequate [redacted]
7.	a) Any significant abnormality altering the anatomy of the nose or throat in a substantial way or nasopharynx or throat that may interfere with the aims of the study and, in particular, any of the nasal assessments or viral challenge, (historical nasal polyps can be included, but large nasal polyps

No.	Exclusion Criteria
	causing current and significant symptoms and/or requiring regular treatments in the last month will be excluded). b) Any clinically significant history of epistaxis (large nosebleeds) within the last 3 months of the first study visit (i.e., quarantine admission) and/or history of being hospitalized due to epistaxis on any previous occasion. c) Any nasal or sinus surgery within 3 months of the first study visit (i.e., quarantine admission).
Prior or Concomitant Medications and Assessments	
8.	a) Evidence of vaccinations within the 4 weeks prior to the planned date of viral challenge. b) Intention to receive any vaccination(s) before the last day of follow-up (NB. no travel restrictions will apply after the Day 28 (±3 days) follow-up visit).
9.	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.
10.	a) Receipt of any investigational medicinal product (IMP) within 3 months or 5 half-lives, whichever is longer, of the IMP used in the other study prior to the planned date of viral challenge. b) Receipt of 3 or more IMPs within the previous 12 months prior to the planned date of viral challenge. c) Receipt of influenza vaccine (or an IMP related to the treatment or prophylaxis of influenza) in the last 6 months prior to the planned date of viral challenge OR a diagnosis of influenza or ILI confirmed by a physician within the last 2 months prior to screening. d) Prior inoculation with a virus from the same virus subtype as the challenge agent. e) Prior inoculation with a virus from the same virus family as the challenge virus in the last 12 months. f) Prior participation in another HVC study with a respiratory virus in the preceding 3 months, taken from the date of viral challenge in the previous study to the date of expected viral challenge in this study.
11.	Use or anticipated use during the conduct of the study of concomitant medications (prescription and/or non-prescription), including vitamins or herbal and dietary supplements within the specified windows, unless in the opinion of the study physician or PI/investigator, the medication will not interfere with the study

No.	Exclusion Criteria
	procedures or compromise participant safety. Specifically, the following are excluded: a) Herbal supplements within 7 days prior to the planned date of viral challenge. b) Any medication or product (prescription or over the counter) for symptoms of nasal congestion within 7 days prior to the planned date of viral challenge. c) Short- and long-acting antihistamines within 7 days prior to the planned date of viral challenge. d) Chronically used medications, vitamins, or dietary supplements, including any medications known to be potent inducers or inhibitors of cytochrome P450 (CYP) enzymes, within 21 days 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 has exceeded the maximum permissible 24-hour dose (e.g., ≥4 g paracetamol per day) over the preceding week. f) Systemic (oral and parenteral) antiviral administration within 4 weeks of viral challenge.
12.	a) Confirmed positive test for drugs of misuse on first study visit (i.e., quarantine admission). [redacted] b) Recent history or presence of alcohol addiction, or excessive use of alcohol (weekly intake in excess of 28 units alcohol; 1 unit being a half glass of beer, a small glass of wine or a measure of spirits). c) Excessive consumption of xanthine containing substances (e.g., daily intake in excess of 5 cups of caffeinated drinks, e.g., coffee, tea, cola). d) Presence of significant signs and symptoms of nicotine withdrawal on first study visit (i.e., quarantine admission). Cotinine testing may be performed at the PI/investigator's discretion.
13.	A forced expiratory volume in 1 second (FEV_1) [redacted]
14.	Positive HIV, hepatitis B virus, or hepatitis C virus test.
15.	Presence of fever, [redacted] on Day -2, Day -1, [redacted]
Other	
16.	Those employed or immediate relatives of those employed at hVIVO.
17.	a) Any other medical, psychiatric, social, or occupational condition and/or responsibility that, in the opinion of the PI/investigator, would interfere

No.	Exclusion Criteria
	with or serve as a contraindication to protocol adherence or the assessment of safety (including reactogenicity) will deem the participant unsuitable for the study. b) Any other reason that in the opinion of the PI/investigator raises a concern that the participant will not be able to cope with quarantine requirements.

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

[redacted]

5.3.2. Caffeine, Alcohol, and Tobacco

[redacted]

5.3.3. Activity

Participants must refrain from strenuous exercise [redacted]

5.3.4. Other Restrictions

Participants will be instructed to avoid close contact with vulnerable people as described in Section 2.3.1.1, Vulnerable Persons, for 2 weeks after they leave the quarantine unit.

Participants will be advised to avoid all but essential travel until the Day 28 (±3 days) follow-up visit.

5.4. Screen Failures

[redacted]

[redacted]

6. STUDY INTERVENTIONS AND CONCOMITANT THERAPY

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

6.1. Study Intervention(s) Administered

Study interventions administered to participants are described in Table 6-1.

Table 6-1: Study Interventions

Intervention Name	**Influenza A/England/7763/2022 H3N2 virus**
Type	Influenza virus
Formulation	Capped cryovial, liquid
Unit Dose Strength(s)	The challenge agent titer is determined in an infectivity assay. The doses are: **Part A** o Dose Arm 1: Medium dose, expected to be approximately $10^{4.5}$ tissue culture infective dose 50% ($TCID_{50}$)/mL (titer may be adjusted based on stock titer) o Dose Arm 2: High dose, expected to be approximately $10^{5.5}$ $TCID_{50}$/mL (titer may be adjusted based on stock titer) **Part B** o Expansion Dose: Expansion of one of the Part A dose arms, to be determined (TBD), depending on outcome of Part A, AND/OR o Optional Dose Arm 3: TBD, depending on outcome of Part A
Dosage Level(s)	A single dose of challenge agent will be delivered.
Route of Administration	Intranasal
Use	Infectious challenge agent
Sourcing	Provided centrally by hVIVO
Packaging and Labeling	Influenza A/England/7763/2022 H3N2 virus will be provided in vials. The details of the challenge agent provision will be provided in the analytical plan (AP).

