receiving IIV4 alone two weeks later at Vaccination Visit 2 (Sequential group) following both Vaccination Visit 1 and 2 Hypothesis: The proportion of participants with moderate or more severe fever, chills, myalgia or arthralgia will be noninferior (not higher) in the Simultaneous group versus the Sequential group.	following both Vaccination Visit 1 and Vaccination Visit 2
Secondary	
1. To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus the Sequential Group following the first vaccination visit	1a. Comparison of the proportion of participants reporting at least one solicited reactogenicity event to include fever, chills, myalgia, or arthralgia of moderate or greater severity in the Simultaneous group with the Sequential group within 1-7 days following Vaccination Visit 1 visit during which participants receive either IIV4 or placebo with an mRNA COVID-19 vaccine
2. To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus Sequential Group following the second vaccination visit	2a. Comparison of the proportion of participants reporting at least one solicited reactogenicity event to include fever, chills, myalgia, or arthralgia of moderate or greater severity in the Simultaneous group with the Sequential group within 1-7 days following Vaccination Visit 2 during which participants receive either IIV4 or placebo without an mRNA COVID-19 vaccine
3. To describe the proportions of participants in the Simultaneous and Sequential vaccination groups with solicited local and systemic reactogenicity events according to severity grade after the first and second vaccination visit and third vaccination visit for those receiving two doses of mRNA COVID-19 vaccine	3a. The proportion of participants in each vaccination group reporting specified solicited local and systemic reactogenicity events of any severity and by severity grade within 1-7 days following the first vaccination visit during which participants receive either IIV4 or placebo with an mRNA COVID-19 vaccine 3b. The proportion of participants in each vaccination group reporting specified solicited local and systemic reactogenicity

days after each vaccination visit for unsolicited adverse events, through Day 120 for serious adverse events (SAEs) and through Day 120 for adverse events of special interest (AESI), as described in Section 5.4.

3.2 Laboratory Studies

3.2.1 Influenza Hemagglutination Inhibition Assay

mRNA COVID-19 vaccine naïve participants will have blood draws on Day 1 (before vaccination) and Day 21 (Pfizer/BioNTech mRNA vaccine) or Day 29 (Moderna mRNA post-IIV4 vaccination to be stored for serum hemagglutination inhibition (HAI) antibody titers. Those receiving a 3rd dose of mRNA COVID-19 vaccine will have blood draws on Day 1 (before vaccination) and Day 29. HAI antibody titers will be compared between groups receiving COVID-19 and IIV4 simultaneously or sequentially for each of the four influenza vaccine strains contained in the respective vaccines for that season. Participants will not receive individual HAI antibody titer results; these are not routinely used in clinical practice.

3.2.2 SARS-CoV-2 Antibody Assay

Participants will have blood draws on day 1 (before vaccination) as noted in 3.2.1
Serum will be assayed for the presence of SARS-CoV-2 antibody using the AdviseDx SARS-CoV-2 IgG II assay and the Alinity I SARS-CoV-2 IgG assay. The assays are intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2 indicating recent or prior infection. Serologic testing will be completed in periodic batches throughout the course of the study and will therefore not be available in real-time or for use in clinical decision-making. The current tests described above are allowed under the FDA's Emergency Use Authorization during the COVID-19 pandemic. Duke will share participant results with each study site as permitted by FDA and CDC. Study sites may share results with participant as permitted by FDA, CDC, and local site regulations.

In addition, pending funding, we are proposing to conduct quantitative SARS-CoV-2 neutralizing antibody titers in a pseudovirus assay to assess antibody levels.

3.2.3 Future studies

In addition to the specified analyses described thus far, there may be other tests or assays that have yet to be identified that may be important for interpreting our study findings or of relevance to vaccine outcomes. Additional laboratory assays may test for antibodies against other bacteria or viruses, markers of inflammation, or used in research on the health of the participants. Specimens banked for use in other studies will be linked to information (including identifying information) that participants provided to the study. Subjects must agree to potential future use of samples in order to be in the study. Because it is unknown if future testing will be of any utility, results of future testing will not be provided.

4 STUDY ENROLLMENT AND WITHDRAWAL

4.1 Subject Inclusion Criteria

Subjects who meet all of the following criteria will be eligible to participate in this interventional study.

