PROTOCOL

Investigation and Comparison of the Antibody Response initiated by Recombinant, Cell-Based and Egg-Based Influenza Vaccines [VAP3]

Protocol Number: VAP00030
Project Number: 2021.247
DSRB Ref Number: 2022/00275
Version: 2.0
Date: 27/10/2022

Author/s:
Sheena Sullivan, Annette Fox, Barnaby Young, Lisa Ng, Ian Barr

Sponsor
Tan Tock Seng Hospital, Singapore

ESC supported by:
Sanofi Aventis

Statement of Compliance

This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee and Health Sciences Authority regulatory approval and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

Table of Contents

1 STUDY SYNOPSIS

Provide brief information

Title:	Investigation and Comparison of the Antibody Response initiated by Recombinant, Cell-Based and Egg-Based Influenza Vaccines
Short Title:	Immunogenicity trial of 3 influenza vaccines
Design:	Randomized controlled trial
Study Centre:	National Centre for Infectious Diseases (NCID), Singapore; WHO Collaborating Centre for Reference and Research on Influenza
Hospital:	National Centre for Infectious Diseases Research Clinic
Study Question:	Does recombinant vaccine result in a better antibody response than either standard egg-grown vaccine or cell-grown vaccine?
Primary objective:	To assess whether vaccination with recombinant quadrivalent influenza vaccine (QIV-R) results in a better antibody response against A(H3N2) viruses than either standard egg-grown vaccine (QIV-E). or cell-based vaccine (QIV-C) as measured by post-vaccination geometric mean titre from the haemagglutination inhibition assay
Secondary objectives	1. To estimate whether antibody responses to all 4 vaccine antigens are greatest in participants who received QIV-R and least in participants who received QIV-E, and if this reflects greater cell- to egg-grown antibody titre ratios. 2. To estimate whether QIV-R induces antibodies against a greater genetic and antigenic range of A(H3N2) viruses than QIV-C and QIV-E. 3. To estimate whether prior vaccination history has less effect on antibody responses induced against all 4 vaccine antigens by QIV-R compared with QIV-C and QIV-E. 4. To estimate whether antibody titres against all 4 vaccine antigens are better maintained up to 12 months after vaccination with QIV-R compared with QIV-C and QIV-E. 5. To estimate whether QIV-R induces higher frequencies of HA reactive B cells and/or B cells with different HA cross-reactivity profiles and phenotypes compared with QIV-C and QIV-E. 6. To assess whether QIV-R offers better protection against infection compared with QIV-C or QIV-E.
Inclusion Criteria:	1. Aged ≥21 years and <50 years. 2. Able to provide informed consent

	3. Willing and able to provide 4 blood samples: 3.1. just prior to vaccination (V1), 3.2. 14–21 days post vaccination (V2) 3.3. 4–6 months post-vaccination (V3) 3.4. 10-12 months post-vaccination (V4) 4. Has not received influenza vaccine for at least 6 months before enrolment 5. Willing to provide current mobile phone number for SMS reminders
Exclusion Criteria:	1. Known contraindication(s) for QIV (e.g. hypersensitivity to vaccine component (including eggs)). 2. Recently (last 7 days) or currently ill or has a fever above 38°C. 3. Cannot recall if they were vaccinated against influenza during more or less than two of the preceding five years. 4. Hypogammaglobulinaemia on immunoglobulin replacement 5. Undergoing immuno-suppressive therapies
Number of Planned Subjects:	360
Investigational products:	FluBlok® FlucelVax® Quad Fluarix® (or equivalent egg-grown QIV)
Safety considerations:	The risk of an adverse event associated with, influenza vaccination, blood collection and collection of nasal swab(s) is low. Any adverse event will be recorded and followed up by the project manager or principal investigator.
Statistical Methods:	Baseline comparisons: Baseline data will be summarised by vaccination group, with each arm. Analysis of the primary objective: The post-vaccination geometric mean titres (GMT) by vaccination regimen will be compared by using mixed-effects linear regression.
Subgroups:	A. Frequently vaccinated (vaccinated during 3-5 of the preceding five years) B. Infrequently vaccinated (vaccinated during 0 or 1 of the preceding five years)

2 GLOSSARY OF ABBREVIATIONS & TERMS

Abbreviation	Description (using lay language)
AE	Adverse event
AR	Attack rate
ARI	Acute respiratory infection At least one respiratory symptom (cough, sore throat) and one systemic symptom (fever, malaise, fatigue, myalgia)
AESI	Adverse event of special interest
GCP	Good clinical practice
GMT	Geometric mean titre Arithmetic mean of the logarithms (base 2) of the last positive dilution of each serum
GMR	GMTR geometric mean titre ratio Arithmetic mean of the logarithms (base 2) of the last positive dilution of each serum
HA	Haemagglutinin The surface protein of the influenza virus that is targeted by vaccines.
HAI	Haemagglutination inhibition assay Laboratory test, which measures anti-haemagglutinin antibodies. These antibodies inhibit attachment of the influenza virus to target cell membrane receptors on red blood cells.
Frequently vaccinated	3 or more prior influenza vaccinations during the past 5 years
IDRL	Infectious Diseases Research Laboratory, National Centre for Infectious Diseases, Singapore
IM	Intramuscular
Infrequently vaccinated	One or no prior influenza vaccinations during the past 5 years
ITT	Intention to treat
NCID	National Centre for Infectious Diseases, Singapore
NPHL	National Public Health Laboratory, National Centre for Infectious Diseases, Singapore

PI	Principal investigator
PICF	Participant informed consent form
PP	Per protocol
QIV	Quadrivalent influenza vaccine
QIV-C	Cell-based quadrivalent influenza vaccine
QIV-E	Egg-based quadrivalent influenza vaccine
QIV-R	Recombinant quadrivalent influenza vaccine
SAE	Serious adverse event
SAP	Statistical analysis plan
Seroconversion	Evidence of a 4-fold increase in antibody titre post-vaccination, as measured using a haemagglutination inhibition or microneutralisation assay
Seropositive	Antibody titre of ≥40, as measured using a haemagglutination inhibition assay
TTSH	Tan Tock Seng Hospital, Singapore
WHO	World Health Organization
WHOCCRRI	World Health Organization Collaborating Centre for Reference and Research on Influenza

3 STUDY SITES

3.1 STUDY LOCATION/S

Site	Address	Contact Person	Phone	Email
WHOCCRRI	792 Elizabeth St, Melbourne	Sheena Sullivan Annette Fox	93429317 93443437	Sheena.sullivan@influenzacentre.org Annette.Fox@influenzacentre.org Annette.Fox@unimelb.edu.au

National Centre for Infectious Diseases	16 Jalan Tan Tock Seng, Singapore, 308442	Barnaby Young	81334132	Barnaby_young@ncid.sg

4 INTRODUCTION/BACKGROUND INFORMATION

4.1 LAY SUMMARY

The proposed study is a prospective randomised trial of 3 influenza vaccine formulations with different manufacturing processes: 1) egg-grown; 2) cell-grown; and 3) recombinant protein. The main objective is to compare the antibody responses following influenza vaccination among these 3 vaccines to determine whether recombinant vaccines offer superior protection over standard egg or cell-based formulations. Because antibody responses to influenza vaccines can differ depending on how many times you have been vaccinated before, we will test these 3 vaccines in 2 groups: A) frequently vaccinated (3 or more vaccinations) and B) infrequently vaccinated (0 or 1 prior vaccinations) participants. Participants will be stratified by their prior vaccination history (frequent or infrequent) and randomised to 3 trial arms receiving one dose of one of the vaccines (egg-grown, cell-grown or recombinant influenza vaccines); i.e. 2 groups × 3 vaccines. Pre- and post-vaccination blood samples will be collected to assess antibody responses to vaccination and to assess changes to the cells that make antibodies. Responses to the 3 vaccines will be compared to assess which vaccine elicits the best antibody response in both infrequently and frequently vaccinated participants.

4.2 INTRODUCTION

Influenza vaccine effectiveness varies from year to year and is generally poorest against A(H3N2), the virus subtype for which genetic and antigenic evolution among circulating strains has been greatest. While vaccination may stimulate robust antibody responses to vaccine antigen, the breadth of antibodies generated may be insufficient to protect vaccinees from infection by all circulating viruses. Furthermore, in persons repeatedly vaccinated vaccine-induced antibody responses may become blunted over time.

In recent years, problems associated with egg-based production of vaccine strains have exacerbated these problems. Influenza viruses generally acquire substitutions within the hemagglutinin (HA) protein to adapt to growth in eggs. In the case of A(H3N2) viruses these adaptations often render them antigenically distinct from the wild-type virus. Subsequently, antibodies induced against egg-adapted epitopes in the vaccine will provide limited protection against infection by circulating viruses, and vaccine effectiveness has been very low. The use of vaccine manufacturing processes that avoid growth in eggs can potentially overcome egg-focusing but may not illicit a broader antibody response.

