DOES REPEATED INFLUENZA VACCINATION CONSTRAIN INFLUENZA IMMUNE RESPONSES AND PROTECTION?

PROTOCOL ADDENDUM FOR FOLLOW-UP OF COVID-19 INFECTIONS AND VACCINATION

4 March 2021

Version 2

[redacted]

Sponsor: Melbourne Health

Funding: US National Institutes of Health (Administrative Supplement to Award:1R01AI141534)

Statement of Compliance

This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

1 TABLE OF CONTENTS

2 GLOSSARY OF ABBREVIATIONS AND TERMS

Abbreviation	Description (using lay language)
ARI	Acute respiratory illness. Defined as one or more of the following symptoms include fever ≥37.8°C, headache, body aches, cough, sore throat, runny nose, sputum.
COVID-19	Coronavirus disease
SARS-CoV-2	Name of the virus causing coronavirus disease
HCW	Health care worker. Any personnel eligible for the free vaccination programme run at participating hospitals or health services. Personnel may include staff, including administrative, research, clinical and support services, employed at the participating hospital. It may also include volunteers, students or honorary staff.
GMT	Geometric mean titre. Arithmetic mean of the logarithms (base 2) of the last positive dilution of each serum.
MN assay	Microneutralization assay. A laboratory test which measures the ability of antibodies to neutralize virus infectivity.
PBMC	Peripheral blood mononuclear cell. A type of white blood cell that contains a single lobed nucleus.
RT-PCR	Real-time reverse transcriptase polymerase chain reaction. A laboratory test used to make many copies of a specific genetic sequence for analysis and can be used to diagnose disease.
Seropositive or seropositivity	Antibody titre of ≥40, as measured using a haemagglutination inhibition assay.
Sero-conversion	4-fold rise in antibody titre, as measured using a haemagglutination inhibition assay.
qPCR	Quantitative PCR
WHOCCRRI	World Health Organization Collaborating Centre for Reference and Research on Influenza.

3 INTRODUCTION

Our existing protocol describes a study to recruit and follow a longitudinal cohort of healthcare workers (HCW) at 6 geographically dispersed Australian hospitals to understand why immunogenicity and effectiveness appear to attenuate with repeated administration of the influenza vaccine. We will recruit 1500 HCW and follow them for up to 4 years. We will collect sera at baseline (prior to influenza vaccination), 14-21 days post-vaccination and at the end of the influenza season (~7 months post). We will conduct weekly syndromic surveillance for acute respiratory illnesses (ARI), and request self-collected swabs from HCWs when they experience ≥2 respiratory or ≥ 1 systemic + ≥1 respiratory symptoms. We will calculate influenza attack rates, assess correlates of protection, conduct detailed immunological assays, and use mathematical modelling to interpret dynamic antibody responses and assess the effectiveness of vaccination programs.

Given the similarities between cases presenting with influenza and cases presenting with COVID-19, our funding agency has requested that we add on surveillance research activities to enhance our understanding of the COVID-19 pandemic. Our cohort presents a number of opportunities for epidemiological, virological and immunological investigations of COVID-19. Other novel coronavirus outbreaks, including SARS-CoV and MERS-CoV, have been characterized by nosocomial transmission,[1-3] and while this does not appear to be the main driver of transmission for COVID-19,[4] it is likely that our ARI surveillance will detect some COVID-19 cases in HCWs.

With the availability of COVID vaccines (CoVax), studies comparing CoVax brands (Pfizer, AstraZeneca, other) are needed and likely to be numerous. Studies that investigate the cellular and molecular basis for any differences in antibody responses against the CoVax brands may be rarer. In addition, there may be limited analysis of responses to CoVax and influenza vaccination, whether the sequence of vaccines matters, and if so why.

