University of BRISTOL

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The LOGIC Study

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Bristol Royal Hospital For Children

NHS University Hospitals Bristol and Weston NHS Foundation Trust

RESEARCH PROTOCOL

Title	**LOGIC Study** **LO**n**GI**tudinal Study of **C**OVID-19: Symptoms, Virology & Immunity
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1 ABSTRACT

The evolving coronavirus disease (COVID-19) pandemic is expected to become the deadliest in over 100 years. The causative agent of COVID-19 is the virus SARS-CoV-2. Preventing the infection is contingent upon understanding the natural history of SARS-CoV-2 infection, to inform the rational design of public health control measures and the development of an effective vaccine. However, at present there are few published data that describe the relationship between the symptoms, viral and immunological dynamics in order to infer the typical characteristics of the infection. We will help address this gap in our understanding by performing a longitudinal cohort study among approximately 135 frontline healthcare workers at the Children's Emergency Department (CED) of the Bristol Royal Hospital for Children. Staff who consent to participate will be asked to complete an online pro forma to record baseline demographic data, and any symptoms suggestive of COVID-19 and/or contact with individuals suspected/confirmed to have COVID-19. In order to track the dynamics of symptoms longitudinally, participants will be asked to complete a brief daily online symptom diary for 3 months and to provide information on their potential exposure to SARS-CoV-2. In order to track viral dynamics, participants will be asked to perform twice weekly self-sampling of saliva, nose and throat swabs when working 'on shift' (i.e. when present in the hospital) for a period of 3 months. During any period of self-isolation and/or illness, sampling will be facilitated by means of "doorstep drop-off and pick-up" of swabs at the participant's home whenever feasible. In addition, blood samples will be collected at weeks 0, 6, 12 and 18 of the study with a follow-up sample at 1 year, to evaluate the immune response to SARS-CoV-2. This study design will allow us to describe the typical relationships between symptoms, mucosal viral load and the development of immunity in a group of individuals likely to be frequently exposed to SARS-CoV-2. The collaborative and multidisciplinary Bristol UNCOVER Group (Bristol University COVID19 Emergency Research Group) is ideally resourced to perform this study having expertise in performing large-scale longitudinal studies of upper respiratory viral and bacterial infections and mucosal immunity (Finn lab) and including one of the few research laboratories in the UK who have the necessary approvals and experience to work directly with live SARS-CoV-2 (Matthews/Davidson lab). The data generated by this study will be a key piece in the jigsaw that informs effective public health and vaccination programmes, to minimise the devastating and far-reaching global effects of COVID-19 and future pandemics.

2 STUDY SUMMARY

Study Title	**LOGIC** (**LO**n**GI**tudinal Study of **C**OVID-19): Symptoms, Virology & Immunity	
Short Title	LOGIC Study	
Study Design	Longitudinal observational cohort study	
Study Participants	Frontline clinical staff at the Children's Emergency Department (CED) of the Bristol Royal Hospital for Children	
Planned Sample Size	135 participants (90% participation of 150 staff pool)	
Planned Study Period	Recruit participants (by mid-May 2020) Sample collection and assay development (by mid-August 2020) Perform viral & immunology assays (by end October 2020) Follow-up blood sampling at 18 weeks and one year (by end April 2021) Completion of all immunology assays (Winter 2021)	
	Objective	**Outcome Measures**
Primary	Describe in detail the temporal relationships between symptoms, mucosal viral load, and immunological responses (potential correlates of protection)	Collect sufficient symptom data and samples to allow temporal correlation analysis of changes in symptoms, mucosal viral load, serum antibody levels, and peripheral antigen-specific T cell enumeration
Secondary	Develop laboratory protocols to safely measure viral load in upper respiratory tract samples; antibodies; antigen-specific T cell frequency and function. Establish whether PCR testing of saliva samples is comparable to that of nose/throat swabs Establish whether HCWs become PCR test positive before developing symptoms; if so for how long and at what viral density Establish predictive value of negative PCR tests in symptomatic health care workers Establish the nature, magnitude & longevity of the antibody and memory T cell response	Protocols yielding reproducible results in line with ACDP/HSE laboratory safety standards for sample collection, transport, storage and laboratory analysis Compare rates of SARS-CoV-2 positivity in saliva with throat and nose swabs Compare changes in viral load and symptoms over time Compare changes in viral load and symptoms over time Measure serum antibody levels and peripheral antigen-specific memory T cells over time
Inclusion Criteria	Frontline clinical staff working regularly at the CED of Bristol Royal Hospital for Children	
Exclusion Criteria	- Staff who on average work less than 2 shifts per week - Staff who anticipate that they will not able to complete at least 6 weeks of the study, excluding annual leave e.g. about to go on maternity leave.	

3 BACKGROUND AND SCIENTIFIC JUSTIFICATION FOR RESEARCH

3.1 The COVID-19 global health emergency

In December 2019, the World Health Organisation (WHO) was alerted to an outbreak of atypical pneumonia in the city of Wuhan, Hubei province, China. Within two weeks, the genetic sequence of the causative pathogen, a novel betacoronavirus was publicly available (1), and subsequently named SARS-CoV-2 (severe acute respiratory distress syndrome coronavirus 2). The clinical infection resulting from SARS-CoV-2 is known as coronavirus-induced disease (COVID-19). As of April 2020, since its emergence, COVID-19 has been responsible for over 100,000 deaths globally, threatening the most significant public health emergency in the last 100 years.

3.2 Reducing COVID-19 mortality

Reducing mortality associated with COVID-19 is dependent on: i) development of a vaccine capable of inducing long-lasting and durable immunity; ii) public health control measures that reduce transmission; and iii) developing treatments that can prevent ARDS associated with COVID-19. Achieving the first two of these will be contingent on understanding the basic tenets of SARS-CoV-2 infection and immunity. However, at present there are no published data that describe the longitudinal relationship between COVID-19 symptoms, and viral and immune dynamics in detail. Understanding such factors during the Ebola outbreak (West Africa 2014-6) was a key factor in developing evidence-based public health control measures and accelerating vaccine development (2).

