Randomized controlled trial, double blind, phase III, to evaluate the immunogenicity of an adjuvanted influenza vaccine among health care personnel (EDUCATE)

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GLOSSARY

SOP	Standard Operating Procedures
NAMRU SOUTH	Naval Medical Research Unit SOUTH
CDC	Centers for Disease Control and Prevention
PI	Principal Investigator
AD	Adjuvanted vaccine
SD	Standard dose vaccine
HCP	Healthcare personnel
IIV3	Trivalent inactivated influenza vaccine
SH	Southern Hemisphere
HI	Hemagglutination inhibition assay
GMT	Geometric mean titer
PII	Personal identifiable information
PDA	Personal Digital Assistant
PIN	Personal Identification Number
EDC/REDCap	Electronic data capture/Research Electronic Data Capture Software
SMS	Short Message Service/WhatsApp
IRB	Institutional Review Board
DCF	Data Collection Form
PBMC	Peripheral Blood Mononuclear Cells

Contents

1. TITLE

Randomized controlled trial, double blind, phase III, to evaluate the immunogenicity of an adjuvanted influenza vaccine among health care personnel (EDUCATE)

2. STUDY SUMMARY

Health care personnel (HCP) frequently receive annual influenza vaccinations and a substantive proportion of them might have attenuated or no response to additional standard dose influenza vaccines. In this population that is seasonally exposed to influenza illnesses and repeatedly vaccinated using standard dose (SD) vaccines, adjuvanted (AD) vaccines might elicit a better immunity than the typically used SD. We therefore propose to conduct a double-blinded randomized controlled vaccine trial among HCPs to determine if AD influenza vaccines generate superior seroprotection against influenza than SD vaccines particularly among previous sub-optimal responders.

The trial will be conducted at two hospital sites in Lima-Peru during the 2022 influenza season. It has annual circulation int the country, but with increases during the months of June to October, which coincides with winter. The study is aimed to HCP who were enrolled in the Cohort study of Influenza and other Respiratory Viruses among HCP in Peru (approximately 1500). The number of participants to be enrolled is 800 approximately. The study design is a randomized, double-blind vaccine trial. Eligible HCP at each site who consent to participate will be randomized 1:1 to receive either a single dose of AD (FLUAD, quadrivalent, Seqirus) or SD (VaxigripTetra, quadrivalent, Sanofi-Pasteur). Participants, researchers and laboratory staff performing the assays will be blinded to the vaccine information.

Participants will be invited to attend the study site for a study screening and assessment of eligibility criteria, if they agree to participate and once consent form is signed, they will visit the site at day 0 for vaccination and medical assessment. Another brief medical adverse event assessment will be performed on days 3 and 7. Participants will be also prompted to begin active surveillance for acute illnesses as soon as US Center of Prevention and Control of Disease (US CDC) and the study site PI confirm that seasonal influenza is circulating locally or there are indicators that circulation is imminent. During the influenza season, participants will be followed up twice a week via SMS or phone calls to assess if they become sick with a respiratory event, if so, they will be asked questions about their illness through an acute illness survey and a swab sample will be requested.
Participants will also be notified when local influenza circulation or the season has ended via SMS message, an email, or a telephone call, based on the same epidemiological data used to trigger the start of the season. A survey will be filled on day 28 post vaccination as well as at the end of the influenza season.
All participants will have blood collected just prior to vaccination (Day 0), 28 days post-vaccination (Day 28), and at the end of the influenza season, to evaluate immune responses to vaccination. We will evaluate differences of seroconversion and seroprotection between AD and SD vaccination groups. In addition, we will use multivariable modelling to assess risk factors for poor immunogenicity and to assess effect of repeated vaccination.

As an optional sub-study, we will examine indicators of cell-mediated immune (CMI) response to influenza vaccination. This part of the study, which is optional to participants, will require collection of additional blood samples at pre-vaccination and days 7 and 28 post-vaccination.

See study timeline which includes the schedules of evaluation per participant.

3. STUDY TIMELINE

STUDY TIMELINE_SCHEDULE OF EVALUATIONS						
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**
Day of Study	**Screening**	**D0**	**D3**	**D7**	**D28**	**D180-200**
Activities						
Informed Consent	x					
Medical History Review		x				
Vital Signs		x	x	x		
Complete Physical Examination		x	x	x		
Targeted Physical Examination		x	x	x		
Pregnancy Prevention Counseling	X*					
Pregnancy Test	x					
Eligibility Confirmation	x					
Enrollment CRF		x				
Randomization		x				
Compensation		x	x	x	x	x
Vaccination /Product Administration (filling form)		x				
Injection Site Evaluation		x	x	x		
Adverse Event Evaluation		x	x	x		
Phone Contact (clinic visit if needed)/filling forms			x	x	x	x
Respiratory surveillance twice a week during influenza season (acute illness detection, sample collection, storage, filling forms)						
Blood sample		x			x	x
PBMC		x		x	x	
Storage of samples		x		x	x	x
Packaging/Shipping						x

*in case a reevaluation is required, it will be scheduled in 1 to 4 weeks

4. STUDY INVESTIGATORS

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5. LOCATION

The study will be performed at two tertiary hospitals in Lima, Peru: Cayetano Heredia National Hospital, and Arzobispo Loayza National Hospital. The two hospitals were selected because have previously participated of an influenza vaccine effectiveness cohort study among healthcare personnel (HCP) and contributed 1,500 participants.

5.1 QUALITY TRAINING

CDC, Influenza Division, NAMRU6 and the National Hospitals Cayetano Heredia, and Arzobispo Loayza have collaborated in influenza research during the past six years. Currently, these institutions are conducting a cohort study in the two hospitals to evaluate influenza vaccine effectiveness among HCP. Each hospital site has at least a Principal Investigator, one physician for enrolment, one nurse for administration of vaccines and 3 nurses for screening and follow-up.

6. HYPOTHESIS

Adjuvanted (AD) influenza vaccine is more immunogenic among HCP, as compared to the non-adjuvanted Standard-Dose (SD) influenza vaccine.

7. OBJECTIVE

Primary objective:

-To determine if AD influenza vaccine generates superior immunogenicity than the non-adjuvanted SD influenza vaccine, at approximately 28 days after vaccination and at the end of season, among HCP

Secondary objectives:

-To compare immune responses post-vaccination, at approximately 28 days after vaccination and at the end of season, among HCP with a history of PCR-confirmed influenza infection during any of the six cohort years (2016 to 2021)
-To compare immune responses post-vaccination, at approximately 28 days after vaccination and at the end of season, among HCP with no history of PCR-confirmed influenza infection during the six cohort years (2016 to 2021), among those with data available for the six cohort years
-To examine whether the number of prior influenza vaccination during the preceding 10 years, as documented by medical records, modifies immune responses after receipt of vaccination with AD or SD influenza vaccine, at approximately 28 days after vaccination and at the end of season
-To evaluate laboratory-confirmed influenza illness rates in the group receiving AD influenza vaccination compared to the group receiving SD influenza vaccination
-To analyze and identify risk factors for sub-optimal response (lack of seroconversion/seroprotection) to SD and/or AD influenza vaccines
- To assess the adverse events among HCP receiving SD and AD influenza vaccines

the studies in reference are those entitled: “Effectiveness of influenza vaccine to prevent infection by influenza virus, loss of hours of work and patient exposure: A prospective cohort study in health workers ” that was carried out during the years 2016 to 2019 and “Effectiveness of the influenza vaccine to prevent infection by the influenza virus, loss of working hours and patient exposure: A prospective cohort study in health workers in the context of the COVID-19 pandemic ”that was carried out between 2020-2021. Both studies were observational epidemiological studies in hospitals in Lima .

8. JUSTIFICATION

An adjuvanted quadrivalent inactivated influenza vaccine (aQIV), FLUAD® Quad (Seqirus) has been licensed in 38 countries including the USA since 2015 for use in persons aged 65 years and older [1, 2].

FLUAD® Quad is a standard-dose influenza vaccine that contains an adjuvant named MF59. This adjuvant is naturally found in humans and enhances immune response to vaccination [1, 3].

Studies have shown a significantly improved immunogenicity and seroprotection among adults aged ≥65 years who received an AD influenza vaccine when compared to those vaccinated with a non-adjuvanted SD [4, 5]. A randomized study by Cowling et al (2019) [4] showed that MF-59 vaccine, among other enhanced vaccines, presented significantly higher proportion of participants who achieved a 4-fold or greater rise in postvaccination titers from day 0 to Day 30, with a postvaccination titer ≥40, a significantly higher geometric mean titer at Day 30, compared to those who received a standard dose vaccine. In addition, they observed a trend rise of vaccine-specific T-cells at 7 and 30 days postvaccination with MF59. Studies have also shown effectiveness of adjuvanted influenza vaccine against influenza-associated outcomes [6].

Studies not noted safety concerns, and while AD vaccine has been associated with increased reactogenicity compared with non-adjuvanted SD vaccine in some studies, these symptoms have generally been reported to be mild or moderate and self-limited [1, 3, 4, 7, 8].

Not all people who receive SD influenza vaccines develop a protective antibody response to influenza virus infections. Vaccinee characteristics can be associated with poor immunogenic response to influenza vaccination [9-17].