We are already collecting sera around April and November, which will enable assessment of the asymptomatic infection rate. We intend to follow up HCW experiencing ARI to document the duration of symptoms and the illness outcome (e.g. GP visit, hospitalization, days absent from work). Importantly, we have already assembled a team ready to follow up HCW experiencing an influenza illness, and require little additional infrastructure to follow up HCW experiencing a COVID-19 illness or a HCW who has been vaccinated for COVID-19

This protocol describes additional surveillance activities directed at leveraging our existing cohort to improve our understanding of the epidemiology of the COVID-19 epidemic, the immunological responses to SARS-CoV-2 infection and vaccination, as well as further understand the virological characteristics.

4 SIGNIFICANCE OF THE PROPOSED RESEARCH

Completion of the proposed research will provide valuable insights into the virological and immunological dynamics of SARS-CoV-2 infection outcomes. We will estimate risk factors for infection and correlates of protection. We will identify drivers of transmission, identify transmission clusters and how they drive the COVID-19 epidemic, and explore virus evolution. We will conduct extensive exploratory immunological analyses to further generate hypotheses about immunological responses to SARS-CoV-2 infection and/or vaccination. An improved understanding of the risk factors and distinct

immunological profiles in people with asymptomatic, mild and severe disease will be useful for developing vaccines and therapeutics.

5 AIMS AND OBJECTIVES

Our overall aim is to increase our understanding of the epidemiological, virological and immunological characteristics of SARS-CoV-2 infections and/or vaccination in HCWs.

5.1 Objective 1: To estimate risk factors and correlates of protection for SARS-CoV-2 infection

Although there is limited understanding of the immune response to SARS-CoV-2, previous work from SARS suggests that protective immunity is induced following the production of protective antibodies.[5] We hypothesize that few HCWs will have antibodies to SARS-CoV-2 at baseline, but that exposure to COVID-19 patients will increase the risk of infection. Progression to severe disease (hospitalization) is expected to be associated with age, and pre-existing conditions, based on analyses from China. We additionally hypothesize that workplace risks, antibody titre to SARS-CoV-2 and other seasonal coronaviruses, the magnitude and kinetic of development of T and B cell responses, and viral load will also predict severity of infection.

5.1.1 Sub-objectives

1. To estimate the asymptomatic, mild and severe attack rates of COVID-19
2. To estimate risk factors for infection
3. To identify differences in antibody titres between HCWs with asymptomatic, mild and severe disease
4. To estimate the antibody titre associated with protection against SARS-CoV-2 infection (correlates of protection)

5.1.2 Outcomes

Completion of this aim will result in estimates of the symptomatic and asymptomatic attack rates, the hospitalisation risk estimates and identification of risk factors for severe disease. We

1. Estimated symptomatic COVID-19 attack rate based on PCR testing
2. Estimated asymptomatic attack rate based on seroconversions
3. Case-hospitalization risk
4. Risk factors for severe outcomes (death or intensive care or hospitalization versus asymptomatic or mild illness)
5. Estimated protective antibody titres
6. Identification of key behavioural drivers of transmission

5.2 Objective 2: To characterize viruses infecting HCW

We will estimate various aspects of within-host virus dynamics in a subset of HCW. We hypothesize that mildly ill HCW will experience a shorter duration of shedding, lower viral loads and less within-host virus evolution compared with severely ill HCWs.

5.2.1 Sub-objectives:

1. To characterize viral kinetics
2. To estimate the degree of within-host virus evolution
3. To identify the presence of transmission clusters

5.2.2 Outcomes

1. Estimated average duration of viral shedding and viral load over time and correlation with severity
2. Estimated speed of within-host virus evolution
3. Within-site similarities in viruses recovered from HCWs

5.3 Objective 3: To characterize immunological profiles following infection by SARS-CoV-2

We will conduct additional immunologic assays on subsets of our cohort to characterize the dynamics and types of innate and adaptive immune responses induced, and how these relate to viral load and severity. This will include analysis of gene expression, focusing on acute transcriptional profiles to characterize and compare innate anti-viral immunity; and quantitation of SARS-CoV-2 reactive B and T cells to determine the kinetics of adaptive immune responses, and to identify proteins and epitopes that are dominant targets of these responses. This will give us an opportunity to understand the contribution of innate and adaptive immune responses to severity. We hypothesize that there will be a greater involvement of memory cells in acute responses to SARS-CoV-2 among HCW with mild versus severe infection. Additional hypotheses will be generated from these investigations as more is learned about this novel infection.