Cell-based vaccines have been developed which can overcome some of the problems associated with egg manufacturing. One cell-based vaccine product currently licensed in Australia is Sequiris' Flucelvax Quad. As with standard dose egg-based vaccines, this product contains 15µg of each of the 4 recommended influenza virus antigens. Early studies suggest that cell-based vaccine may perform better against standard-dose egg-based vaccines (1-4).

Both egg and cell-based vaccines rely on the same production method for >70 years, which depends upon the growth and purification of live viruses that must be inactivated and split before being formulated into vaccines (5-7). The chemical inactivation process requires substances that cause significant disruption to key vaccine proteins (including HA) and have been found to alter the vaccines' antigenicity via crosslinking antigens and disrupting native epitopes (5, 8-12). Disruption of key antigenic structure is likely to impact vaccine efficacy (9). Recombinant vaccines offer an alternative manufacturing pathway. The Sanofi recombinant vaccine Flublok® uses a recombinant technology to produce purified HA from a baculovirus overexpression system. The HA molecule produced in an insect cell line is in an un-cleaved form, known as HA0, i.e. a form that is unable to mediate endosomal and viral membrane fusion. Importantly the manufacturing process does not require any chemical inactivation, meaning that the HA proteins are not exposed to any potential cross-linking agents that may alter antigenicity of the vaccine (6). In addition, Flublok® contains a higher concentration of antigen than standard-dose vaccines with 45 µg of each antigen included.

Recent clinical evidence has shown Flublok® to have superior efficacy over traditional cell-based influenza vaccine (13), an immunogenicity profile comparable to Fluzone® high dose in older adults (14) and superior antibody driven viral neutralisation compared to other cell-based vaccines (1, 3, 4). Another recent comparative analysis of antibody response from healthy adults (18-49 years old), comparing egg-based, cell-based and recombinant (Flublok®) vaccines found that Flublok® resulted in significantly higher titres of neutralizing antibody (1). This study used *in vitro* neutralisation assays to evaluate the antibody function and again indicates that the recombinant vaccine may have properties that allow for better viral neutralisation compared to traditional cell-based vaccines. As such clinical efficacy gains could be associated with differences between the HA in the different vaccines.

4.2.1 STUDY OVERVIEW

This study will be conducted in 360 adults, aged 21–49 years in Singapore to generate immunogenicity data. It will be a randomized, modified double-blind study comparing the immunogenicity of recombinant quadrivalent influenza vaccines (QIV-R) against egg-based (QIV-E), and cell-based (QIV-C) vaccines. This study is powered to primarily assess the immunogenicity (as assessed by hemagglutination inhibition (HAI) geometric mean titres (GMTs) at 14–21 days post-vaccination) of QIV-R compared with QIV-E, and will also assess QIV-R compared with QIV-C.

Given the acceptable safety data generated from the millions of doses of licensed QIV-R administered in the US and elsewhere and the fact that we will source this product from the US, it is considered that the risk/benefit ratio is appropriate for the conduct of a clinical study with QIV-R.

5 AIMS AND HYPOTHESES

5.1 STUDY AIM

The broad aim of the proposed study is to estimate whether vaccination with recombinant quadrivalent influenza vaccine (QIV-R) results in a better antibody response against A(H3N2) viruses than either standard egg-grown vaccine (QIV-E) or cell-based vaccine (QIV-C).

5.2 HYPOTHESES

1. As recombinant vaccine includes a higher dose of antigen and does not use egg-based manufacturing, vaccination with recombinant vaccine results in better antibody responses, particularly against A(H3N2) viruses, than either standard egg-grown vaccines or cell-grown vaccines.
2. Vaccine induced titres against influenza A(H3N2) viruses (~ wild-type), will be highest amongst recipients of recombinant vaccine, and lowest amongst recipients of standard egg-based vaccine because antibody responses are miss-directed towards egg-adapted epitopes in the latter.
3. The dose and form of recombinant vaccine means that it induces antibodies against a broader range of epitopes than the egg- and cell-grown vaccine. In turn, this will increase the range of strains against which vaccine-induced antibodies cross-react because some of these epitopes are conserved.
4. The attenuating effects of prior vaccination (~pre-existing immunity) on vaccine immunogenicity are alleviated using recombinant vaccine because higher doses of antigen allow new B cell responses to be induced in addition to recalling memory responses.

6 OBJECTIVES AND OUTCOMES

6.1 PRIMARY OBJECTIVE

To assess whether vaccination with recombinant quadrivalent influenza vaccine (QIV-R) results in a better antibody response against A(H3N2) viruses than either standard egg-grown vaccine (QIV-E) or cell-based vaccines (QIV-C) as measured by post-vaccination geometric mean titre from the haemagglutination inhibition assay.

Corresponding outcome measures:

1. Post vaccination GMT in each vaccination group, adjusted for vaccination history and baseline titre.
2. Difference in log-titres between the QIV-R and QIV-E, adjusted for vaccination history and baseline titre.
3. Difference in log-titres between the QIV-R and QIV-C, adjusted for vaccination history and baseline titre.

6.2 SECONDARY OBJECTIVES

6.2.1 SECONDARY OBJECTIVE 1

To estimate whether antibody responses to all 4 vaccine antigens are greatest in participants who received QIV-R and least in participants who received QIV-E, and if this reflects greater cell- to egg-grown antibody titre ratios.

Corresponding outcomes:

1. Post vaccination trend in GMT QIV-R > QIC-C > QIV-E
2. Post-vaccination seropositivity and trend in seropositivity of QIV-R > QIC-C > QIV-E
3. Pre- to post-vaccine mean fold-rise in GMT (~ Geometric Mean Ratio (GMR)) and trend in fold rise of QIV-R > QIC-C > QIV-E
4. Post-vaccination seroconversion and trend in the proportion who have seroconverted of QIV-R > QIC-C > QIV-E

6.2.2 SECONDARY OBJECTIVE 2

To estimate whether QIV-R induces antibodies against a greater genetic and antigenic range of A(H3N2) viruses than QIV-C and QIV-E.

Corresponding outcomes:

1. Breadth of antibodies induced by vaccination as measured through pre-vaccination, post-vaccination and post-season serum samples tested against a landscape panel of approximately 30 influenza A(H3N2) strains that have circulated since 1968.

6.2.3 SECONDARY OBJECTIVE 3

To estimate whether prior vaccination history has less effect on antibody responses induced against all 4 vaccine antigens by QIV-R compared with QIV-C and QIV-E.

Corresponding outcomes:

For each vaccine type and vaccination history group (frequently versus infrequently vaccinated) compare:

1. Post-vaccination GMT
2. Post-vaccination seropositivity
3. Post-vaccination fold-rise in geometric mean antibody titre (GMT)
4. Post-vaccination seroconversion fraction
5. Post-vaccination antibody breadth (proportion of landscape antigens with GMT>40)

6.2.4 SECONDARY OBJECTIVE 4

To estimate whether antibody titres against all 4 vaccine antigens are better maintained up to 12 months after vaccination with QIV-R compared with QIV-C and QIV-E

Corresponding outcomes:

1. Post-season GMT
2. Post-season seropositivity
3. Proportion of landscape antigens (as per Objective 2) with GMT>40 post-season

6.2.5 SECONDARY OBJECTIVE 5

To estimate whether QIV-R induces higher frequencies of HA reactive B cells and/or B cells with different HA cross-reactivity profiles and phenotypes compared with QIV-C and QIV-E.

Corresponding outcomes:

1. Heterogeneity of antibody generating B cells in terms of phenotype and reactivity against a panel of well-defined recombinant hemagglutinin probes that represent a range of influenza A(H3N2) virus clades.

6.2.6 SECONDARY OBJECTIVE 6

To assess whether QIV-R offers better protection against infection compared with QIV-C or QIV-E.