In the Americas, health authorities recommend that HCP receive annual influenza vaccination [18] to protect them from influenza illness, its potentially serious complications, and associated absenteeism, as well as to limit contagion from providers to their frequently vulnerable patient population. As such, HCPs frequently receive annual vaccinations and a substantive proportion of them might have attenuated or no response to additional standard dose influenza vaccines. In this population that is seasonally exposed to influenza illnesses and repeatedly vaccinated using SD vaccines [19, 20], AD vaccines might elicit a better immunity than the typically used SD. We therefore propose to conduct a double-blinded randomized controlled vaccine trial among HCPs to determine if AD influenza vaccines generate superior seroprotection against influenza than SD vaccines particularly among previous sub-optimal responders.

9. ANTICIPATED POLICY IMPACT

If our hypothesis is correct, health authorities might want to evaluate the marginal benefit of vaccinating HCP with AD rather than non-adjuvanted SD influenza vaccines to improve their seroprotection and the interruption of transmission to other contacts.

10. STUDY DESIGN

This study is designed as a randomized controlled trial (RCT), double blind, phase III, to evaluate the superior immunogenicity of an adjuvanted influenza vaccine in comparison with the immunogenicity of a non-adjuvanted standard dose influenza vaccine , among health care personnel

10.1 Study population

Eligible adult participants (age 18 or older) will work in one of the selected healthcare facilities (Cayetano Heredia National Hospital, and Arzobispo Loayza National Hospital) and will meet the following inclusion criteria:

Inclusion criteria:

- Be aged ≥18 years old;
- Have participated in the healthcare personnel cohort study between 2016 and 2021.
- Work at the facility full-time (≥30 hours per week);
- Have routine, direct, hands-on or face-to-face contact with patients (within 1 meter) as part of a typical work shift, including, but not limited to, physicians, nurses, respiratory therapists, physical therapists, unit clerks, radiograph technicians, medical assistants, and transporters;
- Work at the facility for ≥1 year prior to enrollment and planning to continue working at the facility for one year after enrollment;
- Willing to receive influenza vaccination (either AD/SD)
-Women of childebaring age have to complete the following criteria:
    - Negative urine pregnancy test performed by the study staff within 24 hours preceding receipt of vaccination
    - Willing to use use another reliable form of contraception approved by the Investigator that they select to use for at least 2 months after receiving the vaccine. If they are not using, we will provide access to contraceptives.
    - Do not be in breastfeeding period

Exclusion criteria:

- Be vaccinated against influenza in the current season (influenza season 2022)
- Have severe, life-threatening allergies against influenza vaccines, eggs or influenza vaccine components
- Have a history of Guillain-Barré Syndrome or other autoimmune diseases
- Received blood or blood products within 3 months before enrollment
- Be pregnant, confirmed by rapid pregnancy test

The participation from each subject will be from the enrollment (March approximately) until the end of the influenza season

10.2 Sample size

We calculate that we will need a minimum sample size of 118 participants per vaccination group for detecting a difference between two proportions (based on seroprotection percentages for influenza A(H1N1p) of a previous study:91% vs 78% [21]) (see below), considering a 95% confidence level (two-sided) and 80% statistical power. To account for loss to follow up, we will increase sample size by 20%, so sample size will be a minimum of 142 subjects per group (Table 1).

Table 1. Sample size calculation based on published seroprotection rates for different influenza viruses

Seroprotection subtypes	P1 (AD group)	P2 (SD group)	n/per group	With additional 20%
Influenza A(H1N1p)	91%	78%	118	142
Influenza A(H3N2)	No information	No information		

Influenza B	No information	No information		

Where P1=percentage of seroprotection for AD group and P2=percentage of seroprotection for SD group

However, although the sample size calculation yields 142 participants per group, there may be different scenarios in which the difference between groups is shortened. In the following table, differences of 10-20% are estimated (Table 2). As a result of this calculation, it is estimated that a sample size of approximately 400 individuals per group will allow us to detect a difference of at least 10% considering a 78% seroprotection rate in the group vaccinated with non-adjuvant vaccine.

Table 2. Different scenarios in seroprotection rates (28 days post-vaccination) per group and sample size calculation

Seroprotection rate in SD group	Relative increase (5) in AD group vs SD group	n per group	Total n	With additional 20%
78%	10%	359	718	862
78%	20%	70	140	168
88g%	10%	131	262	314
88%	15%	58	116	140

10.3 Pre-enrollment procedures

Study staff will contact all HCP who participated of a vaccine effectiveness study at the two participant hospitals to identify potentially eligible subjects and invite them to participate in the vaccine trial. After a brief description of general aspects of the study, field workers will check if potential eligible subjects, aged $\geq$ 18 years, will be available during the study period, been able to attend the study clinic for scheduled visits, have valid personal identification forms, and may be interested to consider participation in the study. (Appendix A) Those interested will be invited to come to the study clinic where the study doctor will explain individually and in detail the study and ask them to sign and written informed consent form if they agree to participate.

10.4 Screening/Enrollment Procedures

Enrollment will begin. during influenza season in Peru[23].

Potential volunteers will come to the study clinic for a screening visit at which time they will be invited to sign a written informed consent form. (Appendix C). Prior to any study procedure the investigator or the health care provider designated, will provide each volunteer sufficient time to read the informed consent document and will fully inform the subject of the nature and scope of the study, potential risks and benefits of participation, the study procedures involved, and will answer all questions for the subject prior to requesting the subject's signature on the informed consent form (ICF). A signed ICF will be obtained prior to performing any study-related procedures.

Once the ICF signature has been obtained, the following screening procedures and assessments will be performed and results recorded on the CRF:

- Assignation of screening number
- Inclusion and exclusion criteria review (Appendix B)

- Demographic documentation including including age, sex, race, ethnic origin, height, and weight. Medical documentation: medical history and nature of contact with patient (Appendix E)
- A complete physical examination including head, eyes, ears, nose, and throat (HEENT), heart, lungs, abdomen, skin, lymph nodes, neurological, musculoskeletal systems
- Vital signs including heart rate(HR), blood pressure (BP), respiratory rate (RR), and oral temperature
- All women will have a reproductive status assessment and women with child bearing potential will have a urine pregnancy test. It will be performed considering a period of 5 weeks after the last menstrual period. Women of reproductive status will be asked about the type of contraception she currently uses. If she is not using any form of secure contraception, she will be offered to attend an appointment in the family planning program from each hospital. As part of that program she will be offer to choose oral or injectable contraception, free of charge, or an alternative secure contraception that could be applied in a family planning clinic (like and intra-uterine device). If the family planning clinic for any reason does not have the method in stock (oral, injectable or barrier), the study will provide it. She can return to be re-screened for the trial once appropriate contraception use has been demonstrated and/or documented. She may be re-cheduled in the range of 1 to 4 weeks after the start of the contraceptive method.
- All screening subjects will be assigned a screening subject identification number and listed in a screening log. Those subjects remaining eligible after the physical exam and evaluation of screening will be enrolled and be given a subject enrollment number prior to be immunized.

Study staff will provide participants with a copy of the informed consent as well as an informative brochure including information of the study, investigational product, and contact information of study staff and schedule of activities (Appendix D). We anticipate to recruit up to a maximum of 800 participants in the study, 400 per group. The informed consent will include permission to access medical records and link data from the previous study with data from the current study.

Subjects who fail screening may be re-screened if the Investigator thinks it is likely the subject will fulfill the inclusion/exclusion criteria requirements at a later time.

10.5 Allocation of subjects

We will randomly allocate consented subjects to either receive standard or adjuvanted influenza vaccination following a blocked randomization methodology to balance the number of participants in two study arms [24]. The electronic data capture system that will be used named REDCap, includes a tool that will allow randomization.

10.6 Intervention

Participants will receive one of the following influenza vaccines: AD influenza vaccination (Southern Hemisphere formulation, FLUAD® Quad (Influenza Vaccine, Adjuvanted), quadrivalent, Seqirus) or non-adjuvanted SD influenza vaccination (Southern Hemisphere formulation, Sanofi Pasteur) (Appendix G). Immediately after vaccination, participants will be asked to remain in observation by a nurse and a physician at the clinical site for a short period of approximately 30 minutes.

10.6.1 Vaccine Storage and Handling

The vaccines will be stored at each site, refrigerated at 2 ºC to 8 ºC. Before the administration, the prefilled syringe or vial has to be shaken. Opened and used vials are not to be reused and they will be identified with the

subject ID number and stored at room temperature at the study clinic for the study monitors to review, and later stored at the site until destroyed at the end of the study. Remained unused vials from 2- 8ºC freezer must NOT be used. At the end of the study, after being reconciled and upon consent by CDC and in coordination with the Peruvian DIGEMID, the used and opened vials will be destroyed.

10.6.2 Vaccine Preparation

On Study Day 0, the investigational nurse or designee will be provided with applicable information for each eligible subject. The investigational nurse or designee will use the randomization list provided by the randomization software in which will indicate the vaccine assignment and the respective randomization code number. The investigational nurse will assign the vaccine to the next available subject. For each subject randomized, the investigational nurse or designee at the time of the vaccination will prepare the vaccine according to instructions that will be provided separately and a label bearing the study subject's identification number, subject's initials, date and randomization code number. After filling in the data, the study nurse will place a transparent sticker covering the label on the syringe, to ensure the preservation of the information.The used vial will be stored at room temperature.

The subject's identification number, initials and randomization code number will be transcribed onto the Vaccine Administration Log.

10.6.3 Vaccine Administration

All vaccines products are to be administered under the supervision of the Principal Investigator or a qualified sub investigator physician designated to CDC in writing prior to the trial and trained in both the protocol and contents of the Investigators' Brochure. Under no circumstances will the Principal Investigator allow investigational products to be used other than as directed by this protocol.

On day 0, before vaccination, subjects will provide approximately 10 mL of venous blood (*pre-vaccination specimen,* Appendix F).