Intervention Name	**Influenza A/England/7763/2022 H3N2 virus**
Current/Former Name(s) or Alias(es)	Not applicable

6.2. Challenge Agent

6.2.1. Preparation/Handling

The challenge agent used in this study is influenza A/England/7763/2022 H3N2 virus.

The challenge agent stock was manufactured under current Good Manufacturing Practice. The challenge agent stock has undergone quality testing performed during manufacturing (identity, appearance, sterility, infectivity, and contaminants) according to pre-determined specifications, and has subsequently also passed an extensive panel of adventitious agent testing.

Each participant will be allocated a unique vial containing the challenge agent and will receive the inoculum intranasally. The inoculum will be prepared and/or provided according to the hVIVO AP and administered in accordance with hVIVO standard operating procedures (SOPs). The time from the challenge agent inoculum thawing to challenge agent administration should be no longer than 4 hours (on wet ice).

Only participants who signed the study-specific informed consent may receive challenge agent and only authorized investigator site staff may supply or administer challenge agent.

All administrations will be made by a member of the study staff and witnessed by a second study staff member. The exact time of challenge agent inoculation will be recorded in the administration log. Accurate records will be kept of when and how much inoculum is prepared and used. The oversight process will be signed off prior to administration of the challenge agent. Any noncompliance or problems with the inoculation will be recorded in the participant's source notes and reported to the PI/investigator.

6.2.2. Accountability/Storage

The PI/investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all challenge agent received and that any discrepancies are reported and resolved before use of the challenge agent.

The challenge agent is stored in a secure -80°C freezer (normal temperature range -60°C to -90°C). All challenge agent must be stored in a secure, environmentally controlled area, and monitored (manual or automated) in accordance with the labeled storage conditions with access limited to the PI/investigator and authorized investigator site staff.

The PI/investigator, institution, or the head of the medical institution (where applicable) is responsible for challenge agent accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study interventions are provided in accordance with hVIVO SOPs.

6.3. Randomization and Blinding

6.3.1. Randomization

Randomization will be applied to Part A of the study and to Part B, if more than one dose arm is enrolled in Part B.

[redacted] In Part A, the randomization number encodes the participant's assignment to 1 of 2 dose arms (2 different doses of the challenge agent) in a 1:1 ratio. If Part B is randomized, the ratio of randomization will depend on the sample sizes.

A designated, unblinded statistician, separate from the conduct or analysis of the study, will be responsible for preparation of the computer-generated randomization code list(s) using SAS.

Randomization numbers will be assigned sequentially in ascending order; and once assigned, that randomization number shall not be reassigned. The study site will keep a log of the randomization number assigned to each participant.

Randomization numbers will follow a specific format, a 3-digit format, e.g., [001]. If participants are replaced as per Section 7.4, Participant Replacement Strategy, the replacement participant will be assigned a new, unique randomization number equaling the randomization number of the replaced participant plus 100, e.g. [101]. This will ensure that the replacement participant receives the same allocated blinded dose as the participant who is being replaced.

6.3.2. Blinding

Both parts will be single-blind; participants will not know the challenge agent dose level (or relative dose level [i.e., higher or lower than other participants]) that they receive.

A limited number of staff designated to prepare the inoculum vials will know the virus titer on the inoculum vials in order to apply the randomization code. The participant will not know which virus titer the participant has been randomized to receive in Part A (and Part B, if applicable). Although this is a single-blind study, the investigator staff performing subjective assessments (e.g., directed physical examination) may also be blinded to which virus titer the participant is randomized to in Part A (and Part B, if Part B is randomized). In Part B, if Part B is not randomized, the virus titer will be known by the staff designated to prepare the inoculum vials and investigator staff performing the assessments.

6.4. Study Intervention Compliance

Participants will receive challenge agent directly from the PI/investigator or a delegated member of the investigator site staff. The date and time of each inoculation at the study site will be recorded in the source documents and recorded in the electronic case report form (eCRF). The dose of challenge agent and study participant identification will be confirmed at the time of dosing by a member of the PI/investigator site staff other than the person administering the challenge agent.

Any noncompliance or problems with the administration of the challenge agent will be recorded in the participant's source notes and reported to the sponsor, if appropriate.

6.5. Concomitant Therapy

Any medications taken and changes in medications will be recorded in the source data from the time of the participant signing the study-specific ICF up to final study contact Day 28 (±3 days). Any medication (including over the counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the quarantine/outpatient stage will be stored, prescribed, and administered in line with their label-specific requirements, and recorded according to the parameters required by the clinical database.

Participants will be reminded to refrain from using any over the counter medication without the approval of the PI/investigator and must notify the study staff as soon as possible if they are prescribed any medication. All medications other than contraceptives must be stopped prior to the planned date of viral challenge unless in the opinion of the PI/investigator, the medication will not interfere with the study procedures or compromise participant safety.

Medications prohibited throughout the study are shown in Table 6-2.

Table 6-2: Prohibited Medication (Healthy Participants)

Prohibited Medication	Washout
Systemic (oral and parenteral) antiviral drugs.	[redacted]
Use or anticipated use during conduct of the study of concomitant medications (prescription and non-prescription), including vitamins or herbal and dietary supplements, unless in the opinion of the PI/investigator the medication will not interfere with the study procedures or compromise participant safety.	[redacted]
Any IMP used in another study.	[redacted]

Prohibited Medication	Washout
	[redacted]
Influenza vaccine (or an IMP related to the treatment or prophylaxis of influenza)	[redacted]
Vaccinations	[redacted]

Any concomitant medication required for the participant's welfare may be given by the PI/investigator. However, it is the responsibility of the PI/investigator to ensure that details regarding the medication and the reason for its use are recorded appropriately in the source notes to permit their transfer to the clinical database.