Corresponding outcomes:

1. Attack rates in each vaccination group
2. Post-vaccination GMT in infected versus uninfected participants for each vaccination group
3. Characterisation of antibody and B cell responses

7 MONITORING OF CLINICAL SYMPTOMS AND SEQUENCING OF INFLUENZA STRAINS

7.1 STUDY DESIGN

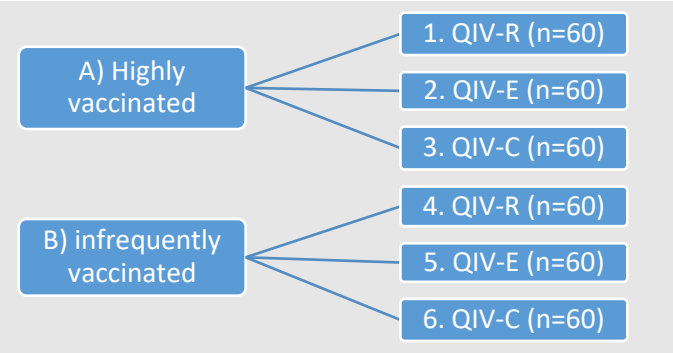
The proposed study is a single season randomized, modified investigator and participant blind study (participants & staff blinded except for clinical trial nurse) clinical trial of 360 adults aged 21-49 years with 3 arms comparing 3 vaccines. Randomisation will be stratified by vaccination history, frequently vaccinated vs. infrequently vaccinated, to compare the responses to each vaccine (Figure 1):

Group A – frequently vaccinated:

1. QIR-R
2. QIV-E
3. QIV-C

Group B –infrequently vaccinated:

4. QIR-R
5. QIV-E
6. QIV-C



	Infrequently vaccinated	Frequently vaccinated	Total
QIV-R	60	60	120
QIV-E	60	60	120
QIV-C	60	60	120
Total	180	180	360

Figure 1. Randomization schema. QIV-E: egg-based quadrivalent influenza vaccine; QIV-C: cell-based quadrivalent influenza vaccine; QIV-R: recombinant quadrivalent influenza vaccine

7.2 JUSTIFICATION OF THE STUDY DESIGN

The objectives of this study will be to assess whether vaccination with QIV-R results in a better immune response towards vaccination compared with either QIV-E or QIV-C.

The vaccines QIV-C and QIV-R used in this study are not licensed in Singapore. However, given the acceptable safety data generated from the millions of doses of QIV-R administered in the USA (under the tradename Flublok Quadrivalent), and the fact that this product will be sourced from the US, we consider that the risk/benefit ratio is appropriate for the conduct of a clinical study with QIV-R.

Some data already exist comparing QIV-R with QIV-E and QIV-C; however early formulations (pre-2013) of QIV-R were susceptible to the formation of disulphide cross-links, resulting in decreased potency during storage (loss of up to 50% potency). This was rectified through the addition of low concentrations of a reducing agent, sodium thioglycolate (STG), in combination with citrate that reduced the rate of potency loss by up to 20-fold (15, 16). HA instability would have led to suboptimal vaccine being used in clinical trials pre-2014 and could explain the initial need for an increased antigen load (45ug / strain), it could also explain why earlier results failed to show any obvious superiority over traditional vaccines. Therefore, new data are required to assess the immunogenicity of this QIV-R over QIV-E and QIV-C.

In addition, investigations to-date have largely only examined antibody titres against the vaccine antigen, and have mostly not considered vaccination history. We will investigate the whether the breadth of antibody responses varies among formulations and whether responses against each vaccine are similarly affected by prior vaccination. Prior studies, by ourselves and others, found that vaccine immunogenicity was clearly attenuated among participants who had been vaccinated during three or more of the preceding 5-years, not attenuated among participants who have one prior vaccination, and somewhat attenuated among participants with two prior vaccinations. Therefore, in this study, we will compare the immunogenicity of each vaccine among infrequently versus frequently vaccinated participants, defined as having 0-1 versus 3+ vaccinations during the preceding five-years. Participants who have been vaccinated twice will be excluded. The number of prior years considered is a pragmatic choice made to minimize the number of years that participants are asked to recall their vaccination status, while still allowing participants to be stratified into groups that are anticipated to have different vaccine immunogenicity. Participants who would normally be vaccinated

annually may have missed vaccinations during 2020-21 due to the COVID-19 pandemic, but would still be classified as highly vaccinated (3+) vaccines if vaccination status over 5-years is considered.

Lower age limit for study participants will be 21 years per the age of majority in Singapore as provided by common law.

7.3 STUDY SCHEDULE

Assessment/Procedure	Screening	Visit 1 (V1) (prior to vaccination)	Visit 2 (v2) (14 days post vaccination)	Visit 3 (V3) (6 months post vaccination)	Visit 4 (V4) (12 months post vaccination)
Study timeline	Day 0	Day 1	Day 14	Day 150	Day 330
Time window	-28 to 1d	NA	+7d	±30d	±30d
Informed Consent	X				
Collection of demographic information	X				
Weight and height measurement	X				
History of seasonal influenza vaccination	x				
Randomization	x				
Blood Collection	x[a]	x[a]	x	x	x
Vaccination		X			
Immediate surveillance (15 min)		X			
ARI surveillance[b]		D14-D360			
Swab collection[b]		D14-D360			
Study termination record					x
Collection of SAEs and AESIs[d]	Throughout study				

AESI = adverse event of special interest, SAE = serious adverse event; ARI: acute respiratory infection

a. Collection of the first blood sample to occur before vaccination, and may be taken at Screening or Visit 1 if the blood draw is not possible for one reason or another. The study team will make every effort to complete all blood-taking in one seating to avoid pricking the participants more than once.
b. Subjects will be contacted weekly by SMS to record information about acute respiratory infections and prompted to collected a nasal swab if symptomatic.
c. AESIs will have the same detailed information collected as SAEs. These include new onset of Guillain-Barré syndrome, encephalitis/myelitis (including transverse myelitis), Bell's palsy, optic neuritis, and brachial neuritis.

7.4 VACCINATION

Participants will be randomized as they are recruited to receive a single injection of one of the 3 vaccines. QIV-R is currently only manufactured to contain influenza virus strains recommended for use in northern hemisphere Influenza Vaccines. Therefore, all vaccines used in this study (QIV-R, QIV-E, QIV-C) will be northern hemisphere vaccines.

7.4.1 QIV-R (FLUBLOK®) BY IM ROUTE AT V1 (D0)

FluBlok is manufactured by Sanofi-Aventis. In the United States, it is registered for use in people aged ≥18 years. Administration is by intramuscular injection. The vaccine antigens are manufactured synthetically and recombined with a baculovirus that infects insects. The recombinant virus is grown in bulk in a qualified insect cell line. Each 0.5mL monodose pre-filled syringe contains 45µg haemagglutinin of each of antigen.

7.4.2 QIV-C (FLUCELVAX® QUAD) BY IM ROUTE AT V1 (D0):

Flucelvax Quad is a registered vaccine manufactured by Seqirus for use in people aged ≥9 years. Administration is by intramuscular injection. The vaccine antigens are prepared in a qualified cell line. Each 0.5 mL monodose pre-filled syringe contains 15µg haemagglutinin of each of the 4 recommended influenza virus strains. Product advice also indicates it may contain traces of propiolactone, cetyltrimethylammonium bromide and polysorbate 80.

7.4.3 QIV-E (FLUARIX) BY IM ROUTE AT V1 (D0)

Fluarix is a registered vaccine manufactured by GlaxoSmithKline (GSK) in Germany for use in people aged ≥6 mths and older. Administration is by intramuscular injection. The vaccine antigens are prepared in a pathogen-specific free embryonated hens eggs. Each 0.5 mL monodose pre-filled syringe contains:

- 15 µg haemagglutinin of each of the 4 recommended influenza virus strains
  Other ingredients:
- polysorbate 80 • octoxinol 10 • dl-alpha-tocopheryl acid succinate • sodium chloride • dibasic sodium phosphate dodecahydrate • monobasic potassium phosphate • potassium chloride • magnesium chloride hexahydrate • water for injections • ovalbumin (≤0.05 micrograms) • formaldehyde (≤5 micrograms) Traces of: hydrocortisone (trace) • gentamicin sulphate(trace) • sodium deoxycholate (trace)

7.4.4 VACCINE COMPOSITION

Influenza antigens included in these vaccines will be:

QIV-E	**QIV-C and QIV-R**
A/Victoria/2570/2019 (H1N1)pdm09-like virus	A/Wisconsin/588/2019 (H1N1)pdm09-like virus
A/Darwin/9/2021 (H3N2)-like virus	A/Darwin/6/2021 (H3N2)-like virus
B/Austria/1359417/2021 (B/Victoria lineage)-like virus	B/Austria/1359417/2021 (B/Victoria lineage)-like virus
B/Phuket/3073/2013 (B/Yamagata lineage)-like virus	B/Phuket/3073/2013 (B/Yamagata lineage)-like virus

7.4.5 SAFETY MONITORING

Participants will be observed for 15 minutes after vaccination for adverse events (AEs), consistent with usual vaccination practices. Participants are asked to contact the project manager to report an AE / SAE throughout the study period. Additionally, participants will be asked if they have experienced an AE at each study visit. See Section 12 for further information about the collection of AE data.