5.3.1 Sub-objectives:

1. To characterise antibody kinetics during the course of infection
2. To examine changes over time in frequency and effector function of the B and T cell populations induced by infection
3. To identify commonly recognised virus epitopes and whether they are targets of neutralizing antibodies
4. To identify genes that are differentially expressed during the acute innate response compared to a post-recovery time-point.

5.3.2 Outcomes

1. Estimated post-infection serum antibody titres over time
2. Enumeration of SARS-CoV-2-reactive B and T cells and identification of dominant epitopes
3. Exploratory immunological findings to characterize the response to SARS-CoV-2 infection

5.4 Objective 4: To characterize immunological profiles following vaccination for SARS-COV-2

We will conduct additional immunologic assays on subsets of our cohort to characterize the dynamics and types of innate and adaptive immune responses induced by vaccination.

5.4.1 Sub-objectives:

1. To measure and compare antibody responses to Adenovirus vector versus RNA formulations of COVID-19 vaccines
2. To characterise and quantify innate immune responses induced by different COVID-19 vaccine formulations as well as influenza protein vaccine
3. To characterize and quantify vaccine specific B and T cell responses induced by different COVID-19 vaccine formulations as well as influenza protein vaccines

5.4.2 Outcomes

1. Estimated post-vaccination serum antibody titres over time for each vaccine formulation.

2. Enumeration of innate immune subsets.
3. Identification of genes that are differentially expressed on d7 compared to d0 for each vaccine formulation, focusing on innate immune associated genes
4. Enumeration of SARS-CoV-2-reactive B and T cells.
5. Estimation of the relationship between antibody responses and innate, and B and T cell responses induced by each vaccine formulation
6. Comparison of antibody (and B and T cell) responses induced against COVID-19 and influenza vaccines among participants who received COVID-19 versus influenza vaccine first or who were co-administered both vaccines.

6 STUDY DESIGN

6.1 Overview of Study Design

The proposed study is a longitudinal cohort study (Figure 1). This study will collect data, blood and respiratory specimens from approximately 1500 HCWs to understand responses to influenza vaccination.