[redacted]

Table 6-3: Permitted Medication

Permitted Medication	Time Period
Paracetamol	[redacted]
Oral contraceptives	Allowed at any time during the study.
[redacted] If, e.g., in an outbreak or pandemic, a newly instated national vaccination program is applicable to an individual participant, the PI/investigator and sponsor will discuss on an individual basis if concomitant vaccination may be allowed, viral challenge be postponed, or the participant be withdrawn from the study.	

6.6. Dose Modification

This is not applicable in this study.

6.7. Treatment of Overdose

This is not applicable in this study.

7. PARTICIPANT WITHDRAWAL / DISCONTINUATION / DISCONTINUATION OF STUDY INTERVENTION

7.1. Participant Withdrawal

[redacted]

7.2. Participant Discontinuation

[redacted]

[redacted]

These participants should continue to be followed for safety. Additional unscheduled assessments or unscheduled visits may be performed for safety reasons. If the participant is not withdrawing consent, assessments as per the early withdrawal visit (see SoE) may still be collected if clinically required. Scheduled follow-up visits may also be attended as per the SoE.

7.2.1. Temporary Discontinuation/Temporary Delay in Enrollment

[redacted]

7.3. Lost to Follow-up

A participant will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the study site 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 the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the PI/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.

Discontinuation of specific sites or of the study as a whole are handled as part of Appendix 1, Regulatory, Ethical, and Study Oversight Considerations.

7.4. Participant Replacement Strategy

Participants may be replaced in this study.

[redacted]

In Part B, if Part B is not randomized, the replacement participant will receive the same allocated blinded dose as the participant who is being replaced.

7.5. Stopping Rules

The PI/investigator and sponsor will perform safety reviews on available clinical and virology data as appropriate during the quarantine period.

Three clinical scenarios relating to the incidence of AEs and SAEs during the study and the procedures that should be performed in each case are presented in Table 7-1.

Table 7-1: Study Stopping Rules

Status	Criterion	Procedure
1.	A report has been received of one (or more) challenge agent-related SAEs in any 1 (or more) participant(s).	If such a status occurs at any point during the study, the PI/investigator will review the data and make decisions on study continuation or termination. Participant follow-up should continue until resolution or stabilization of SAEs.
2.	No challenge agent-related SAEs have been reported but an overall pattern of clinical changes or symptoms exists, which may appear minor or moderate in terms of individual AEs or SAEs, but which collectively represent a concern for safety.	
3.	Unexpected challenge agent-related SAE(s) or unexpected challenge agent-related AE(s) of clinical concern have been reported following viral challenge.	If such a status occurs at any point during the study, then the PI/investigator and the hVIVO safety governance committee will review the data and make decisions on study continuation or termination based on expectedness* of the viral event. If the event is unexpected, further administration of the virus will not take place. The PI/investigator and the safety governance committee will review the data and decide on whether it is appropriate to recommence inoculation (approval of a substantial amendment from the Competent Authorities is required) or terminate the study.

* Expectedness will be assessed by referring to the challenge agent dossier.

A final follow-up visit will be performed on Day 28 (±3 days). Follow-up of any event should continue until resolution, stabilization, it is judged by the PI/investigator to be no longer clinically significant, the participant is lost to follow-up, or an alternative explanation has been provided.

Further enrollment into the study may be either temporarily or permanently discontinued if:

- An unacceptable number of severe or life-threatening exacerbations of AEs take place (as determined by the PI/investigator).
- Any clinically significant life-threatening AEs considered related to the challenge agent as determined by the PI/investigator occur.

7.6. Potential Adaptations to Operational Design

This study is designed to be able to utilize adaptive features to enhance study safety, efficiency, and efficacy. These design elements are predefined in their scope and limit, as detailed in Table 7-2.

The implementation of study-specific adaptive features will be documented in a non-substantial amendment. Generic adaptive features may be implemented at any time at the discretion of the PI/investigator.

Table 7-2: Adaptive Features

Adaptive Design Category	Feature	Limit
Generic		
Cohort(s)	1. Participants who have been withdrawn (for any reason) may be replaced (sponsor and/or PI discretion). 2. Participants who are replacing a withdrawn participant may be randomized for inclusion, and inoculated: a. In an ongoing cohort b. In a new cohort c. Separately. 3. Any study cohort may run at the same time. 4. The number of participants enrolled in each cohort may be reduced or increased (sponsor and/or PI discretion) to best meet the study objectives.	1. The stopping rules of the study must be always adhered to, and replacement participants may not be enrolled to replace participants who have been withdrawn from the study due to the meeting of stopping rules. 2. The total number of study participants will not exceed 80. 3. Replacement participants will be given replacement randomization numbers, where applicable (see Section 7.4, Participant Replacement Strategy). 4. All protocol-defined rules and safety criteria must be met before any study part, cohort, or participant commences the study.
Sample/Specimen	1. The PI/investigator may perform additional safety assessments, at any time, if they believe them to be clinically required. 2. Where clinically required (sponsor and/or PI discretion), participants may be referred for consultation(s) and/or investigation(s) under the care of a specialist physician.	1. The maximum blood volume (described in Section 8, Study Assessments and Procedures) collected from the participant will not be exceeded. 2. Any required additional safety assessments, or specialist referrals, will be conducted on a case-by-case basis. As such the maximum number needed cannot be prospectively defined.

Adaptive Design Category	Feature	Limit
Duration of Inpatient Stay	1. A participant's inpatient stay may be prolonged if discharge criteria of minimal infectiousness is not met (sponsor and/or PI discretion).	1. Must meet the terms and criteria as detailed in the participant information sheet. 2. Participants must always be able to leave the study site unhindered if they wish to do so. 3. The additional stay is triggered based on the minimal infectiousness discharge criteria not being met (as detailed in this protocol), and the participant's suitability for residential stay will be assessed on a case-by-case basis. As such, a maximum length of stay cannot be prospectively defined.