Vaccine will be prepared as described in 10.6.2 and administered after removal from the freezer.

Administrations of the vaccine will be given by intramuscular injection into the deltoid muscle region on Day 0. The vaccination will be given into the non-dominant arm of each subject. Each site should be wiped with an alcohol wipe before injection of the vaccine. The arm used (right or left) and the time when the vaccination was given should be recorded in the CRF and source document.

10.6.4 Post-Vaccination

Emergency equipment will be available on site and procedures instituted in the event of anaphylaxis post vaccination. Medical personnel will be on site for investigational product vaccination and remain at least +30 minutes after administration of the vaccine to the last subject on the day of vaccination. The following procedures and assessments will be performed and results recorded on the subject CRF:

- Vital signs including Heart Rate, Blood Pressure, Respiratory Rate, and temperature
- If indicated by a change in subject health status, an optional targeted physical examination including HEENT, heart, lungs, abdomen, skin, neurological, and musculoskeletal systems may be performed at the discretion of the Investigator.
- Perform vaccination symptom assessment

- Subjects will be queried regarding the occurrence of AEs since administration of study vaccine
- Subject will be asked about their current state of health and be reminded to capture signs and symptoms in the memory aid (Appendix U)
- Subjects will be informed that an auxiliary study nurse will contact them by phone to set a visit on Day 3 (± 2) and Day 7(± 2) to conduct a safety assessment. However, due to pandemic, if for any reason the participant have some impediment in attending the venue in person; it would be possible to set up a virtual visit.
- Further assessments that need to be performed will be recorded on the "Unscheduled CRF(s)".
- A vaccination study card will be provided with the date of the vaccination.
- The time of completion of evaluation and time of discharge will be recorded.

10.7 Follow up

Study staff will contact participants on day 3 (Appendix H) and day 7 (Appendix I) to ask about potential adverse effects or reactions participants might have developed after vaccination.

On day 28 (range 21-35 days), participants will provide another 10 ml sample of whole blood (*post-vaccination specimen,* Appendix F).

At the end of the influenza season, participants will provide a final sample of whole blood (10 ml) (*end of season specimen,* Appendix F) and complete an end of season questionnaire (Appendix J).

10.7.1 End of Season Survey

A brief telephone survey (or in person, if needed, as last method of data collection after internet and telephone) administered at the end of the influenza season will provide a final opportunity to report the occurrence of previously unreported illnesses (especially medically attended illnesses), and the possible use of prophylactic medications that were not reported during surveillance . Brief updates on participants' health status and other infection control and preventive measures may also be included as well as an infection control .

10.8 Surveillance monitoring

An *acute illness*[1] is defined as one or more of the following symptoms within the past 7 days: cough, runny nose, body aches, or feverishness (could be not measured fever). The focus of the study is on acute or newly worsened symptoms; this will be emphasized in the phrasing of screening questions, orientation to

[1]Sources for this method and similar items can be found in the following manuscripts:

Henkle E, Irving SA, Naleway AL, et al. Comparison of Laboratory-Confirmed Influenza and Noninfluenza Acute Respiratory Illness in Healthcare Personnel during the 2010–2011 Influenza Season Infection Control and Hospital Epidemiology, Vol. 35, No. 5 (May 2014), pp. 538-546

Sokolow LZ, Naleway AL, Li D-K, et al. Severity of influenza and noninfluenza acute respiratory illness among pregnant women, 2010e2012.Am J Obstet Gynecol 2014;211:xx-xx.

Irving et al. (2011) Comparison of clinical features and outcomes of medically attended influenza A and influenza B in a defined population over four seasons: 2004–2005 through 2007–2008. Influenza and Other Respiratory Viruses DOI: 10.1111/j.1750-2659.2011.00263.x

participants, and staff training. Nonetheless, participants will be discouraged from assuming they know the source of symptoms or dismissing symptoms they might attribute to allergies or non-infectious sources, since the study intends to assess all acute illnesses regardless of presumed etiology. Following are active surveillance steps.

a. Starting at enrollment, reports of acute illness pre-season will be acknowledged by study staff but will not prompt respiratory specimen collection;

b. During the approximately 10-20 weeks of local influenza circulation, participants will receive twice-weekly communication via cellphone messaging (e.g. *WhatsApp*) or phone call to confirm whether they have acute illness symptoms;

c. During follow up participants will be asked to contact study staff immediately by cellphone messaging (e.g. *WhatsApp*) or telephone when they experience symptoms of acute illness; when possible, some facilities will check staff schedules to identify participants who might have missed work because of illness in order to proactively contact them;

d. Participant reports of symptoms will be logged by study staff in a "Symptom Screening Log" (Appendix K);

e. Participants who report an acute illness (within 7 days of illness onset) will be asked to self-collect a respiratory specimen; a nasal swab will be collected using kits provided by study staff [25-27]. If the participant does not wish to self-swab, study staff will perform the nasal swab through a visit to the participant's workplace or home (Appendix L);

f. Participants who self-swab will either hand deliver their specimen to the study staff located at their facility or contact study staff (by telephone or SMS) for pickup within 24 hours of swabbing whenever possible (but no later than within 72 hours);

g. Participants will complete staff-administered telephone (or in person, if needed, as secondary method of data collection after trying telephone contact) acute (Appendix M) and follow-up interviews (Appendix N);

h. Participants will return to active surveillance at illness resolution (reporting at least one day without symptoms) or two weeks post-illness onset, whichever comes first;

10.8.1 Symptom Screening Log

During study orientation and as part of obtaining informed consent, study staff will emphasize that the study is interested in even mild or minor illnesses. Although each individual has somewhat different definitions of feeling "sick" or "ill," orientation will encourage participants to consider even mild feelings of this nature as constituting feeling sick or ill. Participants will be encouraged to respond to twice-weekly SMS messages to confirm if they are sick with an acute illness. Participants who do not response to SMS will be called on the telephone by study staff. If participants report an acute illness during the active surveillance period by SMS message or direct contact to study staff (as described below), study staff will assess illness symptoms and onset date using the Symptom Screening Log (Appendix K).

10.8.2 Acute Illness Survey

Participants with qualifying acute illnesses that trigger respiratory specimen collection (see below) will be asked questions about their illness through an Acute Illness Survey (Appendix M). The survey items will be administered by study staff by telephone (or in person, if needed, as secondary method of data collection after trying telephone contact) and include date of illness onset, symptoms and subjective severity, missed work and perceived productivity, patient contact and precautions taken to avoid transmission, receipt of medical care, and use of antiviral or antibiotic prescriptions.

10.8.3 Illness Follow-up Texts and Survey

Seven days after the illness onset date, participants will be sent a message to their mobile phone asking if their illness has been resolved. An illness episode will be considered to have been resolved when the participant subjectively reports he or she is “no longer sick.” If the illness has not been resolved, inquiries will be repeated every 48 hours until the illness is resolved or up to 3 contacts (or on days 9, 11, and 13 post illness onset). If symptoms have become worse or a new symptom is added on day 13, a new episode will be considered and steps in section 9.8 will apply.

Regardless of illness resolution, participants will be telephoned approximately 10 days after the illness onset date and asked to complete a brief Illness Follow-up Survey (Appendix N), which addresses symptoms, missed work, productivity, and medical care for the days since completion of the Acute Illness Interview.

10.8.4 Timing of Active Surveillance

We will refer to historical patterns for the start and end of local influenza circulation in planning study milestones. We will also monitor all available sources (e.g. NAMRU-6 influenza surveillance, INS-MoH) of local surveillance for laboratory-confirmed influenza virus infections, allowing for starting surveillance up to two-four weeks prior to typical influenza circulation. Participants will be prompted to begin active surveillance for acute illnesses as soon as US CDC and the study site PI confirm that seasonal influenza is circulating locally or there are indicators that circulation is imminent. Participants will also be notified when local influenza circulation or the season has ended via SMS message, an email, or a telephone call, based on the same epidemiological data used to trigger the start of the season.

10.9 Masking (blinding)

The principal investigators will be in charge of the blinding. This will be a double-blind study, meaning that the participant will be unaware of the type of influenza vaccination that he/she will receive and study staff and serologic tests will be blinded to vaccine type/dose.

10.9.1. Blind opening contingency plan Masking (blinding)

Only in the event of an emergency or serious adverse event or other particular event that alters the risk-benefit profile of the intervention will the principal investigator determine whether unmasking of a participant's study intervention assignment is justified. The safety of the participants should always be the first consideration when making such a determination.

If the investigator decides that opening the blind is warranted, the investigator should make every effort to communicate such a decision by telephone or mail to the sponsor or medical monitor prior to unmasking a participant's treatment assignment, unless this can delay the process of opening the participant's assignment, since it is necessary to have the approval of one of them in order to be able to communicate with the person responsible for the randomization, who has access to the central randomization lists by Code of each participant, and thus request such information.

The date and reason why the blind was broken must be recorded in the source documents and in the CRFe by the investigator of the research center.

If the treatment allocation blind is opened, this information should only be shared with the other medical researchers of the center when it is necessary to know it (especially when the cause is a safety event that corresponds to a serious adverse reaction). Information about treatment allocation should not be shared with other people.

10.10 Withdrawal of Subjects from the Study

Every reasonable effort should be made to ensure that each subject complies with the protocol and completes all study visits. However, a subject may voluntarily withdraw or be withdrawn involuntarily from participation if:

- The subject withdraws consent.
- The investigator recommends discontinuation in the interest of the subject’s safety (i.e., adverse event related) or because of significant and irremediable protocol non-compliance.
- CDC, NAMRU6, the IRB terminates the clinical trial for safety or other reasons.