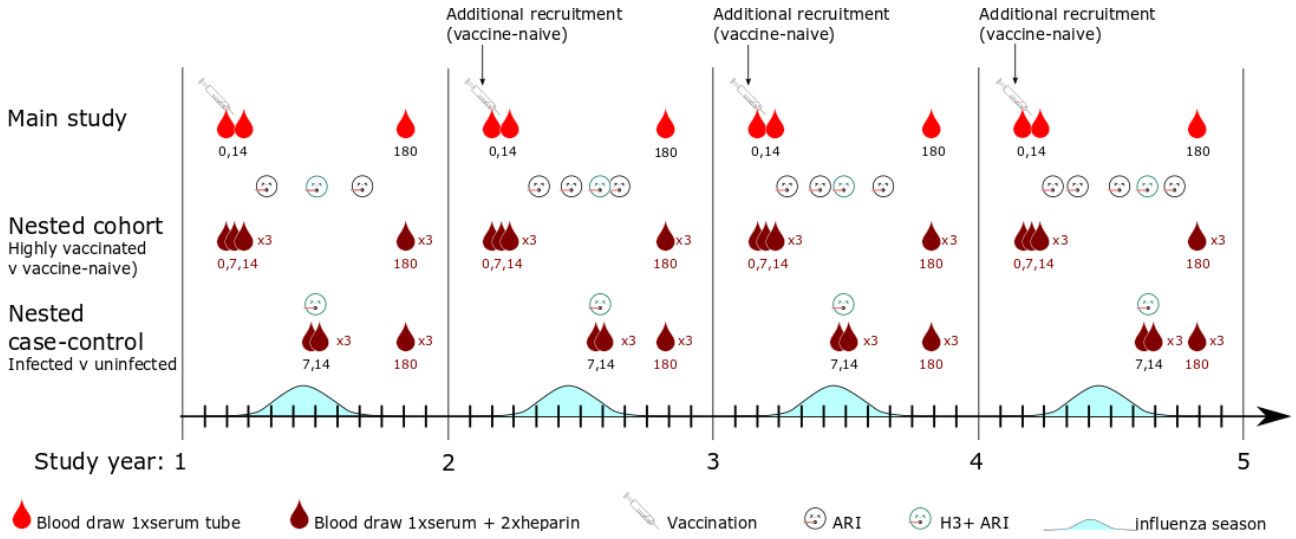


Figure 1. Study flowchart for recruitment in the main study.
We plan to recruit around 1500 HCWs, and expect that this will consist of at least 100 HCWs in each of the vaccination groups; the majority are likely to be frequent vaccinees. The expected proportion of each group is described in 9.1.

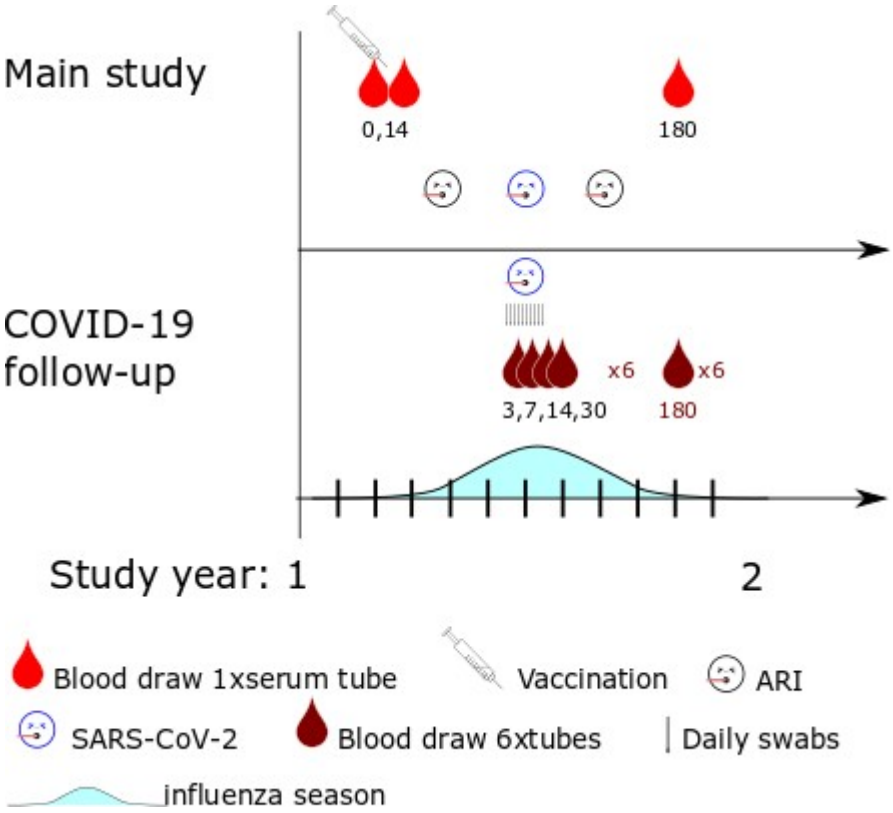


Figure** 2. **Study flowchart for follow-up of COVID-19 infections among HCW.
Up to 4 additional blood draws may be scheduled and additional daily swabs may be requested. These activities only pertain to HCWs identified with SARS-CoV-2 infections.

6.2 Study Schedule

Follow-up of COVID-19 infections will be conducted for at least the first year of recruitment, and may continue into the second year of follow-up if infections continue to be identified.

Follow up of COVID-19 vaccinations will be conducted in year two of the study or when vaccines become available, and may continue into subsequent years if vaccination is required periodically.