8. STUDY ASSESSMENTS AND PROCEDURES

[redacted]

8.1. Demographics and Baseline Characteristics

8.1.1. Demographics

Demographic data will be recorded at the screening visit.

8.1.2. Height, Body Weight, and Body Mass Index

Height and body weight measurements will be recorded in compliance with hVIVO's standard procedures.

Body mass index will be calculated as: $BMI\ (kg/m^2) = \frac{\text{Body Weight (kg)}}{\text{Height (m)}^2}$

8.1.3. Medical and Medication History

Medical and medication histories will be recorded at screening and quarantine admission, including, but not limited to, detailed histories on current contraception, and allergies (e.g., rhinitis, dermatitis, food, aspirin/non-steroidal anti-inflammatory drugs, and asthma).

8.1.4. Challenge Agent Serology Samples

A participant must be serosuitable to take part in the study, i.e., he/she must have no or low pre-existing serum levels of antibodies specific to the challenge agent. This antibody titer cut-off for serosuitability will be described in the applicable hVIVO policy.

Serum levels of pre-existing influenza A/England/7763/2022 H3N2 virus-specific antibodies will be determined using hemagglutination inhibition (HAI) or micro-neutralization assay.

8.1.5. Patient Health Questionnaire (PHQ-9) and Generalized Anxiety Disorder (GAD-7) Questionnaire

[redacted]

8.2. Respiratory Samples

The following exploratory nasal and throat sampling procedures will be performed during the study and are detailed in the sections below:

- Nasal wick (nasosorption)
- Nasopharyngeal swab
- Throat swab

When any nasal sampling time points occur together, the order of sampling will typically be (1) nasal wick (2) nasopharyngeal swab.

8.2.1. Nasal Wick

A nasal wick (Class 1 Device) procedure will be used to collect samples of epithelial lining fluid for:

- Inflammatory markers, e.g., cytokines/chemokines.
- Other protein biomarkers, e.g., antibodies.

Remaining epithelial lining fluid may be stored and used for exploratory purposes.

8.2.2. Nasopharyngeal Swab

Nasopharyngeal swabs will be performed to collect samples of nasal material for:

- Respiratory pathogen screen
- Influenza discharge test (e.g., RVAT)
- Viral load assessments (see Section 8.3.3)
- Exploratory assessments (see Section 8.3.4).

Remaining material from the nasopharyngeal swabs may be stored and used for exploratory purposes.

8.2.2.1. Throat swab

Throat swab swabs will be taken for viral load assessments (see Section 8.3.3, Viral Load Assessment).
Samples may be used and/or stored for exploratory purposes (see Section 8.3.4, Exploratory Assessments).

8.2.2.2. Respiratory Pathogen Screen

On entry to quarantine, a nasopharyngeal swab will be collected and tested to detect the presence of a set of respiratory pathogens, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that could potentially contraindicate a person's participation in the study. The methodology to be used to conduct the respiratory pathogen screen will be documented in the AP. [redacted]

8.2.2.3. Influenza Discharge Test

Where required, an influenza discharge test (e.g., RVAT) will be used to determine the presence of influenza in a nasopharyngeal swab sample taken prior to discharge from the quarantine unit on Day 8. A PCR test may be used as an alternative test for this purpose, details of which will be documented in the AP. Additional RVAT/PCR tests may be performed at the discretion of the PI/investigator.

8.2.3. Breath respiratory sampling

To assess the potential of transmission endpoints additional exploratory assessments of virus in exhaled breath may be performed using the following methods:

- Breath sampling
    - Participants will be asked to wear a single use face mask that has been fitted with a sampling matrix insert, capable of capturing virus. Similar masks have recently also been successfully demonstrated to detect SARS-CoV-2 in exhaled breath (Williams et al., 2020; Zhou et al., 2023). The insert will be harvested from used masks and analysed for virus and virus-related signals.
    - Handheld respiratory samplers
- Air sampling
    - Air sampling to detect airborne virus may be performed using a free-standing mechanical air sampler.

Any of the above assessments may be performed on subset of participants. A subset may include between none and up to all participants in the study.

8.3. Pathogenicity Assessments

8.3.1. Participant Diary Cards

Symptom Diary Card

Participants will report and assess the severity of any challenge agent-related signs and symptoms 3 times per day (except for felt nauseous, vomiting, and diarrhea, which are assessed once per day, in the morning, for the preceding 24 hours) during quarantine, at the same time each day (±1 hour), using the hVIVO symptom diary card. This information will be collected using a paper or electronic form.

The following symptoms are included in the 13-item symptom questionnaire and will be used in analyses of clinical symptom-related endpoints. These symptoms will be graded on a scale of 0 to 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); shortness of breath and wheeze have an additional grade, i.e., grade 4: symptoms at rest.

- Runny nose
- Stuffy nose
- Sneezing
- Sore throat
- Earache
- Malaise/tiredness
- Headache
- Muscle and/or joint ache
- Chilliness/feverishness
- Cough
- Chest tightness
- Shortness of breath
- Wheeze

The following influenza-related symptoms will be graded on a scale of 0 to 3 (grade 0: no symptoms; grade 1: just noticeable and no interference with activity [or 1 episode within the preceding 24 hours]; grade 2: clearly bothersome from time to time but does not interfere with me doing my normal daily activities [or 2 to 3 episodes in the preceding 24 hours]; grade 3: quite bothersome most or all of the time, and it stops me participating in activities [or ≥4 episodes in the preceding 24 hours]):

- Felt nauseous
- Vomiting
- Diarrhea

Additional exploratory analyses may be performed which include these additional symptoms.