5.1.1 Primary Two-Dose COVID-19 Series Schedule of Events

Table 5: BNT162b2 (Pfizer-BioNTech) Schedule of Events

Procedure	Visit 1	Visit 1a	Visit 2	Visit 2a	Visit 3	Visit 3a	Visit 4	Visit 5	Visit 6	Un-Scheduled
	Clinic	Phone/Text /Email/Data Review[1,2,3]	Clinic	Phone/Text /Email/Data Review[1,2,3]	Clinic	Phone/Text /Email/Data Review[1,2,3]	Clinic	Clinic	Phone	Clinic
Estimated Study Day (Relative to Visit 1)	**1**	**1-7**	**15**	**15-21**	**22**	**22-28**	**36**	**50**	**121**	
Time Following Visit 1 (Days) [Window]	0	0-6	14 [+/-2]	14-20	21 [+/-4]	21-27			120 (+/-14)	
Time Following Visit 2 (Days) [Window]							21 [+/-4]			
Time Following Visit 3 (Days) [Window]								28 [+/-4]		
Informed consent & Medical Release of Information	X									
Review Eligibility Criteria	X									
Review Temporary Delay Criteria	X		X		X					
Demographic and Health History	X									
Influenza and COVID-19 Vaccination History	X									
Obtain unsolicited adverse events			X		X		X			
Obtain SAE and AESI information			X		X		X	X	X	X
Concomitant medications	X		X		X		X	X	X	X
HRQOL measure	X	X								
Measure temperature	X		X		X					X
Venipuncture	X				X		X	X		
Randomization	X									
Vaccination with COVID-19 vaccine	X[4]				X[5]					
Vaccination with IIV4 or Placebo	X		X							

EQ-5D-5L
The EQ-5D is a standardized, generic measure of health status that provides information on HRQOL and activities of daily living: mobility, self-care, usual activities, pain/discomfort and anxiety/depression (http://www.euroqol.org/) [35]. In addition, the instrument contains the EQ Visual Analogue Scale (EQ-VAS) which measures the respondent's self-rated health.

The EQ-5D-5L is the new version of the EQ-5D that increases the levels of severity from three to five to significantly increase reliability and sensitivity while maintaining feasibility and reducing ceiling effects (**Appendix A**) [36,37]. The descriptive system comprises 5 dimensions of mobility, self-care, usual activities, pain/discomfort, anxiety /depression. For each of these dimensions, there are 5 response levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The respondent is asked to indicate his/her health state by ticking in the box against the most appropriate statement in each of the 5 dimensions. This decision results in a 1-digit number expressing the level selected for that dimension. The digits for 5 dimensions can be combined in a 5-digit number describing the respondent's health state from 11111 as best health and 55555 as worst health These numbers are converted to a Utility Index that ranges from -0.109 (worst health) to 1.000 (best health) for US specific values. The minimum clinically important difference ranges from 0.05 to 0.1 depending on health conditions being studied.

EQ-VAS
The EQ-VAS records the respondent's self-rated health on a 20 cm vertical, visual analogue scale with endpoints labelled 'the best health you can imagine' (100) and 'the worst health you can imagine' (0). The respondent marks an 'X' on the scale number to indicate how their health is 'today.' The minimum clinically important difference on the VAS is 8.

The EQ-5D-5L and EQ-VAS have several advantages for use in this study. The measure is applicable to a wide range of health conditions and treatments and provide a simple descriptive profile and a single index value for health status. It has been validated in US and international populations and in adolescents and adults of all ages[35,38,39]. The measure is useful for monitoring the health status of patient groups at different moments in time and assessing the seriousness of conditions at different moments in time. The measure is designed for self-completion by respondents. It is simple, straightforward, take only a few minutes to complete and can easily be completed by adolescents and older adults. The instrument was designed to reduce respondent burden while achieving standards of precision for purposes of group comparisons involving multiple health dimensions. It has been widely used throughout the world in many different studies, including randomized controlled clinical trials, vaccine studies, and HRQOL studies.

5.6 Biospecimens Collection & Handling

5.6.1 Serum

Blood specimens will be collected during study visits as described in Tables 5 and 6.
All blood samples (≈10 mL) will be collected into serum separator tubes and processed as follows:

The upper bound of a site-stratified Newcombe binomial confidence interval (Yan and Su 2010) with Cochran-Mantel-Haenszel (CMH) weighting of the difference will be used to make these assessments with no adjustment to the alpha level for multiple comparisons.