3.3 Symptom dynamics in SARS-CoV-2 infection

The spectrum of clinical presentation of COVID-19 is broad. Many infected individuals are asymptomatic or have mild symptoms similar to the common cold, but nonetheless transmit the virus (3-5). Reported symptoms include fever, cough, runny nose, sore throat, sticky/red eye, loss of taste and smell, headache, weakness, muscle pain, drowsiness, abdominal pain, diarrhoea, and vomiting (6). Severely affected individuals exhibit pneumonia associated with acute respiratory distress syndrome (ARDS) resulting in significant mortality, despite prolonged ventilatory support. Observational cohort studies have identified that increasing patient age, chronic respiratory and cardiovascular disease, obesity, diabetes, and hypertension are all risk factors associated with poor outcomes (7). There are currently no good quality population-based longitudinal cohort data dynamically describing symptoms. Various initiatives to crowd-source population level data have begun, including the COVID Symptom Tracker (King's College London) (8). While these initiatives will help inform epidemic progression, they cannot provide detailed dynamic/longitudinal assessment of symptoms in a cohort of specific individuals, nor are they systemically associated with individual assessments of SARS-CoV-2 virology and immunity.

3.4 Viral dynamics in SARS-CoV-2 infection

The diagnosis of acute COVID-19 infection is made by tests that detect SARS-CoV-2 genomic material, namely reverse transcriptase polymerase chain reaction (RT-PCR) in respiratory tract secretions (9, 10). The WHO have endorsed a list of in-house and commercial assays from multiple countries utilising primers against multiple gene targets, including ORF1ab and N, ORF1b-nsp14 and N, spike protein gene and RdRP (11). It appears that SARS-CoV-2 is detectable in multiple human specimens, including oropharyngeal (throat) or nasopharyngeal swabs, saliva, bronchoalveolar lavage, blood (serum/plasma), faecal and cerebrospinal fluid samples (12-14). The detection rate of SARS-CoV-2 does not appear to be significantly different between oropharyngeal and nasopharyngeal swabs (15). In a detailed virological analysis of 9 hospitalised patients, all swabs were RT-PCR-positive by day 5 post-symptom onset (15). Swabs remained positive by RT-PCR for up to 28 days post-symptom onset, but live virus isolation was not possible after day 8 post-symptom onset (15). Such data help clinicians understand the accuracy of virological tests and also help public health professionals design control measures such as self-isolation and shielding. However, there are currently no longitudinal data describing the dynamics of viral detection in a specific cohort of pre-symptomatic and asymptomatic individuals. Furthermore, it is not understood how the character, size, and duration of viral shedding relate to immunity.

3.5 Immune dynamics in SARS-CoV-2 infection

Following infection, the immune system develops specific responses and immunological memory, broadly divided into cellular (T cell) and antibody (B cell) immunity. A range of antigen-specific effector and memory T and B cells are produced specific for different epitopes of the responsible pathogen. Depending on the epitope and timing, antibodies produced by plasma cells (mature B cells) have differing functional properties such as avidity, ability to neutralise virus and opsonophagocytic activity for large pathogens such as bacteria and fungi. Likewise, antigen-specific T cells directed against certain epitopes are more likely to be capable of helping B cells to produce durable antibody responses or promote direct T cell effector function against virus-infected cells. Measurement of the effector and memory responses directed towards specific antigens expressed by pathogens can therefore correlate with protection against re-infection (correlates of protection). Antibody responses are much the most widely used as markers of previous exposure to an infection and correlates of protection following infection or vaccination. Detection of antigen-specific T cells are also important both because B cells depend on CD4+ T cell help for durable antibody responses and because CD8+ T cell responses contribute to viral elimination and long-term immunological memory. T cell and antibody responses to immunodominant epitopes of SARS-CoV-2, their quantitative and temporal relationships with symptoms and viral shedding and correlation with protection have not been characterised in detail at this time. Defining such correlates of protection will assist: i) estimation of the duration of natural immunity; ii) accurate identification of individuals who are immune to COVID-19, and can potentially resume unrestricted activities during the pandemic; iii) evaluation of immunity generated by candidate vaccines; iv) further understanding of the epidemiology of the infection.

It is possible that immunity to SARS-CoV-2 will not prove long-lasting, a problem that would be compounded by selection pressure driving SARS-CoV-2 mutation, as has occurred for seasonal coronavirus OC43 (16). A human challenge infection experiment involving the seasonal coronavirus 229E did not prevent re-infection a year later, but was associated with reduced viral shedding and symptoms (17). Therefore, for SARS-CoV-2, it is important that longitudinal studies of immunity are performed to understand whether protection wanes over time, and to help interpret antibody tests and how they relate to the time course of disease.

3.6 The LOGIC Study - understanding the relationship between COVID-19 symptoms, virology and immunity

At the time of writing, other large observational studies, some in healthcare workers, have commenced across the UK (Table 1). Unlike the LOGIC study, these projects aim to observe trends in seroprevalence over time and do not address detailed questions around the natural history of the infection integrating the chronology of symptoms, virology and detailed immunological evaluation.

Table 1. Comparison of LOGIC study with current COVID-19 sero-epidemiological projects

Study (ref)	Site	Study Type	Population	Number	Symptom Tracking	Virology	Immunology
SAFER (18)	London (UCL)	Longitud. cohort	HCW in 5 clinical areas across 2 Trusts	Unknown	Yes, details unknown	Nose/throat (twice weekly) RT-PCR	Serum (monthly)
COVID19-HCW (19)	London (UCL)	Longitud. cohort	HCW in A&E, AMU, ICU across 3 Trusts	400	Unknown	Nose/throat, (freq. unknown) RT-PCR	Serum (freq. unknown)
Oxford sero-epidemiology (20)	Oxford	Cross-sectional	Healthy individuals aged 0-24y	Unknown	Unknown	Nil	Serum (once)
ESCAPE (21)	PHE	Cross-sectional	HW and PHE staff London/Manchester Army soldiers London	1000 300	Unknown	Nil	Serum (once)
LOGIC	Bristol	Longitud. cohort	HCW in CED	135	Yes daily	Nose/throat & saliva (twice weekly) RT-PCR +/-subgenomic mRNA	Serum & PBMCs (6 weekly) +/- salivary IgA +/- genetic control of immunity

In the LOGIC study, we aim to address the gaps in the literature outlined above by describing the dynamic relationships between COVID-19 symptoms, mucosal viral load and immunological responses which may correlate with protection. Performing such studies during a global pandemic is challenging for several reasons including: i) difficulties in obtaining samples because of the requirement to avoid unnecessary physical contact; ii) limited access to a population that remains exposed to the pathogen despite public health control measures, but in whom the majority have not already been infected; iii) limited access to study subjects who are still able to attend the workplace and participate in research; iv) limited access to a population in which long-term follow-up observations can be made. The LOGIC Study will overcome these barriers by studying frontline healthcare workers at the Children's Emergency Department (CED) of Bristol Royal Hospital for Children, who can safely self-sample from their own upper respiratory tracts. Clinician self-sampling of the upper respiratory tract has been shown in a pre-print study to be both feasible and comparable in diagnostic accuracy with samples collected by clinical care providers (22). Studying infection in children's hospital staff is likely to be relevant to the community acquired infection, because a recent pre-print study of 791 Spanish healthcare workers did not demonstrate any significant differences in the rate of SARS-CoV-2 infection across hospital disciplines and pre-determined risk categories of exposure (23). Finally, at the time of writing, the South West of England has the lowest number of COVID-19 case notifications and deaths, suggesting that the LOGIC study can capture the exponential phase of the epidemic curve among HCWs (24).