Any adverse event leading to early discontinuation will be followed to resolution. No subject prematurely withdrawing/withdrawn will be replaced.

10.11 Halting Rules

The study will be halted for safety review by the Clinical Monitor if: (1) if one subject experiences a serious adverse event (SAE) assessed as possibly, probably, or definitely related to investigational product or (2) if there is a subject death assessed as possibly, probably, or definitely related to investigational product. Interpretation of adverse events that impact on halting rules will be done by the Clinical Monitor, Sponsor and IRB.

The clinical Monitor will review the safety data, after the first 50 vaccinations, and will present the data to the IRB board. In case of serious adverse events, vaccination will terminate immediately.

10.12 Schedule of study activities

Day of Study	Event/Activity	Forms to be completed (Annex)
Day 0	Screening/Enrollment/Vaccination	Screening Form Enrollment Survey Vaccination Form
Day 3	Day 3 Post-Vaccination Follow-up	Day 3 Post Vaccination Follow-up Form
Day 7	Day 7 Post-Vaccination Follow-up	Day 7 Post Vaccination Follow-up Form
Day 28	Day 28 Post-Vaccination Follow-up/blood sample collection	Day 28 Blood collection Form
End of Season	End of Season Survey/blood sample collection	End of Season Survey

10.13 Study outcomes

The primary outcome to be measured will be seroconversion at day 28 after vaccination. Seroconversion will be defined as having a haemagglutination inhibition (HI) geometric mean titre (GMT) of ≥1:40 in the case the pre-vaccination HI GMT <1:10 or having a four-fold rise in titre in the case the pre-vaccination HI GMT ≥1:10. We will also measure HI GMT at the end of season. Seroprotection will be defined as having an HI GMT ≥1:40.

Other outcomes that will be measured include:

-reactogenicity to vaccination
-acceptability of AD influenza vaccination
-acute respiratory illness (ARI)
-ARI-associated outcomes (absenteeism, medical visits, hospitalization, other)
-laboratory-confirmed influenza and other respiratory pathogens

11. ANALYSIS

11.1 Data analysis

Baseline data will be described and analyzed for group comparison, as a check on the adequacy of randomization procedures. Table 3 includes the dependent variables for the primary objective and the first 4 secondary objectives, including the evaluation criteria for each one (Table 3), while tables 4 and 5 detail the dependent variables for secondary objectives 5 and 6, accordingly .

Primary objective: To determine if AD influenza vaccine generates superior immunogenicity than the non-adjuvanted SD influenza vaccine, at approximately 28 days after vaccination and at the end of season, among HCP.

Variable	**Dimensión** (Dimension)	**Indicador** (Indicator)	**Valores** (Values)	**Criterios de medición** (Measurement criteria)	**Tipo de variable/indicador** (Type of variable/indicator)	**Instrumento de medición** (Measurement instrument)
Immunogenicity of AD and SD vaccines among health care workers	Seroconversion (Clinical)	Comparison of HI* GMT† of serum collected at Day 28 (post-vaccination specimen) and at the end of the influenza season (end of season specimen) to serum collected at Day 0 (pre-vaccination specimen) for each vaccine (AD and SD)	Person seroconverted following vaccination	Post-vaccination/end of season HI GMT ≥1:40 if pre-vaccination HI GMT <1:10 OR 4-fold rise in post-vaccination/end of season HI GMT if pre-vaccination HI GMT ≥1:10	Quantitative Continuous	Haemagglutination inhibition assay (laboratory)
			Person did not seroconvert following vaccination	Post-vaccination/end of season HI GMT <1:40 if pre-vaccination HI GMT <1:10 OR <4-fold rise in post-vaccination/end of season HI GMT if pre-vaccination HI GMT ≥1:10		
	Seroprotection (Clinical)		Person is seroprotected following vaccination	Post-vaccination/end of season HI GMT ≥1:40	Quantitative Continuous	Haemagglutination inhibition assay (laboratory)
			Person is not seroprotected	Post-vaccination/end of season		

			following vaccination	HI GMT <1:40		

*HI = haemagglutination inhibition

†GMT = geometric mean titer

Secondary Objective 5: To evaluate laboratory-confirmed influenza illness rates in the group receiving AD influenza vaccination compared to the group receiving SD influenza vaccination.

Variable	**Dimensión** (Dimension)	**Indicador** (Indicator)	**Valores** (Values)	**Criterios de medición** (Measurement criteria)	**Tipo de variable/indicador** (Type of variable/indicator)	**Instrumento de medición** (Measurement instrument)
Laboratory-confirmed influenza illness rates in HCP receiving AD vs. SD vaccine	Clinical	Comparison of persons who did and did not become sick with influenza after receiving the AD or SD vaccine	Person became sick with influenza after receiving AD or SD vaccine	Nasal swab specimen tests positive for influenza by PCR	Quantitative Discrete	PCR assay (laboratory)
			Person did not become sick with influenza after receiving AD or SD vaccine	Nasal swab specimen tests negative for influenza by PCR		

Secondary Objective 6: To assess the adverse events among HCP receiving SD and AD influenza vaccines

Variable	**Dimensión** (Dimension)	**Indicador** (Indicator)	**Valores** (Values)	**Criterios de medición** (Measurement criteria)	**Tipo de variable/indicador** (Type of variable/indicator)	**Instrumento de medición** (Measurement instrument)
Adverse events in HCP receiving AD vs. SD vaccine	Anticipated (Clinical)	Local reactions: pain on vaccination site, swelling, lump itching, bruising	Yes	Person had a local and/or systemic reaction(s) after receiving AD or SD vaccine	Quantitative Discrete	Post-vaccination surveillance via follow-up visits and monitoring (calls, surveys)

		AND/OR Systemic reactions: fever (≥38°C), muscle ache, joint pain, headache, malaise, fatigue, weakness, sweating, shivering	No	Person did not have a local and/or systemic reaction(s) after receiving AD or SD vaccine		
	Serious, Unanticipated (Clinical)	Death, life-threatening adverse event, hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity affecting normal life activities, and other unanticipated event that seriously impacts the health of participants	Yes	Person had a serious adverse event after receiving the AD or SD vaccine	Quantitative Discrete	Post-vaccination surveillance via follow-up visits, monitoring (calls, surveys), and hospitalizations
			No	Person did not have a serious adverse event after receiving the AD or SD vaccine		

Below we describe the statistical tests that we will perform to address each primary and secondary objective, highlighting which study population will be included for each analysis. All statistical tests will be performed with a significance level of 5% and a statistical power of 80%.

Primary objective: To determine if AD influenza vaccine generates superior immunogenicity than the non-adjuvanted SD influenza vaccine, at approximately 28 days after vaccination and at the end of season, among HCP. We will use Wilcoxon signed-rank tests to assess HI GMT differences and differences in proportions of HCP who seroconverted and were seroprotected between AD and SD vaccination groups. All HCP included in the clinical trial will be included as the study population for these analyses.

Secondary objectives:

1) To compare immune responses post-vaccination, at approximately 28 days after vaccination and at the end of season, among HCP with a history of PCR-confirmed influenza infection during any of the six cohort years (2016 to 2021). The outcome variables will be the same as those of the primary objective matrix of variables, but dividing the enrolled study population to include only the HCP with a history of PCR-confirmed influenza infection during any of the six-year cohort (2016-2021). We will use Wilcoxon signed-rank tests to assess HI GMT differences and differences in proportions of HCP who seroconverted and were seroprotected between AD and SD vaccination groups. Only HCP in the clinical trial with a history of PCR-confirmed influenza infection during any of the six cohort years (2016 to 2021) will be included as the study population for these analyses.

2) To compare immune responses post-vaccination, at approximately 28 days after vaccination and at the end of season, among HCP with no history of PCR-confirmed influenza infection during the six cohort years (2016 to 2021), among those with data available for the six cohort years. The outcome variables will be the same as those of the matrix of variables in the primary objective, but dividing the enrolled study population to include only the HCP with no history of PCR-confirmed influenza infection during any of the six-year cohort (2016-2021). We will use Wilcoxon signed-rank tests to assess HI GMT differences and differences in proportions of HCP who seroconverted and were seroprotected between AD and SD vaccination groups. Only HCP in the clinical trial with no history of PCR-confirmed influenza infection during any of the six cohort years (2016 to 2021) will be included as the study population for these analyses.

3) To examine whether the number of prior influenza vaccination during the preceding 10 years, as documented by medical records, modifies immune responses after receipt of vaccination with AD or SD influenza vaccine, at approximately 28 days after vaccination and at the end of season. The result variables will be the same as those of the matrix of variables in the main objective, examining the differences by the number of influenza vaccines received by health personnel (HCP) during the previous 10 years. We will evaluate the possible effects of repeat vaccination on the immunogenicity of AD and SD using multivariate logistic regression models. Other variables to consider in the model include age, sex, presence of chronic diseases, body mass index, smoking, and alcohol consumption due to possible confounding factors with the immune system's response to vaccination against HIV. influenza. All health personnel participating in the clinical trial will be included as a study population for these analyzes.