7.1.4 Secondary Objective 1

- To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus the Sequential Group following the first vaccination visit

7.1.5 Secondary Objective 2

- To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus Sequential Group following the second vaccination visit

Secondary objectives 1 and 2 will be evaluated using a Mantel-Haenszal statistic in a stratified analysis by site at the alpha 0.05 level. No adjustments will be made to the alpha level for these evaluations.

7.1.6 Secondary Objective 3

- To describe the proportions of participants in the Simultaneous and Sequential vaccination groups with solicited local and systemic reactogenicity events according to severity grade after the first and second vaccination visit and third vaccination visit for those receiving two doses of mRNA COVID-19 vaccine

Tables (one for each visit 1, 2, and 3) will be produced that summarize each solicited local and systemic reactogenicity event by classification (none, mild, moderate, and severe), as well as by moderate or severe for each study group. These tables will have the number and percentage for each classification by study group and the confidence interval of the difference between the study groups. (*Note: assessments for solicited local reactogenicity will not be assessed following Visit 2 for the simultaneous group*)

7.1.7 Secondary Objective 4

- To describe the proportions of participants in the Simultaneous and Sequential vaccination groups experiencing at least one serious adverse event and a description of these events

A table will be produced that summarize participants experiencing at least one serious adverse event during the study period by group. This table will have the number and percentage for each outcome by study group and the confidence interval of the difference between the study groups. Listings with the clinical narratives will also be provided. The primary analysis population will be the Full Analysis Population 2.

7.1.8 Exploratory Objectives

The analysis for the exploratory objectives will be detailed in the comprehensive Statistical Analysis Plan. The Immunogenicity Population will be the primary analysis population for exploratory HAI objectives.

7.2 Data Management

The novel Vanderbilt-designed resource developed specifically for online collection of research information, the Research Electronic Data Capture (REDCap) platform (https://projectredcap.org/), will be used to design study forms, including the reaction forms, and short customized questionnaires to collect information from study subjects. REDCap provides: 1) a streamlined process for rapidly building a database; 2) an intuitive interface for collecting data, with data validation and audit trail; 3) automated export procedures for seamless data downloads to common statistical packages; 4) branching logic, file uploading, and calculated fields; and 5) a quick and easy protocol set-up. This system will be used by Duke for data management. All electronic linkages will fulfill regulations for protection of human subjects and requirements to minimize the risk of breach of confidentiality.

All study-related documents containing protected health information, e.g. enrollment logs, case report forms, diaries completed by study participants, will be maintained in secure research offices at Duke, John Hopkins CIR, and Cincinnati Children's Hospital, which are accessible to research staff only.

The study team will utilize a secure, encrypted, file transfer method for sharing study documents and data with the CDC. No personal identifiers will be included in any shared documents or datasets.

7.2.1 Research Electronic Data Capture (REDCap)

REDCap (http://project-redcap.org/), assists with the collection and management of data for diverse clinical and translational research studies. REDCap was designed around the concept of giving research teams an easy method to specify project needs and rapidly develop secure, web-based applications for collection, management and sharing of research data. REDCap accomplishes these key functions through use of a single study metadata table referenced by presentation-level operational modules. Based on this abstracted programming model, databases are developed in an efficient manner with little resource investment beyond the creation of a single data dictionary. The concept of metadata-driven application development is well established, and the critical factor for successful data collection lies in creating a simple workflow methodology allowing research teams to autonomously develop study-related metadata in an efficient manner. Both products include secure institutional data hosting and include full audit-trails in compliance with Health Insurance Portability and Accountability Act (HIPAA) security requirements. The REDCap Consortium is comprised of 647 active institutions. The REDCap currently supports 68,000 projects with over 89,000 users spanning numerous research focus areas across the consortium. The current project will use this software application for the design of electronic forms to collect information from study participants, to link the baseline data, sample collection date, and laboratory results in an automated database family, to perform data cleaning and data quality assurance efficiently, and to design an analytical dataset for the analysis of the project data.