The collaborative and multidisciplinary Bristol UNCOVER Group (Bristol University COVID19 Emergency Research Group) is ideally resourced to perform this study having expertise in performing large-scale longitudinal studies of upper respiratory viral and bacterial infections and mucosal immunity (Finn lab) and including one of the few research laboratories in the UK who have the necessary approvals and experience to work directly with live SARS-CoV-2 (Matthews/Davidson lab). The data generated by this study will help scientists understand the relationships between symptoms, mucosal viral load, and immunity that will inform effective public health and vaccination programmes, to minimise the devastating and far-reaching global effects of COVID-19 and future pandemics.

4 LAY SUMMARY

COVID-19 is the most significant infectious epidemic of modern times, currently in April 2020 killing thousands of people around the world every day. Strategies to prevent spread of infection include social distancing and the development of a vaccine. These require detailed understanding of the disease. There are some basic questions about COVID-19 to which we urgently need answers, including: 1) How often are COVID-19 tests negative during the infection or positive without showing any signs of illness? 2) Can we reliably test for immunity to COVID-19 after infection or vaccination with a blood test? 3) How long after infection does immunity last? To answer these questions, we need to understand the links between the symptoms of COVID-19, swab tests for infection and blood tests for immunity. We will study all these things in doctors and nurses working in the Emergency Department of Bristol Royal Hospital for Children over a 3-month period starting in April 2020. Staff who agree to participate will fill out an online symptom dairy every day. Twice a week, participants will swab their own nose and throat and provide a saliva sample, which we can test for COVID-19 in the laboratory. Blood samples will be taken from participants at the beginning of the study, at 6-week intervals for 18 weeks, and again after a year. The main advantage of studying doctors and nurses is that they can easily and safely obtain samples from their own nose/throat/mouth. After the study has been completed, we will analyse the results and publish them in a scientific journal. This study will help us provide answers to the fundamental questions about COVID-19 infection that are needed to inform public health measures such as social distancing and vaccination.

5 PRINCIPAL RESEARCH QUESTION

What are the dynamic relationships between symptoms, mucosal viral load and immunological responses, which are potential correlates of protection, in SARS-CoV-2 infection?

6 OBJECTIVES & OUTCOME MEASURES

Primary Objective

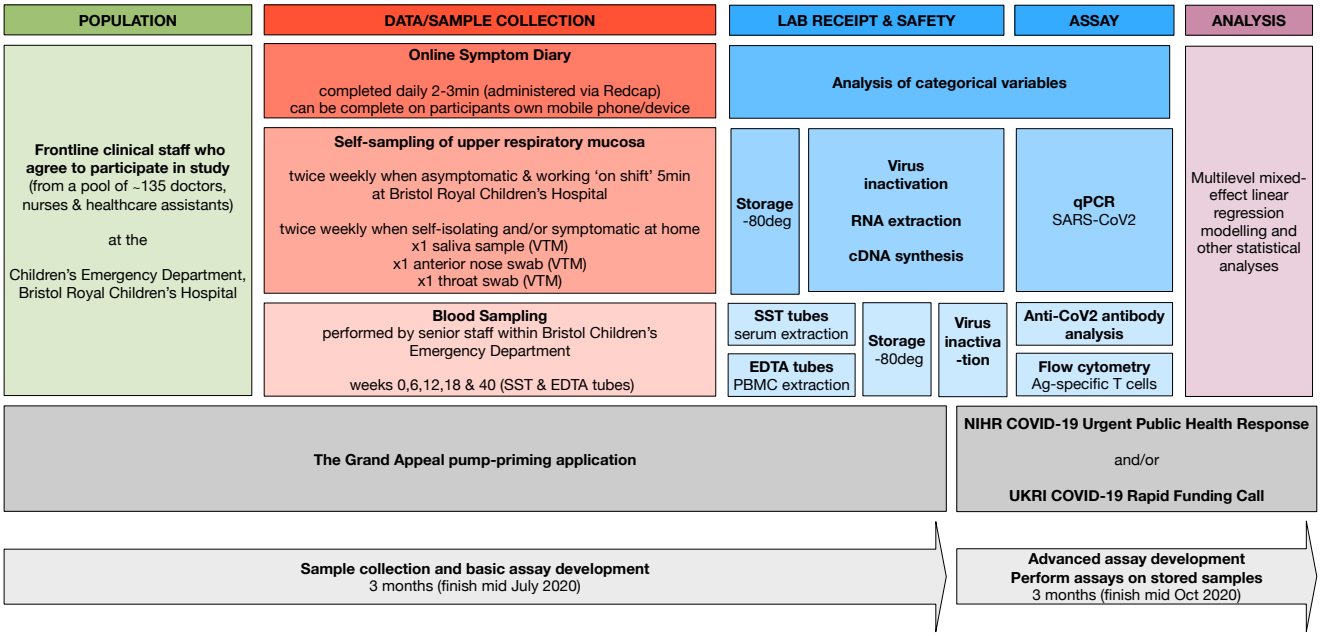
To describe in detail the temporal relationships between symptoms, mucosal viral load, and immunological responses (potential correlates of protection) in an adult population highly and repeatedly exposed to SARS-CoV-2.

Secondary Objectives

1. Develop a toolkit of laboratory protocols to safely and accurately measure: i) viral load in upper respiratory samples; ii) anti-SARS-CoV-2 antibodies in blood; iii) peripheral antigen-specific memory/effector T cell frequency and function.
2. Compare SARS-CoV-2 viral load by RT-qPCR in saliva, and throat/nose swabs.
3. Establish whether SARS-CoV-2 can be detected by RT-qPCR in healthcare workers before they develop COVID-19 symptoms, and if so for how long and at what viral density.
4. Establish the predictive value of a negative test in a symptomatic health care worker i.e. whether one negative test predicts negativity on other simultaneous samples and subsequent repeat test(s).
5. Establish the nature, magnitude & longevity of the anti-SARS-CoV-2 antibody and T cell response in relation to the symptoms and viral dynamics of COVID-19.