Additional to the ordinal symptom diary card, a visual analog scale diary card using a 100 mm scale, with the same symptoms, will be completed by the participants.

Participant Common Cold Perception Questions

Two additional common cold-related questions will be answered by the participant each morning. The first question asks 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.

1. 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:

2. Compared to yesterday, I feel that my cold is:
    - o Very much better
    - o Somewhat better
    - o A little better
    - o The same
    - o A little worse
    - o Somewhat worse
    - o Very much worse

8.3.2. Nasal Discharge Collection from Paper Tissues

Each participant will be given pre-weighed packets of paper tissues. Participants will be asked to place single tissues used for nose blowing or sneezing into a specified collection bag (for that participant only).

A daily 24-hour paper tissue collection will take place throughout the quarantine period. Distribution of paper tissues and collection bags will start in the morning of Day -1, with the first collection on Day 0. Thereafter, distribution and collection of tissues will occur daily at the same time point (±1 hour) each day , with tissues collected 24 hours after distribution, until discharge from quarantine.

24-hour paper tissue collections will be analyzed to determine the following over the quarantine period:

- o 24-hour nasal discharge weight
- o The number of paper tissues used for nose blowing or sneezing over each 24-hour period.

8.3.3. Viral Load Assessment

Viral titer may be determined by qRT-PCR and/or a viral culture assay to investigate the following parameters:

- o Viral load
- o Infectivity status and rate
- o Viral dynamics (e.g., duration, peak, time to peak)

Viral culture will be performed on those samples that have been shown to be quantifiable by qRT-PCR.

8.3.4. Exploratory Assessments

To explore baseline immunology and response to infection with influenza, blood and respiratory samples may be used for exploratory assays related to respiratory viral infection and immunology.

DNA/RNA analysis may be performed on nasal, throat and blood samples. Nasal samples may be tested for suspected community-acquired infections. Blood and respiratory samples may be used and/or retained for exploratory purposes related to the study objectives, respiratory virus infections, and host response to infection.

Analyses on any type of breath/aerosol samples may be performed to assess the viral load released into the environment as a surrogate for viral transmission potential assessments.

8.4. Safety Assessments

8.4.1. Complete Physical Examination

A complete physical examination to include a full systemic assessment.

8.4.2. Directed Physical Examination

Directed physical examinations will be conducted by the PI/investigator and will include examination of the eyes, ears, nose, throat, and respiratory system/chest (via stethoscope). Based upon the presence or absence of clinical signs and symptoms, PI/investigator discretion will be used to determine the requirement to perform further assessments or a complete physical examination.

[redacted]

8.4.3. Vital Signs

Vital signs assessments will be recorded [redacted]

[redacted]

Study-specific normal ranges for vital signs are provided in Appendix 4, Normal Ranges.

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 sponsor requirements using the Food and Drug Administration (FDA) toxicity grading scale September 2007 (FDA, 2007).

[redacted]

8.4.4. Tympanic Temperature

[redacted]

Study-specific normal ranges for tympanic temperature are provided in Appendix 4, Normal Ranges.

Temperature may be monitored more frequently during quarantine, if appropriate.

[redacted]

8.4.5. Electrocardiogram

Study-specific normal ranges are provided in Appendix 4, Normal Ranges.

[redacted]

8.4.6. Clinical Safety Laboratory Assessments

8.4.6.1. Urinalysis

Clinical urine safety analysis will be undertaken using commercially available urine test strips (i.e., dipsticks) that provide an instant result, which will be documented in the source data.

Urinalysis will be performed to evaluate the parameters described in Appendix 2, Clinical Laboratory Tests.

[redacted]

[redacted]

8.4.6.2. Drugs of Misuse and Cotinine

[redacted]

8.4.6.3. Alcohol Breath Testing

[redacted]

8.4.6.4. Safety Blood Assessments and Analysis

[redacted]

8.4.7. Pregnancy Tests and Follicle-stimulating Hormone

[redacted]

8.4.8. Lung Function

Spirometry

[redacted]

[redacted]

8.5. Adverse Events, Serious Adverse Events, and Other Safety Reporting

The PI/investigator is responsible for ensuring that all AEs/SAEs and pregnancies are identified, evaluated, recorded, and reported in a timely manner as per regulatory requirements, hVIVO SOPs, and the study-specific protocol. The PI/investigator is also responsible for ensuring that the medical management (including follow-up) of AEs/SAEs and, where appropriate, pregnancy symptoms/complications is provided by competent investigator site staff.

The sponsor of the study will also perform an evaluation of seriousness, severity, causality, and expectedness of all SAEs.

The definitions of an AE/SAE, as well as 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 Appendix 3, Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

8.5.1. Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

All AEs/SAEs will be collected from signing of the study-specific ICF until the last scheduled follow-up visit at the time points specified in the SoE or until the resolution of the AE/SAE.

Investigators are not obligated to actively seek AEs/SAEs after conclusion of study participation. However, if the PI/investigator learns of any SAE 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 PI/investigator must promptly notify the sponsor.

8.5.2. Method of Detecting Adverse Events/Serious Adverse Events

The method of recording, evaluating, and assessing causality of AEs/SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3, Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

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

8.5.3. Follow-up of Adverse Events/Serious Adverse Events

[redacted]

8.5.4. Regulatory Reporting Requirements for Serious Adverse Events

Any SAE will be reported immediately by the PI/investigator to the sponsor (in practice reporting within 24 hours of the PI/investigator's knowledge of the event). [redacted]

8.5.5. Pregnancy

[redacted]

8.6. Pharmacokinetics

Pharmacokinetic parameters are not evaluated in this study.

8.7. Pharmacodynamics

Pharmacodynamic parameters are not evaluated in this study.