Data will be entered into the REDCap database by members of the study team from Duke, Johns Hopkins CIR, and Cincinnati Children's Hospital using the paper case report forms utilized to record data collected as part of study procedures. Study investigators will be responsible for assuring that all paper records are securely stored according to the requirements of their IRBs. The study investigators will be responsible for assuring the accuracy of the data entered from the paper forms into REDCap. Only the assigned identifiers will be used in REDCap. Therefore, personal health identifiers will not appear in the REDCap database.

In order to perform data cleaning and data quality assurance efficiently, numerous built-in filters and checks for consistency of the data including range and limit checks, branching logic and pull down menus to limit choices for categorical variables to a pre-specified list will be implemented and performed automatically to minimize data entry error. The data will be randomly sampled and checked against source records on a regular basis. The data and related analytical datasets will also be stored at the lead and contributing sites with secured password-protected computers.

7.3 Role of the CDC Investigators in the Project

This study is funded by a CDC contract with Duke University, Johns Hopkins University and Cincinnati Children's Hospital as Task Orders in the CISA Project Contract. CDC staff will collaborate with all sites to develop the protocol, conduct the study, ensure the study is aligned with US Department of Health and Human Services (CDC) public health priorities, and analyze the data and disseminate the results. CDC may receive access to coded data not containing any directly identifying information.

8 HUMAN SUBJECTS

8.1 Human Subjects Involvement, Characteristics, and Design

Duke, Johns Hopkins University, and Cincinnati Children's Hospital investigators will be responsible for submitting the protocol, informed consent, diaries, recruitment letters, flyers, and any written or verbally conveyed materials specific to this project to their institutional review boards. CDC staff will be responsible for submitting materials to the CDC Human Subjects Review for determination to rely on Duke IRB.

To facilitate subject recruitment at the practices, we will request a waiver of consent and HIPAA authorization for ascertainment (identification, selection) and/or recruitment of potential subjects while recording identifiable private health information (PHI) prior to obtaining the subject's consent. This information will be obtained from review of the electronic scheduling and medical record systems in the clinics in order to determine eligibility for study enrollment where available. We will review only the minimum amount of information necessary to determine eligibility, i.e. date of birth, medical and surgical history, and recent laboratory test results. The PHI collected prior to consent will be used to recruit and screen only. Use of PHI in this manner involves no more than minimal risk to subjects and no information will leave the study sites.

Requests for continuing review, when required, will be submitted at each engaged institution in accordance with institutional procedures. Protocol deviations or concerns about study integrity

will be reported promptly to the overseeing IRB or CDC in accordance with institutional requirements.

8.2 Sources of Material

Medical history and immunization history will be obtained from the medical record when available and from patient report. Demographic information will be obtained from the medical record and patient report. Subjects will record solicited adverse reactogenicity events and any medical intervention sought on study days 1-7 following each vaccination visit on the symptom diary. Diary information will either be reported electronically or on paper which will be given to the study team during a study visit. Diary information reported electronically will be preferred but paper reporting will be allowed. The research staff will assess oral temperature.

8.3 Potential Risks and Benefits

mRNA COVID-19 vaccines

Two COVID-19 vaccine received Emergency Use Authorization (EUA) by the FDA in December 2020. BNT162b2 received FDA approval for individuals ≥ 16 years of age and mRNA-1273 received authorization for those ≥18 years of age. The Pfizer/BioNTech mRNA COVID-19 vaccine received additional EUA for adolescents 12 to 15 years in May 2021. These vaccines have been recommended at these ages by the ACIP. Participants will be provided with the respective fact sheets for the mRNA COVID-19 vaccine they receive including the BNT162b2 https://www.fda.gov/media/144414/download and the MRNA-1273 https://www.fda.gov/media/144638/download

Side effects that have been reported with mRNA COVID-19 vaccines include both injections site reactions as well as general side effects. Injection site reactions include: pain, tenderness and swelling of the lymph nodes in the same arm of the injection, swelling (hardness), and redness at the injection site. General side effects include: fatigue, headache, muscle pain, joint pain, chills, nausea and vomiting, fever and feeling unwell. There is a remote chance that an mRNA COVID-19 vaccine could cause a severe allergic reaction, usually occurring within a few minutes to hours after getting a dose of vaccine. Signs of a severe allergic reaction can include: difficulty breathing, facial and throat swelling, tachycardia, total body rash, dizziness and weakness. There have been rare reports of cases of inflammation of the heart—called myocarditis and pericarditis—happening after mRNA COVID-19 vaccination[25-27]. The events have mainly occurred in adolescents and young adults and more often after the second dose of vaccine. Available data from short-term follow-up suggest that most individuals have had resolution of symptoms, but information is not yet available about potential long-term sequelae[25,26]. These may not be all the possible side effects of the mRNA COVID-19 vaccines. Serious and unexpected side effects may also occur. mRNA COVID-19 vaccines are still being studied in clinical trials. Available data support the safety of dose 3 mRNA COVID-19 vaccine in immunocompromised individuals[13].