7 SUMMARY OF DESIGN AND METHODOLOGY

LOGIC is a longitudinal cohort study of approximately 135 frontline staff at the Children's Emergency Department (CED) of the Bristol Royal Hospital for Children. Staff who consent to participate will be asked to complete an online case report form collecting baseline demographic data, recent symptoms suggestive of COVID-19, and contact with individuals suspected or confirmed to have COVID-19. In order to track the dynamics of symptoms longitudinally, participants will be asked to complete a brief daily online symptom diary for 3 months, which includes information on potential exposures to SARS-CoV-2. To track viral dynamics, participants will be asked to perform twice weekly self-sampling of saliva, nose and throat swabs when working 'on shift' (i.e. when present in the hospital) for a period of 3 months. During any period of self-isolation and/or illness, swabs will be performed twice weekly, by means of "doorstep drop-off and pick-up" of swabs at the participant's home whenever feasible. In addition, blood samples will be collected at the start of the study and then after 6, 12, 18, and 52 weeks in order to evaluate immune responses to SARS-CoV-2 over time. Samples will be picked up every day from the CED and transported directly to the Biomedical Sciences Building, University of Bristol. RNA, serum and peripheral blood mononuclear cells (PBMCs) will be stored at -70ºC after processing of samples at the laboratories at the University of Bristol following the appropriate level of biosafety containment and viral inactivation steps as approved by the Health and Safety Executive. Saliva and throat/nose swabs will be tested for SARS-CoV-2. The anti-SARS-CoV-2 antibody profile in serum (and saliva where appropriate) will be profiled by enzyme linked immunosorbent assay (ELISA). Cellular immunological memory and T cell activation will be explored using flow cytometry, in response to stimulation of PBMCs with overlapping peptide pools. The relationships between symptoms, host gene expression, viral (saliva/nose/throat) and immune (antibody/T cell) dynamics will be analysed using multilevel mixed-effect linear regression modelling, alongside other appropriate statistical techniques.



8 PARTICIPANT RECRUITMENT CRITERIA

8.1 Inclusion Criteria

- Any patient-facing clinical staff member working regularly in the Children's Emergency Department (CED) of Bristol Royal Hospital for Children, including doctors, nurses and healthcare assistants.

8.2 Exclusion Criteria

- Staff who, on average, work less than 2 shifts per week
- Staff who anticipate that they will not able to complete at least 6 weeks of the study, excluding annual leave e.g. about to go on maternity leave.

9 STUDY PROCEDURES

9.1 Patient & Public Involvement

We sought input from a broad group of clinical staff who would be potential participants, including a range of grades of medical and nursing (registered and unregistered) staff. Three PPI groups were performed by open Zoom meetings advertised in the Children's Emergency Department and led by the Principal Investigator, in line with current recommendations on virtual meetings. We sought general feedback on the study, including positives or negatives, and what may affect the decision to take part. We also sought specific advice as to whether: (i) they felt the study is deliverable and acceptable to a broad range of staff in its current form; (ii) the information leaflet was clear and the rationale well explained; and (iii) the frequency of testing is likely to be acceptable to participants, both when they elect to take part, and in longer term continued participation. In total 36/150 (24%) of CED staff attended these meetings. Details of these PPI sessions, including how specific discussion and feedback points from CED staff have been integrated into this protocol are included in **Appendix 1**.

9.2 Participant Information Sheets

The appropriate CED clinical line managers will forward an email by the LOGIC investigators to staff to invite them to participate in the study. The invitation email will contain an electronic Participant Information Sheet (PIS) as a pdf attachment, as well as a hyperlink to the e-consent module (described below). In particular, the PIS includes contact details of the research team, giving potential participants the option to ask questions about and discuss any aspect of the study.

9.3 Informed Consent

Following distribution of the Participant Information Sheets, staff will have up to 2 weeks to undertake informed consent. A two-week recruitment period will help 'spread' out day 0 blood sampling so that it is feasible for same-day processing in the research laboratory. Informed consent will be taken using an e-consent webtool, hosted securely on a REDCap database, which will help limit physical contact in line with current pandemic guidance by the Health Research Authority (25). The invitation email and PIS will contain a hyperlink that directs individuals who are interested in participating to the e-consent webtool. This will be accessible from any internet-enabled mobile or desktop device. The REDCap script for the online consent module is attached to this protocol. Consent specifically address issues pertaining to storage and sharing of samples and data, and the possibility of re-contacting participants for any follow-up studies as well as permission to access COVID-19-related medical information from participant medical records should they be admitted to hospital.

9.4 Enrolment into Study

Directly after consenting to take part, while still on online REDCap consent tool, participants will be:

- asked to give their mobile phone number, home email address, and residential address. Home email and phone number will facilitate the delivery of email/SMS text reminders for participants to: i) complete the baseline data collection (daily reminder until completed) ; ii) complete the online symptom diary (daily reminder); iii) self-sample (daily reminder at 8am at least 48 hours

after last sample); iv) blood samples (daily reminder following prescribed intervals set out in protocol). Residential address will facilitate the "doorstep drop off/pick up" of samples, at any time when participants are self-isolating.
- allocated a personal study ID number that will accompany each individual participant throughout the study.
- be directed to the online baseline data collection form.

9.5 Baseline Data Collection

Following consent and enrolment into the study, baseline clinical variables will be collected from participants who complete the secure online REDCap data collection tool. The chosen variables in baseline data collection have been selected from: i) published reports of risk factors for severe COVID-19 disease such as ischaemic heart disease (7); ii) other population-based longitudinal studies which are currently underway in understanding COVID-19 epidemiology, to ensure harmonisation of the dataset with other studies e.g. ALSPAC (Avon Longitudinal Study of Parents and Children) (26). For further details on the questions/variables, please the attached REDCap script (with branching logic).

9.6 Daily Symptom Diary

Following enrolment, participants will receive daily SMS/email reminders to complete a symptom diary via the secure online REDCap portal. The symptom diary takes less than 2 minutes to complete if they have no symptoms, and 5 minutes if they have symptoms. The variables in the symptom diary have been selected from: i) published reports of COVID-19 symptoms (6); ii) population-based longitudinal studies currently underway in understanding COVID-19 symptomatology, to ensure harmonisation of the dataset with other studies e.g. ALSPAC (Avon Longitudinal Study of Parents and Children) (26) and the COVID Symptom Tracker (King's College London) (8). For further details on the questions/variables, please the attached REDCap script (with branching logic). Investigators will have sought specific consent to access a participant's medical records if they are admitted to hospital, to record details of their symptoms and clinical presentation within the symptom diary.