4) To analyze and identify risk factors for sub-optimal response (lack of seroconversion/seroprotection) to SD and/or AD influenza vaccines. The outcome variables will be the same as those of the matrix of variables in the main objective, examining the differences by the following variables: age (subdivided between age groups: 18-49 years, 50-65 years,> 65 years); sex (man, woman); presence of chronic conditions (diabetes, autoimmune disorder, asthma, blood disorder, chronic lung disease, cardiovascular disease, high blood pressure, HIV / AIDS, kidney disorder, liver disorder, metabolic disorder, neurological disorder); body mass index; smoking and alcohol consumption; previous laboratory confirmed influenza diagnosis; previous influenza vaccine. We will use generalized linear models (GLM) with Poisson or binomial link functions as appropriate to assess risk factors. Other variables to consider in the model include age, sex, presence of chronic diseases, body mass index, smoking, and alcohol consumption due to possible confounding factors with the immune system response to vaccination against HIV. influenza. All health personnel participating in the clinical trial will be included as a study population for these analyzes.

5) To evaluate laboratory-confirmed influenza illness rates in the group receiving AD influenza vaccination compared to the group receiving SD influenza vaccination. Using data collected through active surveillance, we will calculate incidence densities and use descriptive statistics and Z-test to assess differences in the proportion of HCPs who had laboratory-confirmed influenza (as determined by PCR on nasal swab samples) during the period of the clinical trial among the HCP who received the influenza vaccines AD vs. SD. A Cox regression model will be used to adjust the risk of developing influenza between both groups according to demographic and occupational characteristics. Health personnel participating in the clinical trial will be included as a study population for these analyzes.

6) To assess the adverse events among HCP receiving SD and AD influenza vaccines. Using data collected through active surveillance, we will use descriptive statistics and the Z test to assess the differences in the proportions of adverse events (anticipated and serious) between the HCP who received the AD and SD

influenza vaccines. All health personnel participating in the clinical trial will be included as a study population for these analyzes.

Table 1: Summary of Analysis by Endpoints and Analysis Populations.

OBJECTIVE	VALUATION CRITERIA	STATISTICAL TEST	POPULATION OF ANALYSIS
Primary Efficacy Analysis			
To determine if the AD influenza vaccine generates superior immunogenicity than the vaccine against SD influence without adjuvant among the HCP.	Immunogenicity - superior serumconversion of AD vaccine versus SD vaccine in HCP.	Wilcoxon signed rank test.	PP population
Secondary Efficacy Analysis			
To compare the post-vaccine immune response, in approximately 28 days after vaccination and at the end of the influenza season, among the HCP with a history of influenza infection confirmed by PCR result during any of the 6 years during the previous cohort study (2016 to 2021).	Post-vaccination immune responses, approximately 28 days after vaccination and at the end of the season, among HCPs with a history of PCR-confirmed influenza infection	Wilcoxon signed rank test.	ITTm1
To compare the post-vaccine immune response, in approximately 28 days after vaccination and at the end of the influenza season, among the HCP who did not have a history of influenza infection confirmed by PCR result during any of the 6 years during the previous cohort study (2016 to 2021); among those with information available during the 6-year cohort.	Post-vaccination immune responses, at approximately 28 days after vaccination and at the end of the season, among HCPs with no history of PCR-confirmed influenza infection.	Wilcoxon signed rank test.	ITTm2
Examine whether the number of prior vaccinations against influenza during the previous 10 years, as documented in the medical records, modifies the immune response after receiving the AD or SD vaccine in approximately 28 days after vaccination and at the end of flu season.	Immune responses after receiving the AD or SD influenza vaccine, approximately 28 days after vaccination and at the end of the season	Descriptive statistics: multivariable logistic regression model	ITTm
Analyze and identify risk factors for a suboptimal response (lack of seroconversion / sero protection) to standard dose (SD) and / or adjuvanted (AD) influenza vaccines	Risk factors for suboptimal response (lack of seroconversion / seroprotection)	Descriptive statistics. Generalized linear models (GLM) with Poisson or binomial functions as appropriate	ITTm

To assess rates of laboratory-confirmed influenza illness in the group that received the AD adjuvanted influenza vaccine compared to the group that received the standard dose SD influenza vaccine.	Laboratory confirmed influenza illness rates among HCP receiving influenza vaccines AD vs. SD	Descriptive statistics., Calculation of incidence densities. Multivariate Cox Regression Model.	ITTm
Security Analysis			
To assess adverse events among HCP who received SD and AD influenza vaccines	Frequency of AE	Descriptive statistics and Z test to evaluate differences in proportions	Security Population

- Population by Protocol: All HCPs vaccinated with any of the research products.
- ITTm population: HCPs who, having been vaccinated, became ill and did not become ill with influenza after receiving the AD or SD vaccine.
- ITTm1 population: HCPs may, having been vaccinated, became ill with influenza after receiving the AD or SD vaccine at approximately 28 days after vaccination and at the end of the season.
- ITTm2 population: HCPs who, having been vaccinated, did not become ill with influenza after receiving the AD or SD vaccine at approximately 28 days after vaccination and at the end of the season.
- Safety population: HCPs that were vaccinated with the investigational product

11.2 Laboratory analysis

11.2.1 Molecular diagnosis

NAMRU-6 laboratory will perform real-time reverse transcriptase polymerase chain reaction (RT-PCR) assays to ascertain infection with influenza A or B viruses and identify influenza A subtype and influenza B lineage. Testing will be completed using US CDC protocol and with primers, probes, and reagents provided by US CDC. A subset of aliquots may be sent to a US CDC reference laboratory in order to confirm and further validate local RT-PCR results.

Additional testing for other respiratory pathogens (e.g. RSV, parainfluenza viruses, human metapneumovirus, adenoviruses, and coronaviruses) might also be conducted. A subset of all viruses will undergo virus isolation. Also, some viruses/specimens will have molecular characterization with genetic sequencing and other assays to detect genetic markers and antiviral resistance. Remaining aliquots of study specimens with future use authorization may be sent to a US CDC-designated facility for additional virus characterization, banking and storage for at least 3 years; no specimens will contain personal identifiers. Samples without future use authorization will be destroyed after the study is finished.

11.2.2 Serology analysis

Samples will be processed and then frozen at -20°C. Specimens will be stored at the local CDC-approved local laboratory for serologic testing and then will be shipped to the US CDC laboratory for serologic testing at the end of the study. Hemagglutination inhibition (HI) assay will be run in duplicate using a CDC-approved protocol. Standard procedures typically include preparing a turkey (for A/H1N1 and influenza Bs) or guinea pig red blood cells (RBC) (for A/H3N2) suspension and treating serum samples with receptor-destroying enzyme to remove nonspecific inhibitors. Nonspecific agglutinins are removed by serum adsorption with packed RBCs. Serum is diluted 2-fold starting from 1:10. The HI titer is the reciprocal of the serum dilution in the last well with complete hemagglutination inhibition.

The panel of influenza viruses to be included in the HI assay will be determined depending on locally circulating viruses during enrollment and historically, including viruses in circulation during the previous 2-6 years.

Samples will be also shipped to US CDC labs for additional testing with additional immunological assays for influenza and other respiratory pathogens (e.g. RSV, parainfluenza viruses, human metapneumovirus, adenoviruses, and coronaviruses) or other agents that cause respiratory diseases such as bacteria, as well as for the biorepository.

11.2.3 Optional Sub-study

As an optional sub-study, we will examine indicators of cell-mediated immune (CMI) response to influenza vaccination. This part of the study, which is optional to participants, will require collection of additional blood samples, pre-vaccination and at 7 and 28 days post-vaccination.

Participants who consent to participate in this optional part of the study, will provide an additional 20mL heparinized whole blood at enrollment before vaccination and at 7 and 28 (in a range from day 21 to day 35) days post-vaccination for extraction of peripheral blood mononuclear cells (PBMCs), which can be used for assays focused on cell-mediated immunity (CMI). Extraction of PBMCs will be performed at NAMRU6 laboratory or at the laboratory assigned by NAMRU6 and then samples will be shipped to a US. CDC approved laboratory to assess cell-mediated immunity.

The separate heparin-treated blood specimens collected for CMI response testing will be kept at room temperature in transit and delivered to the laboratory within 2-4 hours of collection if possible (though standard procedures allow for up to 12 hour delays). PBMCs will be isolated from the heparinized whole blood using standard procedures and cryopreserved in liquid nitrogen until further analysis.

12. INVESTIGATIONAL AND CONTROL PRODUCT

Investigational product:

- Generic name: FLUAD® QuadInfluenza Vaccine, Adjuvanted (Seqirus)
- Dosage and Administration: a single 0.5 mL for intramuscular injection
- Lot/batch/serial number: TBD
- Expiration date: TBD
- Potency: TBD
- Formulations: SH 2022 quadrivalent formulation as per World Health Organization (WHO) recommendation:
  - A/Victoria/2570/2019 (H1N1)pdm09-like virus 15micrograms HA (hemagglutinin per dose);
  - A/Darwin /9/2021 (H3N2)-like virus 15micrograms HA (hemagglutinin per dose;
  - B/Austria/1359417/2021(B/Victoria lineage)-like virus 15micrograms HA (hemagglutinin per dose);
  - B/Phuket/3073/2013 (B/Yamagata lineage)-like virus 15micrograms HA (hemagglutinin per dose).

FLUAD® Quad (Influenza Vaccine, Adjuvanted) is a sterile suspension for intramuscular injection, is a quadrivalent, inactivated influenza vaccine prepared from virus propagated in the allantoic cavity of embryonated hens' eggs inoculated with a specific type of influenza virus suspension. Each dose contains hemagglutinin (HA) from each of the four influenza strains recommended for the 2022 influenza season and also contains MF59C.1 adjuvant (), a squalene based oil-in-water emulsion. Each strain is harvested

and clarified separately by centrifugation and filtration prior to inactivation with formaldehyde. The inactivated virus is concentrated and purified by zonal centrifugation. The surface antigens, hemagglutinin and neuraminidase, are obtained from the influenza virus particle by further centrifugation in the presence of cetyltrimethylammonium bromide (CTAB).