7 DATA COLLECTION

7.1 Overview of data collection

The additional data that will be collected from participants with COVID-19 infection includes:

1. Blood samples
2. Daily respiratory and rectal swab samples

For participants who are vaccinated for SARS-CoV-2, additional blood samples and vaccine details will be collected.

At each recruitment site, the same general procedures for obtaining informed consent and collection of specimens will be followed. A summary of the number of additional visits and procedures at each visit is below.

Table 1. Additional study visits for participants testing positive for SARS-CoV-2 (does not show study visits described in the main protocol).

	Assessment/ Procedure	Daily	d3	d7	d14	d30	End of season
Procedures	Serum blood collection		x	x	x	x	X*
	Heparin blood (Absolute counts, PBMCs, plasma)		x	x	x	x	x
	PAXgene		x			x	
	Symptoms diary	x					
	Respiratory swabs	x					
	Rectal swabs	x					

*Serum will be collected anyway as part of the main study.

Table 3. Additional study visits for participants who receive SARS-CoV-2 vaccination and are in the main COVID-19 vaccination study (does not show study visits described in the main protocol).

Assessment/ Procedure	d0	d14-21 post dose 2 (booster)
Serum blood collection	x	x

Table 4. Additional study visits for participants who receive SARS-CoV-2 vaccination and are in the nested COVID-19 vaccination study (does not show study visits described in the main protocol).

Assessment/ Procedure	d0	D7*	D14*
Serum blood collection	x	x	x
Heparin blood (Absolute counts, PBMCs, plasma)	x	x	x
PAXgene	x	x	

***** post dose 2 (booster)

7.2 Blood sampling

7.2.1 Convalescent blood collection after resumption of hospital duties

We expect that most HCW will be able to return to work within 14 days and that we should be able to safely collect convalescent blood draws. For the majority of SARS-CoV-2-infected HCWs, we will request:

1. 3 post-infection blood draws
    a. d14
    b. d30
    c. end of season
2. Collection of up to 54ml of blood at each time point

7.2.2 Acute blood collection

For a subset of HCWs who test positive for SARS-CoV-2, additional blood samples will be requested during the acute infection period.

1. 2 additional post-infection
    a. d3
    b. d7
2. Collection of up to 54ml of blood at each time point

7.2.2.1 Home visits

In the likely event that a HCW has not returned to work by d3 or d7 these blood collections will be done in the home. Home visits will be prearranged by a member of the study team. PPE will be provided for team members going to a participant's home for blood collection. Blood collection will proceed as normal.

7.2.3 SARS-CoV-2 vaccination blood collection

For HCWs who are vaccinated for SARS-CoV-2, we will request:

1. 1 pre-vaccination blood draw (1 x 8 ml serum tube, +/- 2 x 9 ml Na Heparin tubes, 1 x PAXgene tube)
2. 1-2 post-vaccination blood draws (after the 2nd dose or otherwise completed course of SARS-CoV-2 vaccination)
   a. d7(1 x 8 ml serum tube, 2 x 9 ml Na Heparin tubes, 1 x PAXgene tube)
   b. d14-d21 (1 x 8 ml serum tube, +/- 2 x 9 ml Na Heparin tubes)

7.2.4 Timing of blood collection

For all groups, the exact timing of the post-vaccination bleed(s) and post-infection bleed(s) may change as more data about optimal timing become available. Where the number of days is specified, some leeway is permitted. For example, blood samples taken 7 days post-infection may be taken 6-12 post; samples taken 14 days post-vaccination or infection may be taken 14-21 days post.

7.2.5 Blood collection procedures

Blood will be collected in a combination of serum, sodium heparin and PAXgene tubes.

As outlined in the main protocol, blood collection will be performed in line with each hospital's phlebotomy policy. The use of a butterfly needle connected to a vacutainer tube is recommended as the best collection method to minimize haemolysis and to reduce the risk of needle stick injury. Blood collection will take place in a private room or behind a screen.