SD-IIV4 and HD-4

SD-IIV4 and HD-IIV4 are FDA-licensed vaccines approved for use in persons ≥ 6 months of age and those ≥65 years old, respectively. Both vaccines are standard clinical practice and recommended by the CDC. Participants will be provided with the CDC Vaccine Information Statement (VIS) for IIV4 (https://www.cdc.gov/vaccines/hcp/vis/vis-statements/flu.pdf).

	events of any severity and by severity grade within 1-7 days following the second vaccination visit during which participants receive either IIV4 or placebo 3c. The proportion of participants in each vaccination group reporting specified solicited local and systemic reactogenicity events of any severity and by severity grade within 1-7 days following the third vaccination visit during which all participants receive an mRNA COVID-19 vaccine alone for those receiving two doses of mRNA COVID-19 vaccine
4. To describe the proportions of participants in the Simultaneous and Sequential vaccination groups experiencing at least one serious adverse event and a description of these events	4a. The proportion of participants in Simultaneous and Sequential Groups with at least one serious adverse event occurring during the study period and a description of each event
Exploratory	
1. To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus Sequential Group following the third vaccination visit for those receiving two doses of mRNA COVID-19 vaccine	1a. Comparison of the proportion of participants reporting at least one solicited reactogenicity event to include fever, chills, myalgia, or arthralgia of moderate or greater severity in the Simultaneous group with the Sequential group within 1-7 days following Vaccination Visit 3 during which all participants receive an mRNA COVID-19 vaccine alone for those receiving two doses of mRNA COVID-19 vaccine
2. To further characterize and describe the proportion of participants in the Simultaneous and Sequential groups with local or systemic reactogenicity events of greater severity following each vaccination visit and cumulatively	2a. The proportion of participants reporting at least one moderate or greater or at least one severe or greater solicited local or systemic reactogenicity event in each vaccination group within 1-7 days following the first vaccination during which participants receive either IIV4 or placebo with an mRNA COVID-19 vaccine 2b. The proportion of participants reporting at least one moderate or greater or at least

1. Persons aged ≥12 years if receiving primary two-dose mRNA COVID-19 vaccine series or persons aged ≥18 years if receiving a third mRNA COVID-19 vaccine dose according to FDA authorization or approval and ACIP recommendation. Note: receipt of an mRNA COVID-19 vaccine within 8 hours of enrollment is permitted
2. English or Spanish literate
3. Intention of receiving influenza vaccine and mRNA COVID-19 vaccine based on ACIP-CDC guidelines
4. Willing to provide written informed consent
5. Intention of being available for entire study period and complete all relevant study procedures, including follow-up phone calls and clinic visits

4.2 Subject Exclusion Criteria

Subjects who meet any of the following criteria will not be eligible to participate in this study:

1. Currently pregnant, planning to become pregnant within the first three months of the study per participant self-report or likely to be pregnant per screening criteria as defined in Section 5.1 at Visit 1
2. Prior receipt of IIV4 during the 2021-2022 influenza season
3. Prior receipt of non-mRNA COVID-19 vaccine
4. Prior receipt of more than 2 mRNA COVID-19 vaccines
5. Documented COVID-19 infection within 6 weeks prior to enrollment confirmed by either medical history or lab testing
6. History of severe allergic reaction after a previous dose of any influenza vaccine; or to an influenza vaccine component, including egg protein
7. History of severe adverse reaction associated with a vaccine and/or severe allergic reaction (e.g. anaphylaxis) to any component of an mRNA vaccine
8. Receipt of any licensed inactivated vaccine within 2 weeks prior to enrollment in this study, receipt of any licensed live vaccine within 4 weeks prior to enrollment in this study, or receipt of Shingrix (Zoster Vaccine Recombinant, Adjuvanted) or HEPLISAV-B (Hepatitis B Vaccine (Recombinant), Adjuvanted) vaccine within 6 weeks prior to enrollment in this study or planning receipt of any vaccines following enrollment until 6 weeks after receipt of the second dose of mRNA COVID-19 vaccine
9. Has an active neoplastic disease (excluding non-melanoma skin cancer or prostate cancer that is stable in the absence of therapy) or a history of any hematologic malignancy*
   **Participants with a history of malignancy may be included if, after previous treatment by surgical excision, chemotherapy or radiation therapy, the participant has been observed for a period that in the investigator's estimation provides a reasonable assurance of sustained cure*
10. Thrombocytopenia, bleeding disorder, or anticoagulant use contraindicating intramuscular injection (a daily aspirin may be acceptable).
11. Has immunosuppression as a result of an underlying illness or medications, such as antirejection/transplant regimens or immunomodulatory agents. Stable HIV disease is permitted per the following parameters:
    a. Confirmed stable HIV disease defined as document viral load <50 copies/mL and CD4 count >200 within 6 months before enrollment, and on stable antiretroviral therapy for at least 6 months

Table 5: BNT162b2 (Pfizer-BioNTech) Schedule of Events

Procedure	Visit 1	Visit 1a	Visit 2	Visit 2a	Visit 3	Visit 3a	Visit 4	Visit 5	Visit 6	Un-Scheduled
	Clinic	Phone/Text /Email/Data Review[1,2,3]	Clinic	Phone/Text /Email/Data Review[1,2,3]	Clinic	Phone/Text /Email/Data Review[1,2,3]	Clinic	Clinic	Phone	Clinic
Estimated Study Day (Relative to Visit 1)	**1**	**1-7**	**15**	**15-21**	**22**	**22-28**	**36**	**50**	**121**	
Time Following Visit 1 (Days) [Window]	0	0-6	14 [+/-2]	14-20	21 [+/-4]	21-27			120 (+/-14)	
Time Following Visit 2 (Days) [Window]							21 [+/-4]			
Time Following Visit 3 (Days) [Window]								28 [+/-4]		
Memory Aid, Link to electronic symptom diary given to participant	X		X		X					
Assess for any immediate reactogenicity symptoms	X		X		X					
Electronic Diary Review[2]		X		X		X				
Complete electronic or paper symptom diary[1]	X	X	X	X	X	X				

1 Symptom diary (solicited local and systemic reactogenicity events) to be completed by participant on Days 1-7 after vaccination.
2 Participants completing paper diary only will be called at days 3 [+2], 17[+2], and 24 [+2] as a reminder and to prompt to bring paper diary to next visit
3 Electronic diary records will be reviewed by study staff at days 3, 8, 17, 22, 24, and 29 for completion or issues.
4 Receipt of BNT162b2 within 8 hours of enrollment is permitted
5. Receipt of BNT162b2 within 36 hours prior to study visit is permitted

- Allow blood to clot at room temperature for at least 30 minutes while standing upright in a rack.
- Centrifuge tube within 8 hours of collection at 1100 to 1300 RCF(g) for 10 minutes.
- Gently remove the vacutainer stopper avoiding serum contamination with red blood cells. Using a single-use pipette, transfer 1.0 mL aliquots of serum (top layer) into 1.0mL or 1.8 mL cryovials, up to 5 cryovials are expected. If less than 1 mL of processed serum is collected, it is a protocol deviation
- All cryovial aliquots will be barcode labelled and contain a unique identifier via REDCap. Numbers should be placed lengthwise on the tube.
- Freeze the cryovials at -80°C in the temperature-monitored research center freezer for future shipment.
- Serum aliquots will be stored in the Duke Human Vaccine Institute Accessioning Lab, the Johns Hopkins Center for Immunization Research laboratory, and at CCHMC Schubert Research Clinic Laboratory until planned HAI analyses at which point the samples stored at JHU and Cincinnati will be shipped to Duke for planned laboratory analysis