- Storage conditions: 2-8 degrees Celsius
- Disposal and Accountability: The Principal Investigator or a designee is responsible for maintaining complete investigational products inventory records accounting for receipt, storage, dispensation, and final disposition using forms supplied by (or local equivalents approved by) CDC or designee (See appendix P). These records will be reviewed by CDC representatives or designee. At the conclusion of the trial, all vaccine vials of investigational products, used and unused, will be destroyed at the hospital site upon consent by CDC.

This vaccine has not been assessed and authorized for its use and commercialization in Peru by the regulatory office of the Ministry of Health (DIGEMID by its acronyms in Spanish)

Control product:

- Generic name: VaxigripTetra dose standard Quadrivalent vaccine (Sanofi Pasteur)
- Dosage and Administration: a single 0.5 mL for intramuscular injection
- Lot/batch/serial number: TBD
- Expiration date: TBD
- Potency: TBD
- Formulations: SH 2022 quadrivalent formulation as per World Health Organization (WHO) recommendation:
  - A/Victoria/2570/2019 (H1N1)pdm09-like virus 15micrograms HA (hemagglutinin per dose);
  - A/Darwin /2671/2019 (H3N2)-like virus 15micrograms HA (hemagglutinin per dose);
  - B/Austria/1359417/2021 (B/Victoria lineage)-like virus 15micrograms HA (hemagglutinin per dose);
  - B/Phuket/3073/2013(B/Yamagata lineage)-like virus 15micrograms HA (hemagglutinin per dose).

VaxigripTetraQuadrivalent (Influenza Vaccine) for intramuscular injection is an inactivated influenza vaccine, prepared from influenza viruses propagated in embryonated chicken eggs. The virus-containing allantoic fluid is harvested and inactivated with formaldehyde. Influenza virus is concentrated and purified in a linear sucrose density gradient solution using a continuous flow centrifuge. The virus is then chemically disrupted using a non-ionic surfactant, octylphenol ethoxylate (Triton® X-100), producing a split virus. The split virus is further purified and then suspended in sodium phosphatebuffered isotonic sodium chloride solution.

- Storage conditions: 2-8 degrees Celsius
- Disposal and Accountability: The Principal Investigator or a designee is responsible for maintaining complete investigational products inventory records accounting for receipt, storage, dispensation, and final disposition using forms supplied by (or local equivalents approved by) CDC or designee (See appendix P). These records will be reviewed by CDC representatives or designee. At the conclusion of the trial, all vaccine vials of investigational products, used and unused, will be destroyed at the hospital site upon consent by CDC.

This vaccine has been assessed and authorized for his use and commercialization in Peru by the regulatory office of the Ministry of Health (DIGEMID by its acronyms in Spanish)

13. ASSESSMENT OF SAFETY

Study staff will contact participants on day 3(±2) and day 7(±2) to ask about potential adverse effects or reactions participants might have developed after vaccination. This evaluation may be both face-to-face and virtual, especially considering that, due to the effects of the pandemic, face-to-face assistance could be affected. In addition, participants may report adverse events at any time to study personnel. Contact information will be provided at enrollment. Any adverse event will be investigated (Appendix O - INS Adverse Event Report Format) and participants will be referred to an independent physician for evaluation. The management and reporting of adverse events will be carried out taking into account the definitions and procedures detailed below.

13.1. Adverse events: definitions and procedures for recording, evaluating, monitoring and reporting

13.1.1. Definition of an adverse event (AE)

An AE is any event or situation that is detrimental to the health of the research subject, to whom an investigational product is being administered, and that does not necessarily have a causal relationship with its administration. Therefore, an adverse event (AE) can be any unfavorable and unintended sign; including an abnormal laboratory finding, symptom, or disease temporarily associated with the use of an investigational product; whether or not it is related to it. All AEs fall into one of two categories: "not serious" or "serious".

A. Some examples of an AE include the following:
   - Any abnormal results in laboratory tests (hematology, clinical chemistry, or urinalysis) or other safety evaluations (eg, ECG, X-rays, vital sign measurements), including those that are worse from baseline , deemed clinically significant by the investigator's medical and scientific judgment (i.e. not related to a known comorbid condition).
   - Exacerbation of a chronic or intermittent pre-existing condition, including an increase in the frequency or intensity of the condition.
   - New conditions detected or diagnosed after administration of study vaccine, even though they may have been present before the start of the trial.
   - Signs, symptoms or the clinical sequela of a suspected drug interaction.
   - Signs, symptoms, or clinical sequela of a suspected overdose of study vaccine or a vaccine / concomitant drug.
   - An adverse effect of the study vaccine or a vaccine / concomitant drug.
   - An accident or injury.

B. Events that DO NOT meet the definition of AE:
   - The medical or surgical procedures or other therapeutic interventions themselves are not AE, but the condition for which the surgery / intervention is required is an AE and must be documented accordingly.
   - The planned surgical measures and the condition (s) leading to these measures are not AE if the condition (s) was known prior to the observation period (see below) and it didn't get worse during the trial.

   In the latter case, the condition should be reported as a medical history.

- Situations in which there was no detrimental medical event (eg, social and / or convenience income in a hospital).
- Predicted daily fluctuations of pre-existing disease (s) or condition (s) present or detected at the start of the trial that are not worsening.

Death is not considered an AE, but a result.

13.1.2. Definition of a SAE

If an event is not an EA as defined above, then it cannot be an EAS, even if the seriousness conditions are met.

An SAE is defined as any detrimental medical event that, at any dose:

- Causes death.
- Is life threatening: The term “life threatening” in the definition of “serious” refers to an event in which a subject was at risk of dying at the time the event occurred. It does not refer to an event that hypothetically could have led to death if it had been more serious.
- Requires hospitalization or the extension of an existing hospitalization. In general, hospitalization means that the subject stayed (usually involving a stay of at least one night) in the hospital or emergency room for observation and / or for treatment that would not have been adequate in the doctor's office or in an outpatient setting. Complications that occur during hospitalization are EA. If a complication prolongs hospitalization or meets any other criteria for seriousness, it will be considered serious. If there are doubts as to whether there was a “hospitalization” or if it was necessary, the AE should be considered serious.

Hospitalization for scheduled treatment of a pre-existing condition that did not get worse from the start is not considered an AE.

- Causes a persistent disability / incapacity: The term disability means a significant impairment in a person's ability to perform normal life activities.

This definition does not seek to include experiences of relatively minor medical relevance, such as uncomplicated headache, nausea, vomiting, diarrhea, flu, and accidental trauma (for example, sprained ankle) that could hinder or prevent daily activities. , but not a significant disorder.

- It is a congenital anomaly / It is a congenital anomaly / congenital malformation in the subject's offspring.
- It is a significant medical event: Medical or scientific judgment should be used when deciding whether a SAE report is appropriate in other situations, such as major medical events that may not pose an immediate risk to life or result in death or hospitalization, but which may endanger the subject or may require medical or surgical intervention to avoid one of the other outcomes described in the definition above. In general, these events should be considered serious.

Some examples of such events include invasive cancers or malignant neoplasms, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or seizures that do not require hospitalization, or the development of dependence or drug abuse.

13.1.3. Assessment of intensity and causality

A. Intensity evaluation:

The investigator will assess the intensity of each AE and SAE reported during the trial and assign it to one of the following categories.

- **Absent** (grade 0): no AE
- **Mild** (grade 1): an event easily tolerated by the subject, causing minimal discomfort and not interfering with daily activities.
- **Severe** (grade 3): an event that prevents normal daily activities. An AE that is assessed as severe should not be confused with an EAS. Severe is a category used to rate the intensity of an event; and both AE and SAE can be classified as serious.

B. Causality Assessment

The investigator is obliged to evaluate the relationship between the study vaccine and each incidence of each AE / SAE. Causation will be determined as follows:

- **Related**: there is a reasonable causal relationship between study vaccine and AD.
- **Unrelated:** there is no reasonable causal relationship between study vaccine and AD.

A "reasonable possibility" of a relationship implies that there are facts, evidence, and / or arguments that suggest a causal relationship, rather than a relationship that cannot be ruled out.

The investigator will use clinical judgment to determine the relationship. Alternative causes, such as underlying disease (s), concomitant therapy or vaccine, and other risk factors, as well as the temporal relationship of the event to administration of the study vaccine, will be considered and investigated.

The investigator will also consult the Investigator's Manual in their assessment.

For each AE / SAE, the investigator **should** document in the medical record that he / she has reviewed the AE / SAE and provided an assessment of causation.

There may be situations where an EAS has occurred and the investigator has minimal information to include in the initial report for the sponsor. However, **it is very important that the investigator always performs an assessment of the causality of each event prior to the initial transmission of the EAS data to the sponsor.**

Investigator can change their opinion on causation based on follow-up information and submit SAE follow-up report with updated causation assessment.

Assessment of causality is one of the criteria used in determining regulatory reporting requirements.

All symptoms described by the patient at the express request of the local investigator are considered related to vaccination.

13.1.4. AE and / or SAE registration

The investigator is responsible for recording all AEs / SAEs observed during the trial, that is, from the moment the subject gives informed consent until the end of the trial visit or until the last follow-up visit.

When an AE / SAE occurs, it is the investigator's responsibility to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostic reports) related to the event.

The researcher must then record the information of all relevant AE / SAE in the eCRF.