7.2.6 Blood sample processing and storage

Blood samples will be processed and stored at each recruitment site, as per the main protocol.

7.3 Enhanced swabbing

7.3.1.1 Respiratory specimen collection

Many questions remain unanswered about viral clearance, but longer duration of shedding appears to be associated with severity of symptoms.[6] We will request HCWs to provide daily swabs while they remain symptomatic and for at least 2 days after symptoms have subsided.

Swab kits will either be mailed in express post parcels or delivered by a member of the study team to the HCW's door while s/he is in isolation. Swabs in universal transport medium can be stored in the home refrigerator until the HCW resumes duties. Swabs will be stored at the site and later forwarded to the laboratory in Melbourne for assessment.

7.3.1.2 Rectal and other swabs

SARS-CoV-2 has been detected in the faecal samples of Chinese patients many days after respiratory swabs ceased to recover virus.[7] Therefore, we may request rectal swabs from some of our participants

as these samples may later prove to be important for understanding the role of virus replication in the gut for transmission. Instructions will be provided (Appendix H).

8 LABORATORY PROCEDURES

8.1 Coronavirus testing and virus characterization

Swabs collected from HCWs reporting ARI symptoms will be tested using reverse transcription real-time polymerase chain reaction (RT-PCR), according to standard operating procedures [redacted]. If the hospital cannot test, swabs will be forwarded to [redacted] or another diagnostic laboratory for testing.

All swabs will be forwarded to [redacted] for further virus characterization, including genetic sequencing.

8.2 Sero-response to infection and identifying asymptomatic infections

Pre-vaccination (~April) and post-season serum samples will be tested for antibodies to SARS-CoV-2 using commercial ELISA kits or serological assays developed in-house. Antibodies to other coronaviruses will also be tested.

8.2.1 Reporting of SARS-CoV-2 RT-PCR results

If a HCW is identified as having SARS-CoV-2 through the study, the site manager will receive RT-PCR results from their relevant diagnostic laboratory and enter the results into the study database. Participants will be informed of their test results following the standard protocol at the hospital at the time.

8.2.2 Quantitative PCR

Consecutive swabs taken over several days will be tested by quantitative PCR to assess the viral load.

8.3 B and T cell assays

A combination of ELISPOT and flow cytometry will be used to enumerate SARS-CoV-2-reactive B and T cells, to characterize proportions that produce antibodies or key cytokines, and to identify proteins and epitopes that are dominant targets of these cells. T cells will be detected following brief (re)stimulation with (pseudo)virus or overlapping peptides whereas B cells will be detected using recombinant viral proteins, either directly ex-vivo or following in vitro differentiation into antibody secreting cells.

8.4 Other immunological assays

Other immunological assays will be explored as more information becomes available about the SARS-CoV-2 virus.

8.5 Sero-response to vaccination

Pre- and post-vaccination serum samples will be tested for antibodies to SARS-CoV-2 spike protein using commercial ELISA kits and serological assays developed in-house.

8.5.1 Reporting of SARS-CoV-2 vaccination results

Participants will receive results of their antibody response to vaccination (Appendix I).

9 DATA CONSIDERATIONS

9.1 Sample Size

We have no prior information on which to predict how many HCWs will be infected with SARS-CoV-2. Some groups have estimated 20% infection rates among their staff, which could mean 300 infections in our cohort of 1500.

9.2 Data Analysis

All statistical analyses will be performed in the statistical software package R.

9.2.1 Incidence of COVID-19 disease

9.2.1.1 Symptomatic attack rate

Symptomatic attack (incidence) rates will be calculated as the number of cases testing positive by RT-PCR during the person-time at risk.

9.2.1.2 Incidence proportion

Serological evidence of infection will be based on a 4-fold rise in antibody titres between baseline and any later time point. The incidence proportion will be calculated as the number of HCWs with evidence of sero-conversion among all HCWs followed during the same period.

9.2.1.3 Asymptomatic incidence proportion

The asymptomatic incidence proportion will be calculated as the number of HCWs with evidence of sero-conversion and no ARI reported among all HCWs followed during the same period.