8.8. Immunogenicity Assessments

Blood will be collected for immunological analysis.

Humoral immune responses, e.g., antibodies to influenza A/England/7763/2022 H3N2 and cytokines/chemokines will be evaluated in serum samples collected from all participants according to the SoE. [redacted]

8.9. Genetics

[redacted]

8.10. Biomarkers

Biomarkers may be evaluated in this study.

9. STATISTICAL CONSIDERATIONS

9.1. Statistical Hypotheses

There will be no inferential statistical analysis for the study, the analysis will be purely descriptive.

Primary, secondary and exploratory endpoints may be compared between randomized groups using inference tests, but these results will have to be regarded as illustrative only and no definitive conclusions should be drawn from them, because of the potential risk of type-1 error (false positive).

9.2. Sample Size Determination

No formal sample size calculation has been performed for this exploratory phase study; the sample size is consistent with earlier influenza, respiratory syncytial virus, and human rhinovirus characterization studies conducted at hVIVO.

A total of up to 80 participants may be given influenza A/England/7763/2022 H3N2 challenge agent, up to 20 participants in each of the dose arms in Part A and up to an additional 20 participants in the expansion arm (if enrolled).

9.3. Analysis Sets

For the purposes of analysis, the following analysis sets are defined (Table 9-1).

Table 9-1: Analysis Sets

Participant Analysis Set	Description
Intent-to-treat (ITT) analysis set	All participants that have been randomized (where applicable) and inoculated. Note: The safety analysis set and ITT analysis set will consist of the same participants.
Intent-to-treat infected (ITT-I) analysis set	All participants that have been inoculated with any dose of challenge agent and who are infected with challenge agent as per the definition of laboratory-confirmed infection for this protocol.
Per protocol (PP) analysis set	All participants having received the planned dose of challenge agent who have a valid result for at least 80% of the planned qRT-PCR nasal and throat sample analyses from Day 1 (pm) up to Day 8 (am), i.e., at least 11 out of 14 samples, and who present no important protocol deviations likely to impact the evaluation of the primary endpoint.
Per protocol infected (PP-I) analysis set	All participants in the PP analysis set and who are infected with challenge agent as per the definition of laboratory-confirmed infection for this protocol.
Safety analysis set	All participants that have been randomized (where applicable) and inoculated.

Participant Analysis Set	Description
	Note: The safety analysis set and ITT analysis set will consist of the same participants.

Membership of participants in each analysis set will be determined at a planned data review meeting, prior to any analysis and database lock.

The primary pathogenicity analysis will be on the PP analysis set. The ITT, PP-I and ITT-I analysis sets will be used for supportive analyses on all or part of the primary and secondary pathogenicity endpoints, as defined in the SAP. The safety evaluation will be performed on the safety analysis set. Additional analysis sets may be defined in the SAP.

9.4. Statistical Analysis

This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

Data will be analyzed and reported using SAS® version 9.4 or later.

9.4.1. Statistical Analysis Plan

The SAP will be developed and finalized prior to database lock for the study. The finalized SAP will be signed prior to unblinding the study data. The SAP will provide a more technical, detailed, and comprehensive description of the statistical analyses that will be computed, expanding on the protocol-specified analysis.

Any deviation(s) from the original statistical plan outlined in the protocol will be described and justified in an amendment to the protocol and/or SAP, as appropriate, and referenced in the final clinical study report.

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

9.4.2. General Considerations

9.4.2.1. Descriptive Statistics

The study will be summarized in terms of descriptive statistics; no formal statistical comparisons are planned.

Continuous variables will be summarized using number of available data, number of missing data, mean, standard deviation, median, lower quartile, upper quartile, minimum, and maximum values. When relevant, confidence intervals (CIs) will be computed for the mean and/or the median. Geometric mean and geometric SD may be provided for peak VL and VL-AUC.

Categorical variables will be summarized using number of available data, number of missing values, frequency counts for each category and corresponding percentage. Percentages will be calculated using the number of available data as the denominator (i.e., not including missing values). When relevant, CIs will be computed. If not otherwise specified in the SAP, the Wilson Score Method will be used to compute CIs for proportions.

- Participants will be grouped and presented against the titer of challenge agent they received.

- The number of participants enrolled, randomized (where applicable), withdrawing (also split by reason for withdrawal) from and completing the study, and the number in each analysis population will be summarized for all participants and by group.
- Any missing data will be accounted for, and the way their possible impact on the study analysis will be evaluated will be described within the SAP.

Full details of the planned analysis will be presented in the SAP. Any deviations from the SAP will be documented in the clinical study report.

9.4.2.2. Inferential Statistics and Significance Testing

Inference tests comparing between groups may be provided but should be considered as purely illustrative.

9.4.3. Planned Analysis

The main analysis will occur at the end of Part B and will combine data from Part A and Part B for the common challenge agent dose arm, if applicable.

9.4.3.1. Participant Accountability

The number of participants enrolled, randomized (if applicable), receiving challenge agent, withdrawing from (also split by reason for withdrawal) and completing the study, and the numbers in each analysis set, will be summarized.

9.4.3.2. Protocol Deviations

Participant's data will be reviewed for important protocol deviations prior to database lock at a planned data review meeting, and decisions will be documented within the meeting minutes. At this meeting, participants will be reviewed for their inclusion/exclusion from the analysis sets. Important protocol deviations will be defined in the protocol deviations management plan.

9.4.3.3. Demographic and Baseline Characteristics

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

9.4.3.4. Compliance to Challenge Agent

A table describing presence/absence of any compliance (challenge agent dose) issues will be presented by dose arm and overall.

Any noncompliance or problems with the administration of the challenge agent will be listed.

9.4.4. Primary Pathogenicity Analysis

The primary pathogenicity analysis will be conducted on the PP analysis set.