9.7 Self-Sampling of Upper Respiratory Mucosa (Hospital)

Participants will be asked to 'self-sample' twice weekly immediately before their clinical duty period at the CED in order to obtain swabs of their nose and throat, and saliva. Following enrolment in the study, participants will be sent:

- Detailed written instructions (self-sampling guidelines for hospital.pdf) and a hyperlink to access to a 5-minute video containing a narrated demonstration by one of the investigators undertaking the self-sampling procedure
- A brief self-sampling *aide-memoire* that will be placed inside the lid of the sample box (self-sampling aide memoire.pdf)
- A reminder to self-sample twice per week.

Investigators will also visit the CED regularly during the study to answer questions about self-sampling.

Following acquisition, samples should not rest for longer than 4 hours in the fridge before being transferred to a freezer. Therefore, a member of the research team will collect sample boxes regularly from CED and drop off a fresh sampling kits. They will collect sample boxes in line with infection control and biosafety requirements. Boxes will be transported to the laboratory inside a resealable clear plastic bags housed within a thermal transport bag, to keep samples at or below 4^{o}C.

9.8 Self-Sampling of Upper Respiratory Mucosa (Home)

When a participant is self-isolating and/or symptomatic (ascertained from symptom diary), a research team member will visit the participant's home address by prior arrangement for a "doorstep drop-off and pick-up" of samples. This arrangement will be based on whether the participant feels well enough and willing to self-sample, and whether the distance of the drop-off/pick-up from the laboratory is feasible. A sampling kit will be placed outside the participant's home, inside a resealable plastic bag (to contain any spillages) placed within a specimen collection container. This pack will also contain

written instructions (self-sampling guidelines for home.pdf) The research team member will return to the car and telephone the participant to indicate that the container has been delivered. The participant will then collect resealable plastic bag containing the sampling kit and return inside their home to perform self-sampling in a private room away from other household members, to minimise infection by aerosols. Used items such as the swab-ends and the plastic saliva funnel will be disposed in the household waste within a plastic sealable bag (provided in the kit). After self-sampling, the participant will return the resealable plastic bag containing plastic box (housing the samples) to the specimen collection container on the doorstep. Once the samples are outside the home in the specimen collection container, the research team member will collect the box and return to the laboratory, or if feasible, visit the home of another participant who is self-isolating. A completely separate set of boxes will be used for each participant to eliminate infection control risks of cross-contamination between participants. While in car transport, samples will be kept at or below 4^0C in a sample transport container containing dry ice, and will be taken to the laboratory within 4 hours of acquisition for freezing.

9.9 Blood Sampling

Following enrolment, participants will receive SMS/email reminders to undergo blood sampling. Regular reminders will be sent until the sample has been taken, at each appropriate interval (weeks 0, 6, 12, 18, and 52). Venepuncture will be performed in the CED by a study investigator or appropriately trained clinical delegate from the CED staff pool (an instructional poster of procedure for taking study bloods will be on the wall with needles/sampling tubes). Blood samples will be obtained by study investigators and CED staff because this was the preferred option expressed during the PPI exercise, and because it avoids infection control risks associated with participants travelling from CED to other hospital areas. All delegates from CED staff pool that are authorised to obtain samples for this study will receive instruction from an investigator of this study, detailing which blood samples need to be collected as well as attention to safety.

Blood sampling will involve peripheral venepuncture to obtain approximately 28mL blood (2x10mL EDTA tube and 1x8mL SST tube) using a butterfly needle closed vacutainer system. Blood samples will be labelled with the participant's study ID number and the date on which they were obtained, and placed within a clear plastic resealable bag, and kept at room temperature in a designated box within CED. Blood samples will be collected from CED by a research team member and transported to the laboratory in a resealable clear plastic bag within a plastic transport container at room temperature.

9.10 Benefits for Participants

During our PPI sessions, CED staff explained what they perceived to be the benefits of participating in the study. There was an overwhelmingly positive response including "participating in this study is one of the most useful things we can do to contribute in the fight against COVID-19" and "I am interested in helping more with this study" (**Appendix 1**). There are no specific financial gift incentives for participants. When participants are self-isolating, when we discuss the timing and logistics of "doorstep drop-off/pick-up" of samples, we will also ask if they would like us to deliver essential items (milk, bread etc.) at no cost to the participant.

9.11 Risks and Burdens for Participants

This study conforms to UK Government guidance on physical distancing and Health Research Authority guidelines on performing research during the COVID-19 pandemic (25, 27). Consent, baseline data collection and symptom tracking are being performed using a secure online platform to avoid unnecessary physical contact between participants and researchers. The pandemic biosafety issues pertaining to self-sampling of the upper respiratory tract and venepuncture are outlined above, but these procedures are relatively safe for the participant. Obtaining a throat and nasal swab may cause mild transient discomfort. Risks of venepuncture include: i) fainting or feeling light-headed: all venepuncture will be performed on an examination couch; ii) minor hematoma/bruise: this will be minimised by the application of pressure at the site of the venepuncture using gauze for 3 minutes following the procedure. Venepuncture will only be carried out by appropriately trained professionals.

10 LABORATORY PROCEDURES

10.1 Biosafety

Self-Sampling: Self-sampling will take place in designated clinical areas of the CED, ensuring that other staff and patients are not present. Participants will wear gloves during self-sampling, and after sampling, will dispose of all potentially contaminated sampling material (e.g. gloves, swab ends and saliva funnels) in the appropriate clinical waste bin. Participants will clean the plastic box housing the sample tubes with an alcohol/detergent wipe before returning the box containing the tubes (within a clear plastic resealable bag) to the fridge where it will be stored until collection.

Blood sampling: Venepuncture will be undertaken using a butterfly needle closed vacutainer system to minimise the risk of blood spillage. Blood samples will be placed within a clear plastic resealable bag, and kept at room temperature in a designated box within CED. Blood samples will be collected from CED by a research team member and transported to the laboratory in a resealable clear plastic bag within a plastic transport container at room temperature.