8 DATA COLLECTION

8.1 OVERVIEW OF DATA COLLECTION

The data collected from participants includes:

1. Baseline questionnaire
2. Follow-up questionnaire
3. Additional information – participant contact information
4. Blood samples
5. Respiratory swab samples
6. Symptoms diaries

	Content	**Indicators**
Baseline questionnaire (Attachment 3)	Demographics	Year of birth, sex, co-morbid conditions, current medications, prior confirmed influenza or COVID-19 infection
	Vaccination history	Years vaccinated for seasonal influenza, dates and types of COVID-19 vaccines

SMS reminders (Attachment 5)	Symptoms diaries	Weekly SMS to document any ARI events
Biodata	Blood sample	Serology: HI assay to detect antibodies to most recent circulating strains of influenza, including the 2022 influenza vaccine strains. Microneutralization assay may be performed for some A(H3N2) and B viruses if HI assay is found to have relatively poor sensitivity to detect titre rise. B-cell analysis: abundance and heterogeneity of memory B-cells induced by vaccination.
	Nasal swab	RT-PCR to identify influenza A, B and other respiratory viruses.

Figure 3. Summary of data to be collected

8.2 BASELINE QUESTIONNAIRE

A baseline questionnaire will be used to collect information about the participant including demographics, height, weight, vaccination history, and relevant medical history (Attachment 3). The past 5-years' vaccination history of the participant will be determined through self-report.

Participants will provide their email and mobile phone number, to allow the study team to send weekly SMS reminders from day 14 post-vaccination.

8.3 SMS REMINDERS

Throughout the study, participants will be sent a weekly short message service (SMS) asking whether they have experienced an ARI (at least one acute respiratory symptom or sign and one systemic symptom) in the previous 7 days (Attachment 5). Responses will be recorded in the study database and used to calculate ARI risk in the cohort at the end of the study.

Participants will also receive SMS reminders about any follow-up appointments. The use of SMS reminders has been shown to improve retention in research studies [16]. The SMS reminder will include their appointment time and the location. Participants failing to attend their appointment will be contacted by phone to reschedule the appointment.

8.4 BLOOD COLLECTION

Participants will be requested to provide blood samples at 4 time-points:

1. Pre-vaccination at Screening or V1 (D-28 to D1)
2. Post-vaccination at V2 (D14 [+7 days])
3. Post-season at V3 (D150 [+/-30 days])

4. Post-vaccination at V4 (D330+/-30 days), and prior to receiving the following years annual vaccination,

Blood collection will take place at the study sites. At each visit blood samples will be taken from a vein in the arm. Up to 30mls of blood will be drawn. This will always include 10ml in serum tubes, and for 1/3 of participants (20/arm) will also include 20ml in Sodium Heparin tubes. The serum tubes will be processed at the IDRL at NCID and will consist of centrifuging serum tubes to separate sera from clotted blood. Sodium Heparin tubes will be processed at A*Star Infectious Disease labs with whole blood leukocyte subsets analysis on NA Heparin blood, followed by separation of plasma and peripheral blood mononuclear cells (PBMCs). Sera and plasma will be stored at -20°C and four aliquots of PBMCs per sample will be stored in liquid nitrogen. Stored samples will be shared between NCID and WHOCCRRI.

8.5 RESPIRATORY SAMPLES

Participants will be provided with swab kits (swab plus vial of universal transport medium) at enrolment with instructions on how to use them (Attachment 6). During the 1-year follow-up period, if participants develop an ARI, they should perform a COVID-19 antigen rapid test (ART) and inform the study team within 24 hours of their ARI and COVID-19 ART result. Participants will be instructed to collect respiratory swab samples only if the COVID-19 ART is negative (Attachment 9). The study team will follow up with them to arrange for the respiratory swabs to be sent to the laboratory for influenza testing. Influenza-positive swabs will then be forwarded to the National Public Health Laboratory at NCID for virus characterization. Participants should store the respiratory samples in the home refrigerator until the study team collects them.

9 RANDOMISATION PROCEDURES

9.1 BLINDING AND CODE BREAKING PROCEDURES

To ensure that objective data are obtained, the study is designed as a double-blind study:

- The PI and the study staff who collect the AE data will not know which vaccine was administered;
- The subjects will be blinded with an eye mask during administration;
- The laboratory personnel who analyse the blood samples will not know which vaccine was administered;
- The research fellow performing the randomisation will be unblinded and will be responsible for performing emergency unblinding.
- The study pharmacist and nurse-vaccinator administering the vaccine will be unblinded.

Prior to randomization a dose number will be generated, which will be used to identify each vaccine for the purpose of randomization, vaccination and the recording of the vaccine administered. Dose numbers will be randomly assigned to QIV-E, QIV-C and QIV-R.

The code may be broken in the event of an AE only when the identification of the vaccine received could influence the treatment of the subject. Code-breaking should be limited to the subject(s) experiencing the AE. The blinding can be broken by the PI or a delegate. Once the emergency has

been addressed by the site, the PI will notify the HREC and the Sponsor if a subject's code was broken.

A request for the code to be broken may also be made by the HREC in the case of an SAE. In this case, the code will be broken only for the subject(s) in question. The information resulting from code-breaking (i.e., the subject's vaccine or group assignment) will not be communicated to either the PI or the immediate team working on the study.

The HREC will be notified of the code-breaking. All documentation pertaining to the event will be retained in the site's study records and in the Sponsor files. Any intentional or unintentional code-breaking must be reported, documented, and explained, and the name of the person who requested it must be provided to the Sponsor.

9.2 RANDOMISATION AND ALLOCATION PROCEDURES

Randomisation to vaccine group is stratified based on history of vaccination. Consenting participants will be identified as being in either the infrequently vaccinated group (0 or 1 prior vaccinations) or the frequently vaccinated group (3 or more prior vaccinations) and subsequently randomized within each stratum (see Figure 1)

A computer-generated randomization list will be used for labelling and packaging of doses.

Within each vaccination history stratum (frequently and infrequently vaccinated), subjects who meet the inclusion/exclusion criteria and provide informed consent will be randomly assigned to one of the 3 vaccine groups (QIV-R, QIV-E or QIV-C) in a 1:1:1 ratio. A second round of randomization will be used to assign participants in each of the 6 groups for collection of additional blood tubes in a ratio of 1:2 (1/3) until a target of 20 per group.

Site staff will enter the identification information into REDCap and confirm a minimal amount of data. The unblinded research fellow will perform the randomisation on REDCap. Both the unblinded research fellow and vaccination nurse will have access to the randomisation results on REDCap. Before administering the vaccine, the nurse vaccinator will check the vaccine group allocated to the participant by referring to the REDCap randomisation results and have the site administrator confirm it. The full detailed procedures for group allocation will be described in the manual of procedures.

If a subject is not eligible to participate in the study, then the information will only be recorded on the screening/enrolment log.

Subject numbers that are assigned by REDCap will be unique for each screened potential participant. Subject numbers will not be reassigned for any reason. The randomization codes will be stored securely in REDCap.

9.3 TREATMENT COMPLIANCE

Compliance is only measured at the time of vaccination. Any non-compliance will be documented so that it can be accounted for in the data analyses. To ensure compliance:

- All vaccinations will be administered by a qualified nurse-vaccinator;

- The project manager will maintain accountability records of product delivery to the study site, product inventory at the site, dose given to each subject, and the disposal of unused or wasted doses.

10 LABORATORY PROCEDURES

10.1 SERO-RESPONSE TO VACCINATION

Pre-vaccination, post-vaccination and post-season serum samples will be tested for antibodies to the 4 vaccine strains in QIV-R, QIV-C and QIV-E vaccines. We will primarily use haemagglutination inhibition (HI) assays to measure antibody titres against the 4 vaccine antigens. This assay measures the ability of antibodies in the blood to prevent haemagglutination—the attachment of influenza virus particles to red blood cells. Samples are serially diluted (titrated) and the highest dilution of serum that prevents haemagglutination is the HI titre. Reciprocal titres of 40 or higher are generally accepted as indicating protection against infection ("seropositivity"), with very high titres suggestive of recent infection (17). The HI assay is the standard assay used to measure antibody response to both infection and vaccination, and to assess the sensitivity of circulating influenza viruses to the vaccine.

For some A(H3N2) and B viruses, we will also use a microneutralisation (MN) to assess antibody responses to vaccination. This is a functional assay that measures the ability of antibodies to neutralize virus infectivity. Serum is mixed with virus and residual infectivity of the virus is assessed by adding the mixture to cells. The highest dilution of serum that neutralizes virus infectivity is the MN titre. Concordance between the HI and MN is high (18).

10.2 INFLUENZA TESTING AND VIRUS CHARACTERIZATION

Respiratory swabs collected from participants reporting ARI symptoms will be tested using reverse transcription real-time polymerase chain reaction (RT-PCR), according to standard operating procedures in each hospital laboratory. Influenza-positive samples will be forwarded to the National Public Health Laboratory for virus characterization. The virus subtype (for influenza A) or lineage (for influenza B) will be identified. Viruses will be isolated and tested by HI/MN or similar assay to assess antigenic match to vaccine, and sequenced to assess genetic match to the vaccine and to identify any genetic clusters.