The main estimator of the primary pathogenicity endpoint, the laboratory-confirmed infection as determined by qRT-PCR on nasal and throat samples (virology) collected twice daily starting 1 day post viral challenge (Day 1 pm) up to discharge from quarantine (planned for Day 8 am), will be analyzed on the PP analysis set.

Descriptive statistics and the 95% CI will be presented by dose arm. Further details will be described in the SAP.

9.4.5. Secondary Pathogenicity Analysis

For the secondary pathogenicity endpoints outlined in Section 3, Objectives and Endpoints, descriptive statistics (see Section 9.4.2.1, Descriptive Statistics) will be summarized by dose arm as described in Table 9-2. For a subset of the secondary endpoints (details will be provided in the SAP), the descriptive analyses may be repeated on the ITT population. Further details will be provided in the SAP.

Table 9-2: Methods for Analysis of Secondary Pathogenicity Endpoints

Endpoint	Analysis
VL-AUC	Calculation of the VL-AUC will be performed on log_{10}-transformed qRT-PCR data using the trapezoidal summation rule based on actual time intervals in hours. Descriptive statistics for continuous variables (Section 9.4.2, General Considerations).
VLPEAK by qRT-PCR	Descriptive statistics for continuous variables (Section 9.4.2, General Considerations).
Peak TSS	Descriptive statistics for continuous variables (Section 9.4.2, General Considerations).
TSS-AUC	Descriptive statistics for continuous variables (Section 9.4.2, General Considerations).

9.4.6. Exploratory Analysis

Exploratory endpoints as outlined in Section 3, Objectives and Endpoints, will be summarized by dose arm. Further details are described in the SAP.

9.4.7. Safety Analysis

All safety analyses will be computed on the safety analysis set.

Unless otherwise stated in the SAP, safety endpoints will be presented in terms of descriptive statistics only.

AEs will be coded using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA) and summarized descriptively by system organ class, preferred term, and dose arm for the number of AEs/SAEs reported, the number of challenge-emergent AEs/SAEs reported, and the number and percentage of participants reporting each event.

[redacted]

9.5. Interim Analysis

No formal statistical interim analysis of data is planned. However, after Part A of the study, preliminary analysis of Part A data will be undertaken to identify the optimum challenge agent dose for the use in Part B of the study. Results of Part A will be kept securely and will be limited to a predefined selection of staff. The SAP will describe this in greater detail.

9.6. Data Monitoring Committee

Not applicable.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Ethical and Study Oversight Considerations

10.1.1. Ethical Considerations

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 International Ethical Guidelines.
- Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
- Applicable laws and regulations.

[redacted]

The PI/investigator will be responsible for the following:

- Providing written summaries of the status of the study to the REC annually, or more frequently, in accordance with the requirements, policies, and procedures established by the REC.
- Notifying the REC of SAEs or other significant safety findings as required by REC procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of ICH guidelines, the REC, the REC, UK SI 2004/1031 The Medicines for Human Use (Clinical Trials) Regulations, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2. Financial Disclosure

[redacted]

10.1.3. Confidentiality

The PI/investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

10.1.4. Informed Consent Process

The trained and delegated study staff competent to perform the informed consent procedure will obtain a signed study-specific ICF from each participant before any study-specific procedures are performed.

Historical screening data may be collected through the hVIVO generic screening process, which is a comprehensive assessment of health status including previous medical history. For assessments taken under the hVIVO generic screening, a separate informed consent is obtained.

When historical screening data collected through the hVIVO generic screening process is used for screening, the study-specific ICF will be obtained at the first study visit (i.e., quarantine admission) from each participant before any study-specific procedures are performed.

Potential participants will typically be sent a copy of the REC approved study-specific participant information sheet (PIS)/ICF at the time of invite to the first study visit (i.e., quarantine admission) and at least a day prior to the visit and will be encouraged to read it prior to their appointment. Upon arrival at the screening visit/quarantine admission visit (as applicable), the study-specific PIS/ICF is discussed by the trained and delegated study staff competent to perform the informed consent procedure, and the participants will be given the opportunity to ask any questions and may take the information sheet away to consider their participation.

All participants will be required to have a good understanding of English and the PI/investigator will be responsible for ensuring that the participant understands the information contained in the PIS/ICF. Once the PI/investigator has confirmed that the participant has capacity and has understood the study, including the benefits and risks of participation, the participant and the PI/investigator can sign and date the study-specific ICF.

The study-specific ICF must be signed and dated by the participant and countersigned by the trained and delegated study staff competent to perform the informed consent procedure (whoever conducted the consent discussion). A copy of the study-specific PIS/ICF will be given to the participant, and the original will be held in the hVIVO TMF.

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 in the study-specific PIS/ICF.

The study-specific PIS/ICF will contain a separate section that addresses the use of samples for future research. The PI/investigator or authorized designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate (with no requirements to disclose the reason for withdrawal) and may withdraw their consent at any time and for any reason.

A separate PIS/ICF for genetic testing of samples will be required to document a participant's agreement to allow any specimens to be used for related exploratory genetic research. Participants who decline to participate in this optional research will not provide this separate signature.

10.1.5. Data Protection

Participants will be assigned a unique identifier by hVIVO. 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. The level of disclosure must also be explained to the participant in ICF.

The participant must be informed that his/her medical records may be examined by clinical quality assurance auditors or other authorized study staff appointed by the sponsor, by appropriate REC members, and by inspectors from regulatory authorities.

10.1.6. Committee(s) Structure

This study will not include an early safety data review. However, participant safety will be continuously monitored by the PI/investigator and sponsor, which includes ongoing safety oversight (to include safety signal detection at any time during the study).

10.1.7. Dissemination of Clinical Study Data

The key design elements of this protocol will be posted on publicly accessible registry. [redacted]

10.1.8. Data Quality Assurance

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

The PI/investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF. This can be paper source and/or eSource.