Transport of samples from hospital to research laboratory: Samples will be collected from CED by a research team member. The plastic box of samples will be transported to the laboratory by foot (<400m) inside a resealable clear plastic housed within a thermal transport bag, to keep respiratory samples at or below 4^{o}C, and blood samples at room temperature.

Laboratory biosafety (RT-PCR of saliva and throat/nose swabs): Prior to analysis, samples will undergo viral inactivation in a Class 1 or 2 safety cabinet in a containment level 2 laboratory, as per Health and Safety Executive approvals for this work.

Laboratory biosafety (blood samples & viral culture work): Serum and peripheral blood mononuclear cells will be extracted in a Class 2 safety cabinet at the appropriate containment level laboratory (under the management of Dr. Andrew Davidson) as per Health and Safety Executive approvals for this work.

10.2 Viral Dynamics

Investigators and collaborators: **Davidson, Matthews, Muir, Finn**

Saliva and swabs (nose/throat) will be tested for SARS-CoV-2 using the reverse transcriptase polymerase chain reaction (RT-PCR) assay recommended by the World Health Organisation or adaptations thereof as the technique is refined and in conformity with Public Health England standard operating procedures. Briefly, after a viral inactivation step, RNA will be extracted from samples either manually or using an automated platform, reverse transcribed into cDNA, and stored at -70^{o}C. Samples will be prepared and real time PCR for SARS-CoV-2 will be performed using Thermo-Fisher QIAgility and QuantStudio or similar platforms. Viral detection will be expressed quantitively as cycle threshold (Ct) number for samples amplifying below the cut off for each assay.

10.3 Immunological response - potential correlates of protection

Investigators **& collaborators: Davidson, Matthews, Wooldridge, Rivino, Halliday, Woolfson, Berger, Finn**

Antibody correlates: Serum will be extracted from SST blood tubes after removal of the clot by centrifugation, and aliquoted into vials for storage at -70^{o}C. Antibody ELISAs will be used to detect antibodies specific for the immunodominant antigens of SARS-CoV-2, e.g. the Spike protein and nucleoprotein. Samples with a detectable antibody response will be further profiled to describe the nature of the response by antibody isotype ELISAs using a range of antigens/epitopes of interest, including the 'Achilles Heel' epitope within the receptor binding domain of the Spike protein- an important potential vaccine target. The functionality of antibodies will be determined by conducting neutralisation assays against live virus. The magnitude, duration and nature of SARS-CoV-2 antibody responses in seroconverters will be related to their symptoms and to the detection of the virus in respiratory samples (i.e. the timing, duration and viral load) potentially enabling evaluation of the accuracy of both PCR and antibody testing strategies for identifying infected healthcare workers.

T-cell correlates: Peripheral blood mononuclear cells (PBMCs) will be extracted from EDTA blood tubes by Ficoll density gradient centrifugation. After this separation step, PBMC will be cryopreserved and stored in liquid nitrogen or in a -70^{o}C freezer. After thawing, assays will be done to assess SARS-CoV-2 specific T-cell responses including measurement of activation in response to overlapping peptide pools. This will allow mapping of relevant T-cell epitopes and development of pMHCI tetramers that can be used to study the dynamics of the SARS-CoV-2 specific T-cell response and persistence of post-infection T-cell memory. As part of this work, PBMCs will be analysed by flow cytometry to enumerate and define the phenotypic and functional characteristics of effector and memory T cell populations: CD4+, CD8+, TCR$\gamma\delta$, Treg and Tfh cells.

Genetic control of immunity: An aliquot of EDTA blood (<500uL) will be frozen directly in the appropriate sample tube at -70^{o}C for subsequent host genomic DNA extraction. Blood will not be stored in this way unless participants have specifically consented for this in the REDCap e-consent script. Genetic analyses will be performed to understand differences in the immune response as appropriate i.e. if significant inter-individual variability is observed in the relationships between symptoms, virology and immunity.

10.4 Supplemental Work

In the REDCap e-consent script, consent is specifically sought from participants to perform supplemental laboratory work that enriches the exploration of our primary research question. Therefore, depending on the results of the initial viral and immunology assays outlined above, further laboratory work on stored samples may include (but is not limited to):

- **Viral culture:** of stored upper respiratory samples followed by sequencing to understand the metagenomic relationships of SARS-CoV-2 viral isolates, which could help inform epidemic tracking within the CED healthcare worker cohort, and whether the virus mutates.
- **Viral replication:** in stored upper respiratory samples by RT-PCR of viral subgenomic messenger RNAs which is only transcribed in infected cells (and not packaged into virion) hence indicating actively infected cells (15).
- **Viral co-infection:** in stored upper respiratory samples to understand the relationship of co-infection with other respiratory viruses, and stored samples may undergo multiplex PCR testing for: adenovirus, influenza A viruses (H1N1/09, seasonal H1N1 and H3N2), influenza B, respiratory syncytial virus, human metapneumovirus, rhinovirus, parainfluenza virus types 1-3 and enterovirus.
- **Lateral flow assays:** Antibody detection approaches developed in the ELISA assays performed in this study may be developed further on stored serum samples, into (rapid diagnostic) lateral flow assays.
- **Cross-reactivity with other coronaviruses**: testing stored serum for pre-existing antibody responses to other coronaviruses, as these impact on the outcome of SARS-CoV-2 infection, and/or may be reflective of antibody cross-reactivity
- **Saliva IgA and IgG measurement**: in stored saliva samples may be measured and correlated with serum antibody measurements.
- **Genetic control of immunity**: in stored host DNA, serum and PBMC samples to search for genetic, soluble or immunophenotypic factors that may be correlated with protection or susceptibility to specific clinical/virology phenotypes.

10.5 Future Work

One of the main advantages of our study design is access to a population of participants that can potentially be studied further in the future, to determine the longevity of SARS-CoV-2 immunity and other related research questions. We can only consider these further studies (and what samples might be required) once we have all the results of the current study. Therefore, we ask specifically in the consent form for permission to contact participants in the future to inform them about follow-up studies that they might be interested in (which would require separate REC approval).