10.3 ANTIBODY LANDSCAPES

We will build antibody landscapes (19) by testing sera in HI assay (as described in 8.1) against a range of influenza A antigens. Landscapes will consist of a panel of approximately 30 influenza A(H3N2) viruses that have circulated since 1968. Panels will include currently-circulating strains and candidate vaccine viruses to assess "future" protection, and will probably include 3-4 viruses from each antigenic cluster.

10.4 ANTIBODY ANALYSIS

Antibody analysis:

HI, MN, competitive antibody binding assays will be used to investigate differences in conformational vs non-conformational epitope recognition and stem / cleavage site reactivity. A panel of well-defined

monoclonal antibodies that span key antigenic sites will be used to investigate differences in antibody response to the vaccines. Any key differences between vaccines to be confirmed with genetically modified antigen. Any differences in stem / cleavage site reactivity to be further investigated using fusion inhibition assays.

ADCC assays will be used to investigate differences in non-neutralising antibody functionality.

10.5 B CELL ANALYSIS

PBMC will be thawed and stained with up to four fluorescent labelled recombinant HA probes representing the vaccine strain and prior A(H3N2) vaccine strains, together with monoclonal antibodies against B cell activation and differentiation markers and isotypes (IgG, IgG3, IgM, IgA, IgD). Stained samples will be analysed on a five laser Cytek Aurora. The magnitude of total HA-reactive B cell response and HA cross-reactivity profiles will be compared.

11 STUDY POPULATION

11.1 INCLUSION CRITERIA

Eligible participants will be recruited from staff members of NCID, TTSH and the general public by hospital/University newsletters, social media posts/advertising, emails and the study website, as permitted. Participants will meet the following criteria:

- Aged ≥21 years and ≤ 49 years.
- Able to provide informed consent
- Willing and able to provide 4 blood samples:
    a. just prior to vaccination (V1),
    b. 14–21 days post vaccination (V2)
    c. 4–6 months post-vaccination (V3)
    d. 10-12 months post-vaccination (V4).
- Has not received influenza vaccine for at least 6 months[1]
- Willing to provide current mobile phone number for SMS reminders

1 To maximise the recruitment of healthcare workers before the year-end flu vaccination exercise (Oct-Dec period), participants who have received their influenza vaccine less than 6 months ago may be enrolled but their vaccination visit and blood sample collection will be scheduled at least 6 months after their last influenza vaccine dose.

11.2 EXCLUSION CRITERIA

- Known contraindication(s) for QIV (e.g. hypersensitivity to vaccine component (including eggs)).
- Recently (last 7 days) or currently ill or has a fever above 38°C.
- Cannot recall if they were vaccinated against influenza during more or less than two of the preceding five years. Vaccinated during two of the preceding five years.
- Hypogammaglobulinaemia on immunoglobulin replacement
- Undergoing immuno-suppressive therapies including corticosteroids[2]

2 Received systemic immunosuppressants or immune-modifying drugs for >14 days in total within 6 months prior to Screening (for corticosteroids >/= 20 milligram per day of prednisone equivalent). Topical tacrolimus is allowed if not used within 14 days prior to Day 1. Individuals who are immunocompromised (e.g. active leukemia or lymphoma, generalised malignancy, aplastic anaemia, solid organ transplant, bone marrow transplant, current radiation therapy congenital immunodeficiency, HIV/AIDS with CD4 lymphocyte count < 200).

11.3 RECRUITMENT PROCEDURES

The study will seek to recruit hospital staff working at NCID, TTSH and the general public. It will be promoted via poster advertisements, as well as sharing of digital copies of the poster via emails and on NCID/TTSH and/or A*STAR websites (Attachment 1 and 2). Participants who are interested in participating in the study may directly contact the study team via the contact details provided on the advertising poster.

A study team member will go through the eligibility criteria and informed consent procedures. Baseline data collection and blood draws will take place in a private location located in or adjacent to the clinic. To facilitate recruitment, the baseline blood draw may occur up to four weeks prior to vaccination.

The recruitment of infrequently vaccinated people will be reinforced by using the “snowball sampling” strategy in which existing study participants can refer other potential participants from among their acquaintances.

Participants will be reimbursed 50$ at each visit for their time and travel costs.

11.3.1 ASSIGNING PARTICIPANT IDS

At baseline, participants will be requested to provide their name, surname and their date of birth to enable linkage to clinical records. Once the necessary data has been abstracted, identifying information, including name and date of birth, will be removed from the working data file and records will only be identifiable by their participant ID. A master list linking participant IDs and clinical records will be stored by the PI.

11.4 RETENTION STRATEGIES

To retain participants during the study period, we will use the following strategies:

1. Weekly reminders through SMS or email to report ARI symptoms;
2. MS Outlook calendar invites and SMS reminders for scheduled follow-up appointments;
3. Provide prompt feedback about the results of influenza testing.

11.5 FOLLOW-UP

At enrolment, participants will be requested to provide a mobile phone number and email address. Follow-up appointment reminders will be sent via email and/or SMS.

In addition, participants will be given a participant ID card at enrolment (Attachment 8). This is so that participants will have at hand their participant ID, the date of influenza vaccination (specific to them) and contact numbers for questions/concerns.

11.6 Informed Consent

A copy of the participant information and consent form (ICF; Attachment 4) will be provided to all participants and written informed consent will be sought from all participants prior to enrolment in the study. Participation in the study will be voluntary. Participants will be informed that they are free to withdraw from the study at any time. The concept of voluntary participation will be clearly explained, and ample time will be given to ensure that participants understand the content and have all of their questions answered. Willing participants will sign the informed consent form to indicate assurance of their understanding and voluntary participation in the research. Informed consent will be administered by the PI or delegated Co-I who will also sign their name. A copy of the signed and dated consent form will be given to the participant. This process will be documented in the participant's study file. Personal information will not be released without written permission of the participant, except as necessary for monitoring by the Human Research Ethics Committee. The project manager and site PI's contact details will be made available to participants should they have any queries or concerns during the study. Signed informed consent forms will be stored in the study office in a locked filing cabinet.

12 Participant Safety and Withdrawal

12.1 Potential risks to participants

Study investigators and institutions are committed to protecting personal health information through the maintenance of privacy and security of each subject's personal information in this study. To protect confidentiality, we will use a study assigned number instead of personal information on study forms and we will store data in locked files and/or secured computers. If information from this study is presented publicly or published in a medical journal, results will be presented using aggregate statistics; individuals will not be identified by name or by any other personally identifiable information. Only specific and authorized researchers (e.g. project manager) in this study may see personal health information but will not disclose personally identifying information about individual participants to others. A participant's general practitioner will be notified the participant is enrolled in the study.

12.1.1 Possible Reactions to Blood Draw

There are no major risks associated with giving blood. Having a blood sample taken may cause some discomfort when the needle is inserted, and some people experience bruising, minor infection or bleeding. Blood will be collected by trained healthcare staff using a butterfly and small gauge needle. Any complications related to blood sample collection will be reported to the project manager, and investigated by study staff.

12.1.2 Possible Reactions to Vaccination

Vaccine injection into the deltoid muscle may cause transient discomfort. Immediate and potentially life-threatening allergic reactions to the vaccine could be manifested by adverse events (AEs) such as laryngeal edema, asthma, or hypotension. These types of reactions are exceedingly rare and would most likely occur in persons with a severe reaction to influenza vaccine in the past. Therefore, people with known contraindications, including prior allergic reaction to vaccine, will be excluded from the study. All participants will be required to wait for 15 minutes after vaccination to document any AEs, including AEs of special interest (AESIs), arising during this period.

12.1.3 POSSIBLE REACTIONS TO RESPIRATORY SWAB COLLECTION

Collection of respiratory swabs can be associated with some pain and discomfort, especially if the nostril is already sore or irritated due to illness. Clear instructions will be provided to participants on how to self-collect the swab to minimise discomfort. Any complications related to respiratory swab collection should be reported to the project manager, and will be investigated by study staff.

12.2 RISK MANAGEMENT AND SAFETY

12.2.1 DEFINITIONS

Adverse Event (AE): Any untoward medical occurrence in a participant enrolled into this study regardless of its causal relationship to study treatment. An adverse event can therefore be any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Adverse events are classified as serious or non-serious.

Serious Adverse Event (SAE): An SAE is defined as any event that:

- results in death; or
- is immediately life threatening; (the term "life-threatening" refers to an event/reaction in which the participant was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe); or
- requires inpatient hospitalisation or results in prolongation of existing hospitalisation; or
- results in persistent or significant disability/incapacity; or
- is a congenital anomaly/birth defect; or
- is a medically important event or reaction. or
- requires inpatient hospitalisation; or
- results in persistent or significant disability/incapacity.