6 LABORATORY ANALYSES

6.1 Baseline SARS-CoV-2 antibody

Serum samples obtained at the Visit 1 (prior to vaccination) will be assayed for the presence of pre-existing SARS-CoV-2 antibody using the AdviseDx SARS-CoV-2 IgG II assay and the Alinitiy I SARS-CoV-2 IgG assay. The AdviseDx SARS-CoV-2 IgG II assay is a chemiluminescent microparticle immunoassay intended for the qualitative and semi-quantitative detection of IgG spike antibodies to SARS-CoV-2 in human serum. The Alinity I SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay intended for the qualitative detection of nucleocapsid IgG antibodies to SARS-CoV-2 in human serum. All results will be interpreted as either positive or negative. A positive result on any assay will be interpreted as a positive antibody test result. The assays are intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2 indicating recent or prior infection. Both assays are currently only for use under the FDA's Emergency Use Authorization.
SARS-CoV-2 antibody assays will be performed in the Immunology Virology Quality Assessment Center (IVQAC) at the DHVI. The IVQAC is a GCLP laboratory which is also accredited by CAP, CLIA and ISO-17043.

6.2 Influenza Hemagglutination Inhibition (HAI) Assay

Influenza Hemagglutination Inhibition (HAI) Assays will be performed on sera collected, contingent on additional funding. Briefly, reference wild-type, reassortant, or vaccine virus strains representative of the specific viral antigens included in the 2021-22 influenza vaccine will be used to evaluate the relative levels of all four influenza strain-specific antibodies in participant serum samples collected pre- and 21 or 28 days post-vaccination from all study participants. To accomplish these activities, all participant samples will be interrogated for influenza antibodies against the strains of interest using the influenza hemagglutination inhibition assay (HI). This assay is considered the "gold-standard" measure by which to evaluate seroconversion/seroprotection in response to seasonal influenza vaccination. This assay will be performed in accordance with the Duke Regional Biocontainment Laboratory Virology Unit's fully optimized and approved SOP (RVUSOP004 Influenza HI of Serum

IIV4 risks include minor problems such as soreness, redness, swelling, or pain where the shot was given, hoarseness, sore, red or itchy eyes, cough, fever, aches, headache, itching, fatigue, all of which usually occur within 1-2 days of vaccination and are self-limiting. More serious problems including a small increased risk of Guillain-Barré Syndrome estimated at 1 or 2 additional cases per million people vaccinated can occur. This is much lower than the risk of severe complications from influenza infection, which can be prevented by IIV4[43]. In addition, any medication can cause a severe allergic reaction, or anaphylaxis, which is estimated at ~ 1 in one million doses of IIV4 administered[44].

General Vaccine Risks
Some people get severe pain in the shoulder and have difficulty moving the arm where a shot was given. This happens very rarely. Syncope (fainting) can occur in association with administration of injectable vaccines. Sitting or lying down when space is available for about 15 minutes can help prevent fainting, and injuries caused by a fall, as recommended in the ACIP General Recommendations on Immunization [8]. Subjects should inform their doctor if they feel dizzy or have vision changes or ringing in the ears.

As with any licensed or authorized vaccine, protection may not occur in 100% of vaccinated persons for either COVID-19 or influenza vaccines.

Blood Drawing
Risks of blood drawing include pain, swelling, bleeding, or bruising at the site where the blood sample is collected. Subjects may also experience dizziness or fainting. There is a small risk of infection around the vein where the blood was collected. Each study subject will be asked to have up to 4 blood samplings with the total volume not to exceed 60mL over an approximately 4 month period of time.

Delay of Influenza Protection
There is a potential risk of a short delay in influenza protection by delaying the receipt of influenza vaccine 2 weeks.

Confidentiality
An additional risk of study participation is the potential for loss of confidentiality.

8.4 Adequacy of Protection Against Risks

8.4.1 Protections against Risk
To decrease the possibility of infection at the site of blood drawing, the area on the arm above the vein where blood will be taken will be prepped with 70% isopropyl alcohol antiseptic prior to venipuncture.

Subjects will be counseled on possible side effects following vaccination and followed closely in the immediate post-vaccination period and during the following week for assessment of moderate to severe local or systemic reactogenicity. In the immediate post-vaccination period, all subjects will be monitored in a sitting or lying position for 30 minutes following vaccinations to help prevent fainting, and injuries caused by a fall. Subjects with a prior history of severe allergic reaction after a previous dose of any influenza vaccine or COVID-19 vaccine, or to a vaccine component, including egg protein, will be excluded from study enrollment. Epinephrine and