9.2.1.4 Hospitalization risk

The hospitalization risk (or incidence proportion) will be calculated as the number of HCWs hospitalized due to COVID-19 among all HCW with either asymptomatic or symptomatic evidence of infection during the same period.

9.2.2 Risk of severe illness

The predictors of severe infection will be estimated using a Cox proportional hazards regression model comparing the risk of COVID-19 illness (coded as 1 for hospitalised or 0 for infected but not hospitalised) among HCWs. If there are sufficient cases, various predictors of severity will be explored in either univariate or multivariate analysis. Predictors may include age, presence of comorbidities, and viral load.

9.2.2.1 Duration of illness and predictors of illness duration

The number of days ill with COVID-19 (count) will be calculated and the risk factors for longer durations of illness will be explored. Because of the excess of 0 counts (people who never get infected), zero-inflated negative binomial regression will be used.

9.2.3 Correlates of protection

We will compare post-season geometric mean titres between those with asymptomatic and symptomatic infections. We will attempt to establish serological correlates of protection for SARS-CoV-2, using a Bayesian implementation of logistic regression that we have used for influenza cohort studies.[8]

9.2.4 Social contacts analysis

Using social contacts data, we will attempt to infer the transmission dynamics for our HCW participants between each round of sample collection. We will use mathematical models social mixing data with infection risk to untangle specific behaviours/contact scaling that may be driving transmission.[9,10] These models may be extended to include genetic sequencing data, which has been previously used to reconstruct transmission clusters.[11]

9.3 Analyses relevant to Aim 2

9.3.1 Duration of viral shedding

For HCW identified as SARS-CoV-2-positive, we will request frequent self-collected swabs while in self-isolation to estimate the duration of shedding up to day 15 post-infection. We will compare the estimated average duration of shedding among HCW with different risk profiles and illness outcomes, using regression methods.

9.3.2 Viral dynamics

We will use quantitative PCR (qPCR) to estimate the viral load over time to understand how long people remain infectious and corroborate these data with daily symptoms diaries to better understand whether viral load is positively associated with symptoms.

Viral load will be included in analyses comparing asymptomatic, mild and severe infections. If possible we will explore the interactions of viral load with demographic (e.g. age) or medical (e.g. heart disease) characteristics.

9.3.3 Viral evolution

Using samples collected from individual HCWs over time, we will use deep sequencing and bioinformatics models to examine the within-host evolution of SARS-CoV-2 viruses.

9.4 Analyses relevant to Aim 3

9.4.1 Antibody kinetics

Sera collected more frequently will be assessed for antibody titre and the titres compared over time. Geometric mean titres will be calculated and plotted to allow visual inspection of the antibody kinetics, overall and within groups (e.g. age groups, severity of infection). The mean rate of decay will be calculated using linear regression. Because little is known about the decay kinetics, various models will be explored to identify the model with best fit, based on visual inspection of the data and model fitting diagnostics.

9.5 Analyses relevant to Aim 4

Mean antibody concentration will be calculated and compared for vaccine groups (CoVax vs influenza vaccine). Seroconversion post-vaccination will be calculated and compared between vaccine groups by logistic regression. Antibody levels will be correlated with fold changes in innate immune cells and in vaccine specific B and T cells detected at d14-21 versus d0.