SAE must be reported to the sponsor within 24 hours.

It is not acceptable for the investigator to send photocopies of the subject's medical records to the sponsor instead of completing the AE / SAE screen in the eCRF.

There may be instances in which copies of medical records for certain cases are requested by the sponsor. In this case, all the identification data of the subject, with the exception of the subject number, will be deleted in the copies of the medical records before they are sent.

The investigator should try to establish a diagnosis of the event based on the signs, symptoms, and / or other clinical information. Whenever possible, the diagnosis (not individual signs / symptoms) will be documented as AE / SAE.

The investigator should document and medically evaluate Adverse Events of Special Interest (AESIs) and overdose cases, and the result should be described on the SAE / AESI / overdose / misuse report form.

The investigator should document and medically evaluate the pregnancies, and the outcome should be described in the pregnancy notification form to be sent to the sponsor.

13.1.5. Monitoring of AE and SAE

The investigator is obliged to perform or coordinate the performance of supplementary measurements and / or evaluations as medically indicated, or as requested by the sponsor to elucidate the nature and / or causality of the AE or SAE as completely as possible. This may include laboratory tests or investigations, histopathological examinations, or additional consultation with other health care professionals.

If a subject dies during trial participation or during the follow-up period, the investigator will provide the sponsor with a copy of all post-mortem findings, including histopathology.

New or updated information will be recorded in the eCRF that was originally completed.

The investigator will send the updated data on the SAE to the sponsor within 24 hours of becoming aware of the information.

13.1.6. AE report

It is the investigator's responsibility to document all AE that occur during the trial in the source documents. AEs should be obtained by non-leading questions to the participant, for example, "Have you had any new or changed symptoms since we last asked / since your last visit?".

The researcher must document all AE that occur during the observation period established in this protocol on the eCRF screens provided.

The following approach to documentation will be taken:

All adverse events (whether serious or non-serious) to be reported should be documented on the “Adverse Event” screen of the eCRF. All AE will be described using the sign, symptom, or medical diagnosis of AE in the eCRF with standard medical terminology, in order to avoid the use of imprecise, ambiguous or colloquial

expressions. Each AE will be defined as serious or not serious according to the definitions in the previous section. The investigator will evaluate the severity of each AE and the causal relationship of the event with the study vaccine.

13.1.7. SAE report

If the AE is **serious**, the investigator will need to complete and sign, in addition to the "Adverse Event" screen of the eCRF, an "SAE / AESI / Overdose / Misuse Report Form" at the time the SAE is detected.

Transmission of the SAE / AESI / Overdose / Misuse report form by email or fax is the preferred method of transmitting this information to the sponsor / medical monitor or coordinator.

This form should be marked as an **"initial"** report and sent **immediately (ie within 24 hours of becoming aware of the SAE)** to the sponsor.

The investigator will document the date that any employee / co-investigator first became aware of the report. All SAE reports (initial and follow-up reports) will be sent to the regulatory authority National Institute of Health (Instituto Nacional de Salud – INS) within 7 days after the SAE.

Initial notification by phone does not replace the need for the investigator to complete and sign the EAS report form within designated reporting periods.

The **"initial SAE report"** should be as complete as possible, including an assessment of causation, details of the current illness and AEs, the reason the event was considered serious, the date of start and end date (if applicable), diagnostic procedures and treatment of the event, relevant medical history and concomitant medications and vaccines, as well as actions taken with the study vaccine (s). The SAE report form **must be signed by the investigator or his / her authorized designee (s).**

Investigator should inform the sponsor of the Adverse Events of Special Interest (AESI) and overdose cases by applying the same SAE reporting rules and timelines.

a. Determination of predictability, baseline safety information: The sponsor will determine the predictability in accordance with the baseline safety information provided in the current Product Data Sheet. Any substantial update or amendment will be considered accordingly.
b. Observation periods: For the purposes of this trial, the observation period for the collection of AE that are required to be reported in the CRFe extends from the time the subject gives informed consent to the end of the trial.

All AEs that occur in the course of the clinical trial, regardless of causal relationship, should be controlled and followed up until the result is known or it is clear that no further information can be obtained.

There must be documented reasonable attempts to obtain the monitoring information and results.

It is the investigator's responsibility to ensure that any necessary additional therapeutic measures and follow-up procedures are performed.

c. Post-trial events: If the investigator becomes aware of any SAE that occurred after the end of the trial, but is considered to be a consequence of the study vaccine (s), this should be reported to the sponsor.

Sponsor will process these SAE.

13.1.8. Reports of other events

A. *Pregnancy reporting and monitoring*: Pregnancy is an exclusion criterion for enrollment in this trial, but participants could become pregnant during their active participation in this trial.

Any pregnancy in a participant who has received a study vaccine must be reported to the sponsor within 24 hours of the time the center becomes aware of its incidence through a pregnancy report form. If the participant becomes pregnant during the trial, she will not receive any additional doses of any study vaccine provided by the sponsor. The pregnancy should be followed to determine the outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of congenital malformations, congenital anomalies, or maternal and / or newborn complications. This follow-up should be done even if the expected duration of the safety follow-up for the trial has ended.

The trial site should keep in contact with pregnant participants to obtain information on the outcome of the pregnancy.

Any complication during pregnancy (eg, gestational diabetes or eclampsia) should be considered an AE; however, these complications could result in the event being considered an SAE. Miscarriages, stillbirth, stillbirth, and reported birth defects in the baby are always considered SAE. The pregnancy itself will not be processed as SAE. The researcher will follow up the participant until the end of the pregnancy and should evaluate the outcome in the shortest time possible, but no more than 30 days after the end of the pregnancy. The investigator should notify the sponsor of the pregnancy outcome by submitting a pregnancy follow-up report.

B. *SUSAR and other regulatory reporting and monitoring*: Any Suspected Unexpected Serious Adverse Reaction (SUSAR) will be promptly reported.
The sponsor will ensure that all relevant information about a SUSAR that is fatal or life-threatening is notified to the competent authorities and the relevant IRB or IEC (s) within 7 days after the sponsor is aware of it. of such event and that the relevant follow-up information is communicated within an additional 8 days.

The sponsor will report all serious and unexpected AE, which the investigator or sponsor considers to have a presumed reasonable causal relationship (suspected serious and unexpected adverse reaction [SUSAR]), to the competent authority, to the Independent Ethics Committee involved and to the researchers, in accordance with current law.

The investigator should inform the sponsor of post-trial SUSARs that occur after the subject has completed the clinical trial.

C. *Reporting and monitoring of misuse and overdose*: Drug misuse and drug overdose should always be reported in the same format (i.e., on the SAE form) and within the same timeframes as an SAE, even if possible that do not lead to an adverse outcome.

When an “overdose” or “drug misuse” of the study vaccine occurs without an AE, the investigator will also need to complete a “SAE / AESI / overdose / misuse report form” and send it to the safety contact. of the sponsor. It should be clearly stated that no AE was observed. If there is no associated SAE, the misuse / overdose will be assessed as non-serious.

In this case, it is not necessary to complete the "Adverse Event" screen of the eCRF.

14. QUALITY ASSURANCE AND QUALITY CONTROL

14.1 Vaccine products

Records will be kept of the batch/lot, quantity and dates that investigational and control vaccine products are received and used. All unused vaccines and all empty or partially used containers will be destroyed. This disposition of the investigational and control vaccine products will be accounted for by using form "Accounting for IVP" (Appendix P).

Vaccine products will be purchased and transported with data loggers for monitoring the temperature throughout transportation and storage. CDC will test vaccine products at the beginning and at the end of the trial for vaccine potency validation.

14.2 Study procedures

All questionnaires will be piloted and refined before the study start date. CDC will follow-up every week with in-country partners to ensure that activities are taking place according to the protocol. CDC and NAMRU6 will evaluate the data collected every month for missing data, data errors and inconsistencies.

14.3 Amendments and deviations

Any amendment to the protocol should be discussed with the Sponsor Representative (e-mail, phone or SMS), recorded on Appendix Q (for internal records), then signed and dated by the Study Investigator and the Sponsor Representative BEFORE implementation. Protocol deviations should be recorded on Appendix R (for internal records), then signed and dated by at least two signatories to the protocol and reported to IRB.

15. ETHICS/PROTECTION OF HUMAN SUBJECTS

The study will undergo a local review by the Institutional Review Board (IRB) of NAMRU6 and will be registered at *ClinicalTrials.gov*. CDC will rely on NAMRU6 IRB review, under reliance agreement between CDC and NAMRU6. All participants will complete informed consent, in which they state that they understand the risks and benefits of participating in the study. Participants may withdraw from the study at any time. Informed consents will be stored at NAMRU-6. Study sites must use access control mechanisms to ensure that only uniquely identifiable, authenticated, and authorized users have access to data to which they have been granted explicit access rights. Credentials (e.g., login names and passwords) must never be shared. Any personal identifiable information (PII) must remain on local servers and is not to be downloaded onto any other device such as a personal digital assistant (PDA), laptop, or portable storage device, nor will it be included in the datasets provided to CDC (except for dates of birth). Study sites may, however, use the REDCap or other mobile application for electronic data collection so long as the following provisions are met:

1. Devices running the application are fully encrypted;
2. Devices running the application require a PIN to be entered to unlock the screen.

All study data, laboratory specimens, and reports, as well as study data collection, process, and administrative forms, will be identified by a coded subject ID in order to maintain participant confidentiality. All study data will be stored separately from study records that contain names or other PII (such as locator forms and informed consent forms).