Important medical events that might not be immediately life-threatening or result in death or hospitalisation may be considered an SAE when, based upon appropriate medical judgement, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

New Safety Finding: means any (other than reportable individual case safety report (ICSR)) safety issue that may require expedited reporting because providing information that may lead to a change in the known risk-benefit balance for the product and as mentioned, but not limited to, in the following regulatory texts: Europe: Good Pharmacovigilance Practices, modules VI and VII; and US: FDA: 21 CFR Parts 312 Investigational New Drug Application- Section 312.32, (c) (1) IND safety reports.

Adverse Event of Special Interest ("AESI") means an adverse event (serious or non-serious) of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the Investigator may be appropriate. Such events may require further investigation in order to characterise and understand them. AESIs may be added or removed during a Study by protocol amendment.

12.2.2 Eliciting Adverse Event Information

Adverse events will be recorded from the time the participant signs the Informed Consent Form until 14 days after each visit.

12.2.3 Assessment and Documentation of Adverse Events

The project manager is responsible for recording all adverse events, regardless of their relationship with the exposure, with the following exceptions:

- Conditions that are present at screening and do not deteriorate will not be considered adverse events.
- Abnormal laboratory values will not be considered adverse events unless deemed clinically significant by the investigator and documented as such.

Adverse events will be recorded using the Adverse Events Report Form, which will record:

- A description of the AE;
- The onset date, duration, date of resolution;
- Severity (mild, moderate or severe);
- Seriousness (i.e. is it an SAE?)
- Any action taken (e.g. treatment, follow-up tests);
- The outcome (e.g. recovery, death, continuing, worsening);
- The likelihood of the relationship of the AE to the exposure being investigated (e.g. Unrelated, Possible, Probable, Definite).
- Whether and when reported to the HREC

All AEs will be followed to adequate resolution.

12.2.4 Serious Adverse Event Reporting

Any SAE occurring in a study participant will be reported to study site. The SAE reporting form will be completed, signed and submitting by an investigator. All SAE reporting forms will be reported to the HREC by email to HrecCorrespondence@mh.org.au in line with the Melbourne Health Research policy.

All SAEs and AESIs, pregnancy and overdose, regardless of the investigator's causality must be reported to Sanofi within one (1) business day of becoming aware of the event.

Sanofi Global Pharmacovigilance
Email: PV.outsourcing@sanofi.com or Fax: to +33 (0) 4 37 37 71 32

And copy in the Australian Sanofi Product Safety Department:
Email: ae@sanofi.com

All SAEs should be submitted to the appropriate Regulatory Authority (TGA) in accordance with the applicable timelines and reporting requirements.

12.3 Handling of Withdrawals

Participants may withdraw from the study at any time for any of the following reasons: participant has chosen to withdraw from the study, protocol violation, or participant has experienced an adverse event.

Information collected to that point will be kept unless the participant explicitly requests otherwise. This information will be used for comparison with participants who complete all study procedures.

All participants will be included in the study analyses, so it is important to have information on as many participants as possible. If a participant wishes to withdraw from the study, every reasonable effort will be made to complete a final evaluation of participants who exit the study early and the reason(s) for withdrawals will be recorded in the participant's study records.

12.4 REPLACEMENTS

The sample recruited allows for some attrition. However, we will attempt to replace participants who fail to attend their first follow-up (V2).

12.5 POTENTIAL BENEFITS TO SUBJECTS

All participants will receive a quadrivalent influenza vaccination. Participants should benefit from coverage against influenza and may be less likely to catch influenza or develop complications during the study period.

Participants will be vaccinated against the influenza A/H1N1, A/H3N2 and the B strains from both the Victoria and Yamagata lineages chosen and recommended by WHO for the composition of QIVs for the 2022–2023 NH season.

Regarding immunogenicity, QIV-R is expected to induce a higher immune response against the 4 influenza strains compared to QIV-C or QIV-E. Therefore, QIV-R is likely to bring an increased benefit versus QIV-C or QIV-E in terms of immunogenicity against the 4 influenza strains with a risk-benefit profile that is expected to be favourable.

13 STATISTICAL METHODS

13.1 RANDOMISATION

Subjects will be stratified based on history of vaccination and randomised into the three vaccine groups using a block-randomisation scheme.

13.2 SAMPLE SIZE ESTIMATION & JUSTIFICATION

The sample size is calculated considering the primary objective to test whether QIV-R offers superior immunogenicity independent of vaccination history. Our estimates for post-vaccination HI titres are based on a study among elderly adults in Hong Kong, comparing QIV-R and QIV-E (20) and is similar to the estimated sample size based on similar HI titres in a study of 18-49 year olds (21).
A sample was simulated under the following model, informed by the published estimates:

$$titre_{t=2} = titre_{t=1} + vax_R + vax_C + priorvax + vax_R \times priorvax + vax_C \times priorvax$$

($titre_{t=2}$ = post vaccination log titre; $titre_{t=1}$ = post-vaccination log titre for QIV-E infrequent vaccination; vax_R = QIV-R vaccinated; vax_C = QIV-C vaccinated)

For the test of a superior A(H3N2) response among vaccinees receiving QIV-R versus QIV-E or QIV-C (one-sided test for the parameter vax_{recomb}) and assuming α=0.05, β=0.2, the sample needed to test these effects is around 55 per arm per vaccination history. Accounting for 10% attrition, the target sample for recruitment will be 60 per arm per vaccination history.

	Infrequently vaccinated	Frequently vaccinated	Total
QIV-R	60	60	120
QIV-E	60	60	120
QIV-C	60	60	120
Total	180	180	**360**

13.3 POWER CALCULATIONS

Power calculations were simulation-based. The following model was used for the simulations:

$$logtitre_{t=2} = b_0 + b_{baseline}\, logtitre_{t=1} + b_{priorvax}\, priorvax + b_C\, vax_C + b_R\, vax_R + b_{C\text{-}add}\, vax_C\, priorvax + b_{R\text{-}add}\, vax_R\, priorvax + e$$

The following table contains the interpretation of all model terms.

Term	Interpretation
$logtitre_{t=2}$	Post-vaccination log-titre.
b_0	Expected post-vaccination log-titre for baseline log-titre 0, infrequent prior vaccination, vaccinated with QIV-E.

$b_{baseline}$	Expected increase in the post-vaccination log-titre for a 1-unit increase in the pre-vaccination log-titre. Adjusted for prior vaccination frequency and vaccine received.
$logtitre_{t=1}$	Pre-vaccination log-titre.
$b_{priorvax}$	Expected difference in the post-vaccination log-titre between the frequently and infrequently vaccinated. Adjusted for baseline titre.
priorvax	0=Infrequent prior vaccinations. 1=Frequent prior vaccinations and vaccine received.
b_C	Expected difference in post-vaccination log-titre between QIV-E and QIV-C. Adjusted for baseline titre and prior vaccination frequency.
vax_C	0=Not vaccinated with QIV-C. 1=Vaccinated with QIV-C.
b_R	Expected difference in post-vaccination log-titre between QIV-E and QIV-R vaccine. Adjusted for baseline titre and prior vaccination frequency.
vax_R	0=Not vaccinated with QIV-R. 1=Vaccinated with QIV-R.
$b_{C\text{-}add}$	Expected additional effect of QIV-C in the frequently vaccinated.
$b_{R\text{-}add}$	Expected additional effect of QIV-R in the frequently vaccinated.
e	Random error

The following table contains the values used for the parameters in the model (subsequent tables will show deviations if there are any).

Term	Value
b_0	1.1
$b_{baseline}$	0.8
$b_{priorvax}$	-0.2
b_C	0.25
b_R	0.5
$b_{C\text{-}add}$	0
$b_{R\text{-}add}$	0.2

In each simulation there was an equal number of subjects in each arm and vaccination history group. Baseline log-titres were simulated from N(3.7, 1) which produced a distribution representative of the baseline titre data of published studies. The error term was sampled from *N(0, 1)* which produces post-vaccination titre distribution representative of published studies.

The model simulates a subject's post-vaccination titre given their pre-vaccination titre, prior vaccination status (frequent or infrequent) and the vaccine they were given (QIV-R, QIV-C, QIV-E). The effect of the vaccines was allowed to vary between frequently and infrequently vaccinated.

Simulations were performed with different per-group sample sizes and different effects of QIV-R (b_R). For the purposes of fitting the model to the simulated data, all titres were censored to the appropriate interval (<10, 10-20, 20-40 up to 5260-10240 on the original titre scale) to simulate real measurements. The model was then fit using the midpoints of the intervals except <10 which was censored to 5 (as it would be for real data).