11 TIMELINE OF INVESTIGATION

STUDY DESIGN & RECRUITMENT Month -1	DATA/SAMPLE COLLECTION Months 0, 1 & 2	LABORATORY ASSAYS Months 3, 4, 5, 6	ANALYSIS & DISSEMINATION Months 7, 8, 9, 10, 11	FOLLOW-UP Month 12	FURTHER ANALYSIS
- PPI & collaborative/inclusive study design - Email invitation with Participant Info Sheet - Discuss participant questions as necessary - REDCap e-consent	- Symptom diary (daily) - Upper resp. self-sampling (twice weekly) - Blood sampling (6 weekly between 0-18 weeks)	- Upper resp. samples (SARS-CoV2 RT PCR) - Serum (antibody assays) - PBMCs (memory T cell assays)	- Correlation analysis - Writing-up of report - Submission to journal	- Blood sample for further serum PBMC assays	- Analysis of 1y samples & decision whether need further follow-up studies

Based in observed trends to date in Bristol and elsewhere, it is expected that this natural history study, will capture an adequate number of new infections occurring among participants within the 3-month sampling period to permit useful conclusions to be drawn. To evaluate this, blood samples will be tested for SARS-CoV-2 antibodies at baseline and again at the 6-week time point to check whether new infections have been occurring. Depending on these results and other available data on the epidemiology of the epidemic locally and nationally (24), the sample collection period may be extended beyond 3 months and/or recruitment to include staff in the adjacent adult ED department.

12 RATIONALE FOR STUDY POPULATION, PROCEDURES & ENVIRONMENT

At the time of writing, the South West of England is the best place to undertake a natural history study of COVID-19 because rates of disease are among the lowest in the UK, and therefore a significant proportion of participants will not already be infected at the start of the study (24). The study design was chosen to address the primary research question primarily because of its feasibility during pandemic public health control measures. Staff at the CED of Bristol Royal Hospital for Children are a relatively healthy population, who are tractable in terms of their availability, work in a single physical area, can self-swab, and will remain in contact with infected patients regardless of pandemic public health control measures. Proximity of Bristol Royal Hospital for Children to the research laboratories of University of Bristol's Biomedical Sciences Building (<400m) makes transfer of samples simple and pragmatic. Finally, the collaborative and multidisciplinary Bristol UNCOVER Group (Bristol University COVID19 Emergency Research Group) is uniquely placed to perform this study having expertise in performing large-scale longitudinal studies of upper respiratory viral/bacterial infection and mucosal immunity (Finn lab) and including one of the few research laboratories in the UK who have the necessary approvals and experience to work directly with live SARS-CoV-2 (Matthews/Davidson lab).

13 SPECIFIC ETHICAL CONSIDERATIONS

This is an observational study and there are few study-specific ethical implications, outside of confidentiality and data protection (governed by GDPR) and the handling and storage of samples (governed by the Human Tissue Act and Health & Safety Executive). The study has been designed in participation with representatives from all groups of frontline CED healthcare professionals as well as the NIHR R&D service. NHS REC approval will be sought via the current expedited process for COVID-19 studies. Participant recruitment and informed consent will take place in accordance with the principles of the Declaration of Helsinki. Results from individuals will not be fed back to participants for several reasons: i) HCW testing is being rolled out across the NHS including Bristol Royal Children's Hospital through occupational health services; ii) samples in this research study will be processed in batches sometime after acquisition so will no longer have any acute clinical relevance; iii) our research lab does not hold the necessary NHS accreditation to provide clinical results.

14 STATISTICAL CONSIDERATIONS

This is a study of the natural history of a novel infection. As such, a power calculation to determine the number of participants that will meet specific endpoints is not appropriate. Data generated by this study will be both categorical (baseline clinical information and symptom diary) and continuous (viral load, antigen-specific T cell frequency, and serum antibody concentration).

To describe the strength of longitudinal associations between symptoms, mucosal viral load, and immunological correlates of protection (the primary objective), we will use multilevel mixed effect regression models to account for repeated measures of the independent and dependent variables and the effect of time. These analyses will involve flexible parametric modelling if this is deemed to be required to better model the longitudinal effects of these variables.

Relating to the first of the statistical secondary objectives, we will investigate the correlation of viral load in swabs of saliva and throat/nose using Pearson's correlation coefficient.

To investigate whether HCWs become PCR test positive before they develop COVID-19 symptoms, we will use a survival analysis framework stratifying follow-up at baseline, date of first positive PCR test, and first development of symptoms (where applicable). The HCWs will be split into two groups - those who developed PCR test positivity before symptoms and vice versa. For each group, rates will be calculated for how long it takes before either they develop symptoms or have a positive PCR test (depending on whether they start with symptoms or a positive PCR test), with sensitivity analyses performed choosing different viral densities.

A survival analysis framework with PCR test result as the outcome will also be used to establish whether a negative PCR test in a symptomatic participant predicts future PCR test results. Start of follow-up will be defined as the first reported symptoms at the time of a negative test and follow-up time will be stratified at time of subsequent PCR test results.

To identify the immunodominant peptides of SARS-CoV-2 associated with memory T cell responses we will use separate multilevel linear models for each variable relating to memory T cell responses as the outcome measures and markers of peptides as the independent variables. Multilevel linear models will also be used to investigate the nature of the antibody response in relation to the symptoms and viral dynamics of COVID-19 by estimating associations between the relevant variables, with separate analyses performed on different outcomes relating to responses e.g. magnitude or length of antibody response.

15 CONFIDENTIALITY AND DATA PROTECTION

Storage and Use of Personal Data: Data will be collected and retained in accordance with the Data Protection Act 1998. The study team will be responsible for data collection, recording and quality control. Study documents (paper and electronic) containing details of demographic data, documentation of inclusion and exclusion criteria, and medical history will be retained for a period of 20 years following the end of the study.

Confidentiality: Study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participant number. All documents will be stored securely and will only be accessible to study staff and authorised personnel. The study will comply with the General Data Protection Regulation (GDPR) which requires data to be anonymised as soon as it is practical to do so.

Access to Data: The Chief Investigator will allow monitors (from UHB on behalf of the Sponsor), persons responsible for the audit, and representatives of the Regulatory Authorities to have direct access to source data / documents. This is reflected in the Participant Information Sheet (PIS). Study monitoring will be undertaken on behalf of the Sponsor by UHB using their monitoring standard operating procedure. Access to study documents will only be available to the investigators, monitors and auditors directly involved in the study. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/initials, not by name.

Notification of Other Health Professionals of Participant Involvement: This is a longitudinal natural history study that does not involve any testing that will be of clinical significance for participants, and is

not a clinical trial of an investigational medical product. Therefore we will not routinely inform the participant's general practitioner of their involvement in this study.