15.1 Compensation

Participants will receive an economic compensation for potential additional expenses of transportation, refreshments among others that derive from their participation for a total amount of USD 30-50 (170 solesl approximately) throughout the different study steps.

16 DATA HANDLING AND RECORD KEEPING

Data will be collected primarily electronically, via REDCap or other EDC system. Paper records will be used if deemed necessary. Raw data whether handwritten or electronic, will be attributable, original, accurate, contemporaneous and legible. NAMRU6 will maintain the database on site and will manage a separate database with PII. Raw data must be maintained and stored in an organized and safe manner. Corrections should not obscure the original entry, and should be dated and marked with initials of the individual(s) making the correction, and reason for change should be given (Appendix T, for internal records). De-identified data will be shared with CDC for analyses. The analyses for the primary objectives of the study will be conducted by CDC. All prepared manuscripts will be submitted to CDC Clearance and will be published in peer-reviewed journals.

16.1. Publication and report of the study results

The results of the study will be documented in a study report to be signed by the sponsor's representatives and a researcher or study coordinator. The results of the study may be requested by the participants from the principal investigator of the study at any time. These results will only be shared by the principal investigator at the express request of the research subject participating in the study and confidentiality measures regarding the randomization of data from other participants will be kept.

In accordance with standard editorial and ethical practice, study results will be published.

The investigator (s), ethics committees, will have the opportunity to review the data analyzes and discuss with the sponsor the interpretation of the study results prior to publication.

Any article or abstract related to the study written independently by the investigators must be submitted to the sponsor for review at least 60 days prior to submission for publication or presentation.

The list of authors of any publication or formal presentation of study results may include, as appropriate, representatives of the sponsor and will be determined by mutual agreement. Regardless of the results of the study, they will be published in an indexed journal.

By signing the protocol, the researcher agrees to maintain the confidentiality of all the information provided by the sponsor and to request the same confidentiality from his staff and the Institutional Research Ethics Committee. Study documents provided by the sponsor (investigator manuals, protocols, FRC and other protocol-related documents) will be properly preserved to ensure their confidentiality. Information provided by the sponsor to the investigator may not be disclosed to others without the direct written authorization of the sponsor, except to the extent necessary to obtain the informed consent of the research subjects who wish to participate in the trial.

17 SAFETY MONITORING

1. The study will have an independent safety monitor that will look for the appropriate execution of the study and the occurrence of adverse events or on the magnitude of the perceived risks. If a safety issue occurs that would halt the study, the IRB and Peruvian INS will be notified.

17.1. Monitoring and Inspection

The sponsor does not plan to conduct any study audits, only monitoring visits will be carried out in accordance with the provisions of the monitoring plan for this trial. The possibility exists that this trial could be inspected by regulatory agencies, including those of local or foreign governments. If a regulatory body contacts the trial site to arrange an inspection, the sponsor should be notified immediately. The investigator and the institution ensure direct access by quality assurance inspectors and auditors to all trial documents and source data.

The monitor will contact and visit the investigator regularly and they will be allowed access to all source documents necessary to verify the CRF entries and other documents related to the protocol; provided that the confidentiality of the research subjects is preserved in accordance with local laws. It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, in order to verify the safety of the subjects, compliance with the protocol, and the integrity, consistency, and precision of the data being entered into them.

The monitoring standards of the sponsor or someone designated by the sponsor (in this case, Peruvian Clinical Research S.A.C.) require full verification of the presence of informed consent, compliance with the inclusion / exclusion criteria, documentation of an SAE and the registry. of the primary efficacy, safety, and tolerability endpoints. Additional verifications of the consistency of the source data with the CRFs are performed according to the specific monitoring plan of the study.

The researcher must ensure that the anonymity of the research subjects is maintained. Research subjects should be identified in CRFs and other documents sent to the sponsor only with the subject's code, and never with the name. The investigator should maintain a list of research subject identification codes showing the randomization number, date of birth, or any other locally accepted identifier. Documents identifying research subjects (for example, signed informed consent forms) should not be sent to the sponsor and should be kept by the researcher in strict confidence.

The investigator and sub-investigators agree to cooperate with the monitor to ensure that any issues detected in the course of these monitoring visits are resolved. If the research subject is hospitalized or dies in a hospital other than the study center, the investigator in charge should contact the hospital in order to document that SAE.

The investigator will provide the sponsor upon request, the background of the study documentation or medical records. This is particularly important when the CRFs are incomplete or when data transcription

errors are suspected. In case of special problems and / or government requests, it is also necessary to have access to complete medical records, provided that the confidentiality of the research subject is protected.

The monitor will be in charge of reviewing the accounting of medication, medication used and medication returned. It will be responsible for observing the percentage of compliance with the treatment. An initial visit will be conducted before the first research subject is included. Monitoring visits and contacts will be made at regular intervals thereafter, according to the frequency defined in the study's specific monitoring plan. The closing visit will take place after the study closes.

18. STUDY ADMINISTRATION

The steering committee will be composed of all the study investigators listed in this protocol or at least one representative of each institution. The steering committee will discuss project progress once a month by teleconference and meet in person at least once at the beginning and at the end of the study.

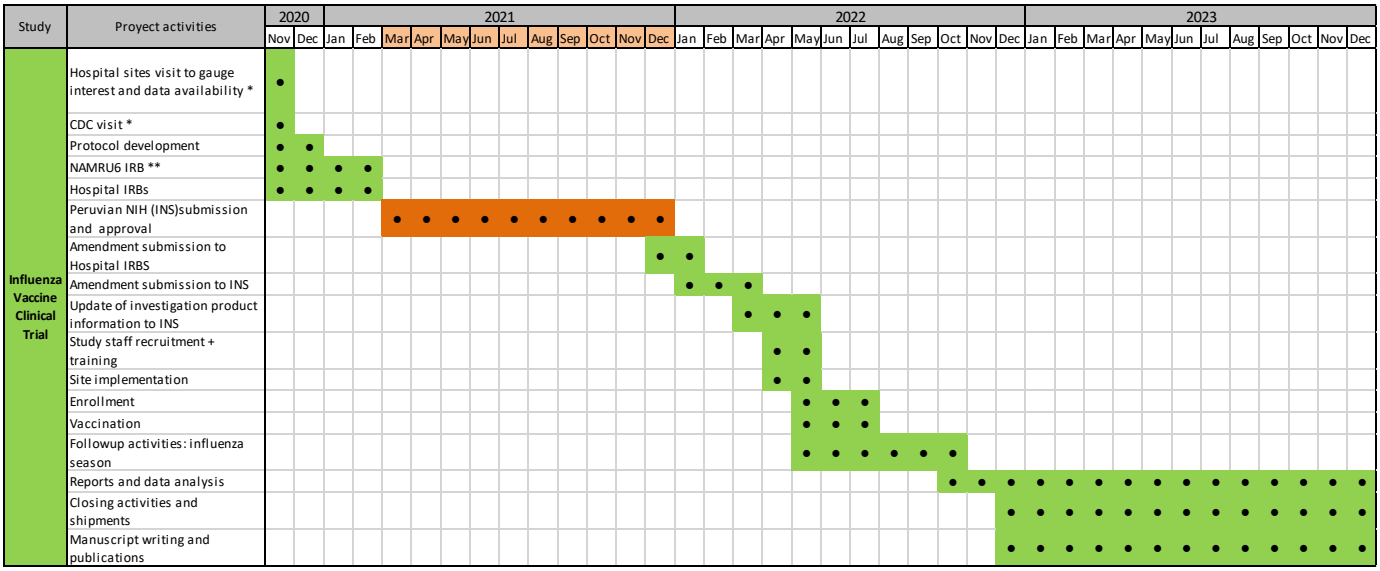
19. CONFLICT OF INTEREST POLICY

Study investigators do not present conflict of interest. Study staff will disclose any potential conflicts of interest to the Institutional Review Board (IRB). This study is funded by the CDC and will be executed in collaboration with NAMRU6 and the participant hospitals.

20. OTHER CONSIDERATIONS

Clinical trial insurance will be purchased before the beginning of the study. Insurance would pay a compensation to participants who suffer any harm as a result of the clinical trial.

21. PROJECT TIMELINE



* All this activities will be starting previously on FY19 and FY20

** review and updates

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23. LIST OF APPENDICES

1. Appendix A Recruitment Form - Version 4- 09072021

2. Appendix B Eligibility Form - Version 05 13122021

3. Appendix E Enrollment Survey - Version 05 13122021

4. Appendix F Blood Specimen Collection Form - Version 4- 09072021

5. Appendix G Vaccination Form - Version 4 - 09072021

6. Appendix H Day 3 Post-Vaccination Follow up Form - Version 4- 09072021

7. Appendix I Day 7 Post-Vaccination Follow up Form - Version 4- 09072021

8. Appendix J End of Season Questionnaire - Version 05 13122021

9. Appendix K Symptom Screening Log - Version 4- 09072021

10. Appendix L Swab Collection Form - Version 4- 09072021

11. Appendix M Acute illness Survey - Version 4- 09072021

12. Appendix N Illness Follow up Form - Version 4- 09072021

13. Appendix O Severe Adverse Event Report - Version 4- 09072021

14. Appendix P Product Accountability Log per study site – Version 02 12082022

14. Appendix P Product Accountability Log per subject – Version 02 12082022

15. Appendix Q Amendment Form Versión - 3 23112020

16. Appendix R Protocol deviation Form Versión - 3 23112020

17. Appendix T. Data correction Form Versión - 3 23112020

18. Appendix U Participant's Daily Record Card Versión 4- 09072021

19. Appendix D Information Broschure – Version 02 12082022