There were 5000 simulations for each sample size and value of b_R. Each simulated dataset was used to fit a model (the same one that was used to simulate it). The primary outcome measure was taken to be the effect of the QIV-R as compared to the QIV-E (i.e., the expected difference in post-vaccination log-titres between the QIV-R and QIV-E,) which corresponds to the b_R parameter in the model. A one-sided and a two-sided test was performed on the b_R estimate for each simulation. The "power" is the proportion of simulated studies that showed that b_R is significantly greater than 0. The results are in the table below.

Per-arm per-vaccine history sample size	True b_R (expected ratio between QIV-R and QIV-E)	Proportion with significant (one-sided) positive b_R	Proportion with significant (two-sided) positive b_R
50	0.5 (1.65)	0.772	0.665
55	0.5 (1.65)	0.810	0.712
60	0.5 (1.65)	0.832	0.741
70	0.5 (1.65)	0.889	0.807
80	0.5 (1.65)	0.920	0.858
200	0.25 (1.28)	0.775	0.676
220	0.25 (1.28)	0.810	0.717
240	0.25 (1.28)	0.834	0.742

Power of 80% can be achieved (for a one-sided test) with approximately 55 subjects per arm/per vaccine history given the true effect of 0.5 (true expected ratio 1.65). The total sample required would be approximately 330 subjects. This equates to 74% power for a two-sided test.

13.4 STATISTICAL ANALYSES

The stratification variable of vaccination history will be included as a covariate in models for all primary and secondary objectives.

13.4.1 PRIMARY OBJECTIVE

To assess whether vaccination with recombinant quadrivalent influenza vaccine (QIV-R) results in a better antibody response against A(H3N2) viruses than either standard egg-grown vaccine (QIV-E) or cell-based vaccine (QIV-C) as measured by post-vaccination geometric mean titre from the haemagglutination inhibition assay

Post-vaccination GMTs will be calculated for each group using the model described in 13.3, in which the outcome is an individual's post-vaccination titre, estimated given their pre-vaccination titre, prior vaccination status (frequent or infrequent) and the vaccine they were given (QIV-R, QIV-C, QIV-E). A t-test (on a model parameter) will be used to assess statistical significance of the effects of QIV-R versus QIV-E and QIV-C.

13.4.2 SECONDARY OBJECTIVE 1

To estimate whether antibody responses to all 4 vaccine antigens are greatest in participants who received QIV-R and least in participants who received QIV-E, and if this reflects greater cell- to egg-grown antibody titre ratios.

The post-vaccination vs pre-vaccination **log-titre difference** (geometric mean ratio) will be compared using linear regression with the explanatory variable being the type of vaccine (reference QIV-E, separate parameter for QIV-R and QIC-C). A t-test will be used to assess statistical significance of the effects of QIV-R and QIC-C (as compared to reference) as well as the difference in those effects.

The post-vaccination **seropositivity** (coded as 1 if the post-vaccination titre is at least 40, 0 otherwise) will be compared using logistic regression with the explanatory variable being the type of vaccine (reference QIV-E, separate parameter for QIV-R and QIC-C). A t-test will be used to assess statistical significance of the effects of QIV-R and QIC-C (as compared to reference) as well as the difference in those effects.

Seroconversion (coded as 1 if the post/pre vaccination titre ratio is at least 4, 0 otherwise) will be compared using logistic regression with the explanatory variable being the type of vaccine (reference QIV-E, separate parameter for QIV-R and QIC-C). A t-test will be used to assess statistical significance of the effects of QIV-R and QIC-C (as compared to reference) as well as the difference in those effects.

The trend will be determined using the estimate of the effect of the cell vaccine and the estimate of the effect of the recombinant vaccine for each of these outcomes. The expected trend is the cell vaccine producing better outcomes than the egg vaccine (estimate of the effect of the cell vaccine should positive) and the recombinant vaccine producing better outcomes than the cell vaccine (the difference of estimates of the effect of recombinant and cell vaccines should be positive).

13.4.3 SECONDARY OBJECTIVE 2

To estimate whether QIV-R induces antibodies against a greater genetic and antigenic range of A(H3N2) viruses than QIV-C and QIV-E.

The breadth of antibodies induced by vaccination will be measured using pre-vaccination and post-vaccination serum samples tested against a landscape panel of approximately 30 influenza A(H3N2) strains that have circulated since 1968. A metric for response breadth (e.g., the proportion of viruses with post/pre vaccination titre ratio of at least 4) will be calculated for each individual and compared using linear regression as for secondary objective 1.

13.4.4 SECONDARY OBJECTIVE 3

To estimate whether prior vaccination history has less effect on antibody responses induced against all 4 vaccine antigens by QIV-R compared with QIV-C and QIV-E.

All analyses for objective 1 and 2 will be repeated including a covariate to indicate the vaccination history group (0=infrequently vaccination; 1=frequently vaccinated). An interaction term between vaccine received and prior vaccination will be included to allow vaccine effect to vary by history of vaccination. Infrequently vaccinated is defined as 0 or 1 prior vaccinations; frequently vaccinated is defined as 3 or more prior vaccinations with any influenza vaccine.

13.4.5 SECONDARY OBJECTIVE 4

To estimate whether antibody titres against all 4 vaccine antigens are better maintained up to 12months after vaccination with QIV-R compared with QIV-C and QIV-E

Post-season log-titres and seropositivity will be compared the same way post-vaccination log-titres and seropositivity are compared in objective 1.

Post-season response breadth will be using the same methods described for objective 2.

13.4.6 SECONDARY OBJECTIVE 5

To estimate whether QIV-R induces higher frequencies of HA reactive B cells and/or B cells with different HA cross-reactivity profiles and phenotypes compared with QIV-C and QIV-E.

The percentage of B memory phenotype (CD27+ and/or IgD-) cells that are HA probe reactive on d14 will be estimated for each vaccine arm, accounting for pre-vaccination percentage and vaccination history as described in 13.3. The HA probe cross-reactivity, isotype, and phenotype of HA reactive B cells for each vaccine group and time-point will be visually compared using t-distributed stochastic neighbor embedding (t-SNE), a statistical method for visualizing high-dimensional data by giving each datapoint a location in a two or three-dimensional map.

13.4.7 SECONDARY OBJECTIVE 6

To assess whether QIV-R offers better protection against infection compared with QIV-C or QIV-E.

Evidence of influenza infection will be based on RT-PCR-confirmed infection, only, as serological evidence may be biased in vaccinees who elicit a good antibody response to vaccination (22).

Attack rates will be calculated for each vaccination group as the number of cases during the person-time at risk. If there are sufficient cases, the proportion testing positive for each vaccine will be compared using logistic regression with influenza infection as 1 if test-positive and 0 if test-negative or not tested and no ARI. The explanatory variable with be the type of vaccine (1=QIV-R, 2=QIC-C, 3=QIV-E). Samples size permitting, we will also explore whether attack rates were associated with the post-vaccination GMT.

14 STORAGE OF BLOOD AND TISSUE SAMPLES

14.1.1 DETAILS OF WHERE SAMPLES WILL BE STORED, AND THE TYPE OF CONSENT FOR FUTURE USE OF SAMPLES

14.1.1.1 Serum samples storage and shipping

Blood samples collected in serum tubes will be centrifuged within 24 hours of collection to separate the clotted blood from the serum and the serum removed to a clean tube. The clotted blood may be discarded. Sera will be aliquoted into separate tubes, the number of which will depend on the volume recovered but is expected to be around 3 aliquots of approximately 500-1000µl each. One serum aliquot (~1 ml will be shipped to WHOCCRRI for serology). One will be stored at -20°C, while the remaining aliquots will be stored in a -70°C freezer or in liquid nitrogen. All freezers are locked and located in secured areas.

Written consent will also be obtained from participants for use of their samples for future studies. All participant information will be de-identified and a Human Research Ethics Committee would have to approve such future studies before stored samples can be analysed.

14.1.1.2 Peripheral Blood Mononucleocytes (PBMCs) storage and shipping

Blood samples collected in Sodium Heparin tubes will be processed for recovery of PBMCs on the same day or within 24 hours of collection. The recovered cells will be aliquoted in to ~4 tubes. Each aliquot will be labelled and cryogenically stored in liquid nitrogen at the recruitment site until the end of each study year. The cryogenic storage facility is in secured area at A*Star Infectious Disease Labs. Half of the aliquots for each sample will be shipped to WHOCCRRI for B cell analysis.