16 USE OF HUMAN TISSUE

Samples will be stored within the School of Cellular & Molecular Medicine (University of Bristol) in locked -70^{o}C freezers fitted with temperature probes/alarms. Laboratory team members will have access to the samples for the purposes of carrying out experiments/analysis. The Chief Investigator will be responsible for all samples. Where appropriate consent is offered, residual samples will be retained after the end of the study in the Bristol Biobank to permit further research to be undertaken. The regulations and approvals of the Biobank permit samples to be retained indefinitely.

17 SAFETY

This study conforms to UK Government guidance on physical distancing and Health Research Authority guidelines on performing research during the COVID-19 pandemic (25, 27). Consent, baseline data collection and symptom tracking are being performed using a secure online platform to avoid unnecessary physical contact between participants and researchers. The pandemic biosafety issues pertaining to self-sampling of the upper respiratory tract and venepuncture are outlined above, but these procedures are relatively safe for the participant. Obtaining a throat and nasal swab may cause mild transient discomfort. Risks of venepuncture include: i) fainting or feeling light-headed: all venepuncture will be performed on an examination couch; ii) minor hematoma/bruise: this will be minimised by the application of pressure at the site of the venepuncture using gauze for 3 minutes following the procedure. Venepuncture will only be carried out by appropriately trained professionals.

18 INDEMNITY

The University of Bristol has arranged Public Liability insurance to cover the legal liability of the University as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University. The University of Bristol holds Professional Negligence insurance to cover the legal liability of the University, for harm to participants arising from the design of the research, where the research protocol was designed by the University. The University of Bristol's Public Liability insurance policy provides an indemnity to our employees for their potential liability for harm to participants during the conduct of the research. In addition, investigators have the protection of medical malpractice indemnity with the Medical Protection Society or Medical Defence Union.

19 REPORTING & DISSEMINATION OF RESULTS

The results of the study will be published in appropriate peer-reviewed academic scientific journals, and will be presented at scientific conferences. No identifiable personal data will be published, all data will completely anonymised. We will not inform participants of the results of the study, for reasons outlined above in "Specific Ethical Considerations".

20 POTENTIAL IMPACT

In this study, we aim to address the gaps in the literature outlined above by describing the dynamic relationships between COVID-19 symptoms, mucosal viral load, and immunological correlates of protection. Performing such studies during a global pandemic is challenging for several reasons including: i) difficulties in obtaining samples because of the requirement to avoid unnecessary physical contact; ii) limited access to a population that remains exposed to the pathogen despite public health control measures; iii) limited access to a population still able to attend the workplace and participate in research; iv) limited access to a population in which long-term follow-up observations can be made. The LOGIC Study will overcome these barriers by studying frontline healthcare workers at the Children's Emergency Department (CED) of Bristol Royal Hospital for Children, who can safely self-

sample from their own respiratory tract. The data generated by this study will help scientists understand the relationships between symptoms, mucosal viral load, and immunity that will inform effective public health and vaccination programmes, to minimise the devastating and far-reaching global effects of COVID-19 and future pandemics.

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22 APPENDIX 1 Patient and Public Participation Sessions

Date of session	Number of attendees (and professional role in CED)
26th March 2020	Consultants x5 Senior Nurse x2 Emergency Nurse Practitioner x1 Research Nurse x1
31th March 2020	Staff nurse x4 Senior Nurse x2 Emergency Nurse Practitioner x1 Senior Registrar x1 Nursing Assistant x1 Registrar x1 Senior House Officer x1
1st April 2020	Senior Nurse x4 Staff nurse x5 Registrar x2 Nursing Assistant x2

Question/Comment	Investigator response and whether protocol modified
How many blood tests?	Clarified sampling protocol
If the swab can only be kept a few hours how does this work?	Clarified hours during which sampling can be performed (7am-4.30pm) and discussion that this should be compatible with most shift times
Logistics of swabbing – how, where and sample storage?	Clarified that specific guidance will be available through a video Swab taking is potentially aerosol-generating procedure so should be performed in a clinical area
Is the symptom tracker daily?	Clarified yes for 3 months
Are there exclusion criteria?	Initially said no, but staff member pointed out what if know are pregnant and going on maternity leave or leaving Trust within a few weeks **[amended protocol in response]**
When self-isolating how will we get swab/sample from/to you?	Asked participants, and agreed that investigators need to come to participant's homes & collecting samples you've taken Brief discussion about whether better to give participants a swabbing kit at the start of the study to take home, or whether to "drop-off" & "pick-up" **[amended protocol in response]**
How will you obtain blood from participants? Can we ENPs in CED take blood from participants?	Acknowledged that we were still working out details of this, but thanks staff member for kind offer of help. **[amended protocol in response]**
What about doctors who are seconded out to the adult hospital during the pandemic?	Acknowledged that we had not thought about this, and asked doctors, who said they would not mind continuing in study if working in adult hospital. Did not find a feasible way of planning this in protocol.
Are we trying to understand results in terms of exposure?	Yes, symptom diary includes qualitative measures that may correlate with exposure
How long does it take to complete symptom diary?	Clarified 2-3 minutes

If someone wants to take part but doesn't want to have bloods taken can they still take part?	Clarified that extent of participation is voluntary **[amended protocol in response]**
We would like to take each other's blood samples, it would be 'good practice'	Acknowledged comment as a discussion point within our own team. Discussed ethical implications of this within our own team after PPI. Agreed that for this research project, we could only support senior staff members (consultants & ENPs) to take blood from participants
I live 1 hour away from hospital, will you be able to visit my house if I am self-isolating?	Discussion with participants that might have difficulty visiting participants who live far away. Point made that traffic low. Another participant responded that need to assess feasibility day-to-day i.e. one day could go to 5 local addresses, and next day go to 1 distant address. **[amended protocol in response]**
Can we take part in other studies?	Clarified yes unless the other studies state otherwise
Will you sample over weekend?	Clarified yes
What is the timeframe to start and finish the study?	Clarified timescale
Will we receive our results e.g. seroconversion?	Clarified no, and explained rationale for this condition as per the protocol
Can I participate if I are taking immunosuppressive treatment?	Clarified yes Also clarified that investigators will not see identifiable medical records of any participant
What if blood tests reveal something unexpected and concerning?	Explained that will not be testing anything that would reveal anything clinically actionable in real-time **[amended participant information leaflet in response]**
Participating in this study is one of the most useful things we can do to contribute in the fight against COVID-19	Open discussion acknowledging issues Resources & support for mental health and wellbeing: - BMA wellbeing - NHS Practitioner Health - www.yougotthiswellness.com - Headspace - www.welldoctors.org
I am interested in helping more with this study	
The pandemic is very overwhelming and mentally exhausting	