10 REFERENCES

1. Chowell G, Abdirizak F, Lee S, et al. Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. *BMC Med* 2015; **13**: 210.
2. Lau EH, Hsiung CA, Cowling BJ, et al. A comparative epidemiologic analysis of SARS in Hong Kong, Beijing and Taiwan. *BMC Infect Dis* 2010; **10**: 50.
3. Varia M, Wilson S, Sarwal S, et al. Investigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto, Canada. *CMAJ* 2003; **169**(4): 285-92.
4. World Health Organization. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) Beijing: World Health Organization, 2020.
5. Liu W, Fontanet A, Zhang P-H, et al. Two-Year Prospective Study of the Humoral Immune Response of Patients with Severe Acute Respiratory Syndrome. *The Journal of Infectious Diseases* 2006; **193**(6): 792-5.
6. Liu Y, Yan L-M, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19. *The Lancet Infectious Diseases*.
7. Wu Y, Guo C, Tang L, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *The Lancet Gastroenterology & Hepatology*.
8. Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. *BMC Med Res Methodol* 2010; **10**: 18.
9. Kucharski AJ, Kwok KO, Wei VW, et al. The contribution of social behaviour to the transmission of influenza A in a human population. *PLoS Pathog* 2014; **10**(6): e1004206.
10. le Polain de Waroux O, Flasche S, Kucharski AJ, et al. Identifying human encounters that shape the transmission of Streptococcus pneumoniae and other acute respiratory infections. *Epidemics* 2018; **25**: 72-9.
11. Robert A, Kucharski AJ, Gastañaduy PA, Paul P, Funk S. Probabilistic reconstruction of measles transmission clusters from routinely collected surveillance data. *medRxiv* 2020.

11 APPENDICES

Appendix H Rectal swab collection instructions

HCW Cohort Study

Rectal Swab Collection Instructions

If you have tested positive for COVID-19, please read the instructions below carefully on how to collect a rectal swab.

You may take a swab using the home kit provided and either return it when you come to work or send via mail.

Included with this kit

- Swab
- Transport tube
- Specimen bag
- Participant ID labels

Before starting

- Wash your hands before taking the swab
- Peel open the package and remove the swab and transport tube
- Label the transport tube with your name, date of birth, participant ID number (use label provided) and the specimen collection date

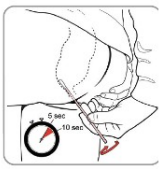
Rectal swab

1. Remove the swab from the plastic and be sure not to touch the tip or lay the swab down.

2. Do not hold the swab past the breakpoint

3. Carefully insert the swab into your rectum about 3-5cm past the outside of the anus and gently rotate the swab for 5 to 10 seconds

4. Withdraw the swab without touching your skin



5. Place the swab in the transport tube and bend to snap at the breakpoint. Tightly screw the cap onto the tube.

What to do next?

1. Place the specimen transport tube in the specimen bag
2. Wash your hands thoroughly using soap and warm water
3. Store in the fridge until ready to deliver or post it to the study staff, preferably within 72 hours
4. Phone or email the study team to let them know you have collected the specimen

Remember, if you have any questions or problems with collecting the specimen, call your study team on [*enter contact number*]

Appendix I Template of COVID antibody results for participants

Insert site specific logo(s) here

[redacted]

SARS-CoV-2 Serology Analysis

Summary of Sera Received and SARS-CoV-2 Antibody Results

Participant ID: _____ **Report date:** _____

Thank you for your participation in this study. Below are the results of your response to vaccination with the SARS-CoV-2 vaccine.

Vaccine received	Pre-vaccination result	Post-vaccination result
	Sera date: dd/mm/yy	Sera date: dd/mm/yy
[*insert vaccine name*]		

Interpreting your antibody results

SARS-CoV-2 antibody levels were measured using an Enzyme-Linked Immunosorbent Assay (ELISA). Cut-offs for classifying assay values as positive (>1.1), negative (< 0.8) or indeterminate (0.8-1.0) are designed to maximize assay sensitivity and specificity, but are not absolute. It is therefore possible that antibody is present in some samples with values below 1.1.

- **Negative result**: SARS-CoV-2 antibody test value is below 0.8 (low)
- **Indeterminate result**: SARS-CoV-2 antibody test value is between 0.8 and 1.0 (intermediate)
- **Positive result**: SARS-CoV-2 antibody test value is above 1.1

These results are provided for research/surveillance information only and should not be used for clinical management.

If you have any queries regarding this report please contact the study coordinator or principal investigators listed below.
[*insert site coordinator, PI names, contact phone number and email*]

HCW Cohort Study – Template of COVID antibody results, Version 1, Date: 4 March 2021