Respiratory swabs will be tested for influenza at the National Public Health Laboratory at NCID. Media from swabs that test positive for influenza virus will be aliquoted into two tubes and stored at -70C. One aliquot will be inoculated into established cell lines to obtain influenza isolates

Influenza isolates will be shipped to the WHOCCRRI for antigenic and genetic testing. Isolates will be assessed using antigenic assays such as the HI assay (see 10.2). Both original specimens and isolates may be genetically sequenced (note that viral RNA will be sequenced, not human DNA). Both the original specimen and any isolates recovered will be stored in -70°C freezers, according to NATA-approved SOPs at the WHOCCRRI. All freezers are locked and located in secured areas. Samples will be stored indefinitely using study numbers.

14.1.1.3 Consent to storage of specimens for future related research

Samples will be stored indefinitely and may be retested as new technologies for understanding immunological responses to respiratory virus infection and/or vaccination become available. During the informed consent process participants will be asked if their samples can be stored for future studies.

15 DATA SECURITY & HANDLING

15.1 DETAILS OF WHERE RECORDS WILL BE KEPT & HOW LONG WILL THEY BE STORED

15.1.1 STUDY DATABASE

A study database will be established and managed using the REDCap electronic data capture tool developed by Vanderbilt University and hosted at NCID. REDCap provides detailed information about the security measures they have taken to secure data storage https://projectredcap.org/software/mobile-app/. Briefly, data are transmitted using secure, encrypted transmission (SSL/HTTPS). Additional validation checks are also used when communicating with the server. The REDCap Mobile App employs encryption-at-rest on the mobile device's hard drive so that all important data and information stored on the device is properly protected from unauthorized or malicious users.

If during the course of the study it is determined that other software may better meet the needs of the study, the team may migrate the study database to different software.

15.1.2 DATA ENTRY

Data collected in questionnaire and generated from laboratory assays will be collected in paper form or electronically. Participants will be able to enter responses to the questionnaire directly using a mobile device. This will avoid the need for transcribing data from paper questionnaires and enable the use of validity checks to ensure that impossible or improbable entries are avoided. Paper questionnaires may also be used if there are technical issues at the study site. The project manager will enter the data into the study database.

15.1.3 DATA STORAGE

Hard copies of any information collected (e.g. informed consent) will be stored in locked filing cabinets in the PI's office. Electronic copies of data collected will be stored using the password protected study database.

All research data will be stored for a minimum of 15 years after completion of the project. After fifteen years electronic records will be erased unless further approval for retention is obtained. Individual participants will not be identifiable from the presented or published material. The PIs will be responsible for all data management.

15.2 CONFIDENTIALITY AND SECURITY

Participant confidentiality is strictly held in trust by the participating investigators, research staff, and the sponsoring institution and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party, without prior written approval of the sponsoring institution. Authorized representatives of the sponsoring institution may inspect all documents and records required to be maintained by the PI, including but not limited to, medical records (office, clinic or hospital). The clinical study site will permit access to such records. All laboratory specimens, evaluation forms, reports and other records that leave the site will be identified by the participant ID number only to maintain participant confidentiality. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by HREC or regulatory agencies.

16 ATTACHMENTS

Document Name	Version Number	Date
1. Poster	V1.0	08 Jun 2022
2. NCID poster	V1.0	05 Aug 2022
3. CRF	V4.0	16 Sep 2022
4. ICF	V4.0	27 Oct 2022
5. Symptoms questionnaire	V1.0	15 Aug 2022
6. Nasal swab instructions	V1.0	10 Aug 2022
7. Acknowledgement form	V1.0	15 Aug 2022
8. Participant ID card	V1.0	06 Sep 2022
9. ARI reporting guide	V1.0	28 Sep 2022

17 REFERENCES

1. Gouma S, Zost SJ, Parkhouse K, Branche A, Topham DJ, Cobey S, et al. Comparison of Human H3N2 Antibody Responses Elicited by Egg-Based, Cell-Based, and Recombinant Protein-Based Influenza Vaccines During the 2017-2018 Season. Clin Infect Dis. 2020;71(6):1447-53.
2. Izurieta HS, Chillarige Y, Kelman J, Wei Y, Lu Y, Xu W, et al. Relative Effectiveness of Cell-Cultured and Egg-Based Influenza Vaccines Among Elderly Persons in the United States, 2017-2018. J Infect Dis. 2019;220(8):1255-64.
3. Levine MZ, Martin ET, Petrie JG, Lauring AS, Holiday C, Jefferson S, et al. Antibodies Against Egg- and Cell-Grown Influenza A(H3N2) Viruses in Adults Hospitalized During the 2017-2018 Influenza Season. J Infect Dis. 2019;219(12):1904-12.
4. Wang W, Alvarado-Facundo E, Vassell R, Collins L, Colombo RE, Ganesan A, et al. Comparison of A(H3N2) neutralizing antibody responses elicited by 2018-2019 season quadrivalent influenza vaccines derived from eggs, cells, and recombinant hemagglutinin. Clin Infect Dis. 2020.
5. Kelly HG, Kent SJ, Wheatley AK. Immunological basis for enhanced immunity of nanoparticle vaccines. Expert Rev Vaccines. 2019;18(3):269-80.
6. Cox MM, Hashimoto Y. A fast track influenza virus vaccine produced in insect cells. J Invertebr Pathol. 2011;107 Suppl:S31-41.
7. Luo M. Influenza virus entry. Adv Exp Med Biol. 2012;726:201-21.
8. Bonnafous P, Nicolai MC, Taveau JC, Chevalier M, Barriere F, Medina J, et al. Treatment of influenza virus with beta-propiolactone alters viral membrane fusion. Biochim Biophys Acta. 2014;1838(1 Pt B):355-63.
9. Furuya Y, Regner M, Lobigs M, Koskinen A, Mullbacher A, Alsharifi M. Effect of inactivation method on the cross-protective immunity induced by whole 'killed' influenza A viruses and commercial vaccine preparations. J Gen Virol. 2010;91(Pt 6):1450-60.
10. Hegde NR. Cell culture-based influenza vaccines: A necessary and indispensable investment for the future. Hum Vaccin Immunother. 2015;11(5):1223-34.
11. Kon TC, Onu A, Berbecila L, Lupulescu E, Ghiorgisor A, Kersten GF, et al. Influenza Vaccine Manufacturing: Effect of Inactivation, Splitting and Site of Manufacturing. Comparison of Influenza Vaccine Production Processes. PLoS One. 2016;11(3):e0150700.
12. She YM, Cheng K, Farnsworth A, Li X, Cyr TD. Surface modifications of influenza proteins upon virus inactivation by beta-propiolactone. Proteomics. 2013;13(23-24):3537-47.
13. Dunkle LM, Izikson R, Patriarca P, Goldenthal KL, Muse D, Callahan J, et al. Efficacy of Recombinant Influenza Vaccine in Adults 50 Years of Age or Older. N Engl J Med. 2017;376(25):2427-36.
14. Ng TWY, Cowling BJ, Gao HZ, Thompson MG. Comparative Immunogenicity of Enhanced Seasonal Influenza Vaccines in Older Adults: A Systematic Review and Meta-analysis. J Infect Dis. 2019;219(10):1525-35.
15. Hickey JM, Holtz KM, Manikwar P, Joshi SB, McPherson CE, Buckland B, et al. Mechanism of a decrease in potency for the recombinant influenza A virus hemagglutinin H3 antigen during storage. J Pharm Sci. 2014;103(3):821-7.
16. Holtz KM, Robinson PS, Matthews EE, Hashimoto Y, McPherson CE, Khramtsov N, et al. Modifications of cysteine residues in the transmembrane and cytoplasmic domains of a recombinant hemagglutinin protein prevent cross-linked multimer formation and potency loss. BMC Biotechnol. 2014;14:111.
17. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond). 1972;70(4):767-77.
18. Laurie KL, Engelhardt OG, Wood J, Heath A, Katz JM, Peiris M, et al. International Laboratory Comparison of Influenza Microneutralization Assays for A(H1N1)pdm09, A(H3N2), and A(H5N1) Influenza Viruses by CONSISE. Clin Vaccine Immunol. 2015;22(8):957-64.

19. Fonville JM, Wilks SH, James SL, Fox A, Ventresca M, Aban M, et al. Antibody landscapes after influenza virus infection or vaccination. Science. 2014;346(6212):996-1000.
20. Cowling BJ, Perera RAPM, Valkenburg SA, Leung NHL, Iuliano AD, Tam YH, et al. Comparative Immunogenicity of Several Enhanced Influenza Vaccine Options for Older Adults: A Randomized, Controlled Trial. Clinical Infectious Diseases. 2019;71(7):1704-14.
21. Dunkle LM, Izikson R, Patriarca PA, Goldenthal KL, Muse D, Cox MMJ. Randomized Comparison of Immunogenicity and Safety of Quadrivalent Recombinant Versus Inactivated Influenza Vaccine in Healthy Adults 18-49 Years of Age. J Infect Dis. 2017;216(10):1219-26.