The PI/investigator must permit study-related monitoring, audits, REC review, and regulatory agency inspections and provide direct access to source data documents. [redacted]

[redacted]

10.1.9. 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.

[redacted]

10.1.10. Study Discontinuation

The sponsor reserves the right to temporarily suspend or terminate the study for any reason at any time. In addition, the study may be temporarily suspended or terminated at any time if, in the opinion of the PI/investigator, the safety data suggests that the medical safety of participants is being compromised.

[redacted]

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

10.1.11. Publication Policy

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

The publication policy and clauses will be covered in the clinical trial agreement.

10.2. Appendix 2: Clinical Laboratory Tests

[redacted]

10.3. Appendix 3: Adverse Events – Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting

10.3.1. Adverse Event

Adverse Event Definition

[redacted]

Events Meeting the Adverse Event Definition

[redacted]

Events NOT Meeting the Adverse Event Definition

[redacted]

Events NOT Meeting the Adverse Event Definition

[redacted]

10.3.2. Serious Adverse Event

Serious Adverse Event Definition

An SAE is defined as any untoward medical occurrence that, at any dose:

a) Results in death

b) Is life-threatening

[redacted]

c) Requires inpatient hospitalization or prolongation of existing hospitalization

[redacted]

d) Results in persistent disability/incapacity

[redacted]

Serious Adverse Event Definition

[redacted]

e) Is a congenital anomaly/birth defect

f) Is an important medical event

[redacted]

10.3.3. Recording, Assessment, and Follow-up of Adverse Events/Serious Adverse Events

10.3.3.1. Adverse Event/Serious Adverse Event Recording

All AEs/SAEs will be collected from the time of written study-specific informed consent until study completion/final study contact or until the resolution of the AE. [redacted]

[redacted]

The PI/investigator will record all relevant information regarding an AE/SAE in the source documents and evaluate AEs/SAEs using the following guidelines:

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

10.3.3.2. Assessment

Description

[redacted]

Onset and End

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

Assessment
Challenge Agent-related Symptoms [redacted] [redacted]
Physical Examination [redacted]

Assessment
Directed Physical Examination
Vital Signs
Temperature
Spirometry

Assessment
Laboratory Values
C-reactive Protein

10.3.3.3. Assessment of Intensity

10.3.3.4. Frequency

10.3.3.5. Assessment of Causality

The relationship of an AE to the IMP will be categorized as shown in Table 10-2.

Table 10-2: Classification of Adverse Event Relationship

Classification	Definition
Not related	
Unlikely related	
Possibly related	
Probably related	
Definitely related	

[redacted]

10.3.3.6. Action Taken

[redacted]

10.3.3.7. Outcome

[redacted]

Table 10-3: Classification of Adverse Event Outcome

Classification	Definition
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

10.3.3.8. Follow-up

[redacted]

[redacted]

10.3.4. Reporting of Serious Adverse Events

Serious AEs must be documented and reported as per hVIVO SOPs.

[redacted]

Table 10-4: Contact Details for Reporting All Serious Adverse Events

Contact	Details
SAE email address:	SAE_HRD-VCS-006@hvivo.com

[redacted]

[redacted]

10.4. Appendix 4: Normal Ranges

[redacted]

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]		
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]		[redacted]
[redacted]	[redacted]		[redacted]
[redacted]	[redacted]		[redacted]

[redacted]

[redacted]

10.5. Appendix 5: Genetics

[redacted]

10.6. Appendix 6: Abbreviations

Abbreviation	Term
AE	Adverse event
AP	Analytical plan
ATS	American thoracic society
AUC	Area under the curve
β-hCG	beta-human chorionic gonadotrophin
BMI	Body mass index
BP	Blood pressure
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
(e)CRF	(Electronic) case report form
ERS	European Respiratory Society
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume (in 1 second)
FRNA	Foci reduction neutralization assay
FSH	Follicle-stimulating hormone
FVC	Forced vital capacity
GAD	Generalized Anxiety Disorder
GCP	Good Clinical Practice
GP	General practitioner
HAI	Haemagglutination inhibition
HIV	Human immunodeficiency virus
HR	Heart rate
HVC	Human viral challenge
ICF	Inform consent form
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
ILI	Influenza-like illness
IMP	Investigational medicinal product
IRAS ID	Integrated Research Application System identification
ISRCTN	International Standardised Randomised Controlled Trial Number
ITT	Intent-to-treat
ITT-I	Intent-to-treat infected
IUD	Intrauterine device
LLOD	Lower limit of detection
LLOQ	Lower limit of quantifiation
LRT	Lower respiratory tract
MedDRA	Medical Dictionary for Regulatory Activities
NPS	Nasopharyngeal swab
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PHQ	Patient Health Questionnaire
PI	Principal investigator
PIS	Participant information sheet

PP	Per protocol
PT	Prothrombin time
qRT-PCR	Quantitative reverse transcriptase-polymerase chain reaction
REC	Research Ethics Committee
RNA	Ribonucleic acid
RVAT	Rapid viral antigen test
SAE	Serious adverse event
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SBP	Systolic blood pressure
SoE	Schedule of events
SOP	Standard Operating Procedure
SpO_2	Peripheral arterial oxygen saturation
SUSAR	Suspected unexpected adverse reaction
TBD	To be determined
TMF	Trial Master File
TSS	Total symptoms score
UK	United Kingdom
URT	Upper respiratory tract
WHO	World Health Organization

10.7. [redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

10.8. Appendix 8: Protocol Amendment History

Amendment 2 dated 20 December 2024 has been updated with the following changes:

- Administrative updates throughout
- Section 1.2: Schedule of events
    - Included twice daily throat swab assessment.
- Endpoints
    - Throat samples included with nasal samples within Endpoints.

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