Study Title: Sero-epidemiological survey of England in 2019/2020

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Sponsor:	University of Oxford
Funder:	National Institute for Health Research (NIHR)

Chief Investigator Signature:

Statistician Signature:

Conflicts of Interest

Professor's Snape and Faust act, on behalf of their employing institutions, as Chief and/or Principal Investigators on research studies funded and/or sponsored by vaccine manufacturers including Novavax, Glaxosmithkline, Sanofi-Pasteur, Medimmune and Janssen. These investigators receive no personal financial benefit for this work.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

Protocol signature page

The undersigned has read and understood the research study protocol detailed above and agrees to conduct the research study in compliance with the protocol.

Principal Investigator (Please print name)	**Signature**	**Site name or ID number**	**Date**

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1. KEY CONTACTS

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2. LAY SUMMARY

Public Health England has an ongoing sero-prevalence programme to assess how well the population is protected from vaccine preventable and emerging infectious diseases. The current way to check this is by testing left over blood samples from participating healthcare laboratories around the country. However, these samples may not be representative of the general population, particularly in younger age groups who are often most at risk from vaccine preventable diseases.

In the Netherlands, they use a different system to assess how well the population is protected from vaccine preventable diseases, actively collecting blood samples from a representative cross section of society. This type of approach would address the limitations of using residual serum samples and allows the collection of additional relevant history e.g. number of family members and previous vaccines received.

Having a large number of blood samples from a range of age groups is also useful when gathering information about an emerging disease such as the current novel coronavirus (SARS-COV-2). These samples can help provide answers regarding the true number of infections in the population. This allows us to work out the severity of the infection on a population basis.

We are therefore conducting a pilot study to assess the feasibility of establishing a national sero-epidemiological survey in England in individuals aged 0 – 24 years. We will be focusing initially on diphtheria, Group C meningococcus and COVID-19.

Given the increased risk of COVID-19 disease in the BAME communitythis data would, in turn, be invaluable in understanding whether higher rates of disease in the BAME community are a result of

- Greater exposure to COVID-19 contacts
- A higher likelihood of being infected once exposed
- Greater risk of disease once infection occurs

This will involve collecting at least one blood sample (+/- saliva samples) from participants who will allocated into three groups:

Group 1: 2300 participants aged 0 to 24 years selected to be representative of their test site region based on post code and associated index of material deprivation (IMD).

Group 2: up to 1200 individuals aged 0 to 19 years. The selection criteria for these participants will be less restrictive than Group 1 in terms of age band and postcode/IMD.
Group 2 can be enhanced by the samples received from other ethically approved research projects where participants have consented for their samples being used outside of the study

Group 3: up to 300 participants aged 0-19 from the Black, Asian and Minority ethnic population.

All participants in group 1 must be resident at the study-specified representative post-codes, and all those in group 2 be aged 19 years or younger. Beyond this, allocation to group 1 and 2 will be managed dynamically during (and potentially, after) the study to ensure those in group 1 are representative of their region in terms of geographic distribution and IMD.

While blood samples from participants in group 1 will be analysed for both vaccine responses and COVID-19 seroprevalence, participants in groups 2 and 3 are primarily being used for COVID-19 seroprevalence testing in the first instance. Samples will be collected at a steady monthly rate to allow assessment of changes in the proportions of participants with antibodies against SARS-CoV-2 (the virus responsible for COVID-19) with time, and a sub-set of participants will provide repeat blood and saliva samples for further analysis of immune responses to SARS-CoV-2. This subset who will provide repeat blood and saliva samples will comprise of both seropositive and seronegative participants.

3. SYNOPSIS

Study Title	**Sero-epidemiological Study of Vaccine Preventable Diseases in England**	
Internal ref. no. / short title	2019/01. What's the STORY	
Study registration	NCT04061382	
Sponsor	University of Oxford	
Funder	National Institute for Health Research (NIHR)	
Study Design	Prospective, cross-sectional sero-prevalence study	
Study Participants	Individuals living in England aged 0– 24 years	
Sample Size	A total sample size of 2800 to 3800 across all study sites. Additional samples can be provided by other ethically approved research projects	
Planned Study Period	June 2019 – August 2021	
Planned Recruitment period	Beginning of July 2019 – June 2021	
	Objectives	Outcome Measures
Primary	• To evaluate the feasibility and added public health benefit of an England, population based sero-epidemiological programme in 0 to 24 year olds	• Representativeness of participants sampled, in terms of the local population's ethnicity, community identity, migrant population and socioeconomic background • Comparison with serological markers of immunity for vaccine preventable diseases as measured in an age matched cohort in current residual sera programme
Secondary	• To evaluate the effectiveness of recruitment methods employed • To assess, in relevant age groups, antibody concentrations against infections and vaccine preventable diseases including, but not limited to diphtheria, group C meningococcus and novel coronavirus (COVID-19)	• Recruitment rate per month, recruitment rates as percentage of potential participants contacted, • cost per sample obtained • disease specific correlates of protection/markers of immunity, e.g. : o Anti-Diphtheria Toxoid IgG concentrations o Capsular Group C meningococcal Serum bactericidal activity (SBA) titres o Serum IgG to SARS-CoV-2 antigens, including spike protein and/or nucleocapsid

		(as measured by ELISA and/or neutralising assay) • A collection of anonymised sera from participants with appropriate consent and known demographic details and immunisation history' • Serum IgG to SARS-CoV-2 antigens, including spike protein and/or nucleocapsid (as measured by ELISA and/or neutralising assay) • Serum IgG to SARS-CoV-2 antigens, including spike protein and/or nucleocapsid (as measured by ELISA and/or neutralising assay) • Salivary IgG to SARS-CoV-2 antigens, including spike protein and/or nucleocapsid (as measured by ELISA)
	• To develop a store of sera from a representative section of 0 to 24 year olds available for future testing of immunity against other infectious diseases of relevance to UK immunisation schedule and public health. • To determine the prevalence of SARS-CoV-2 infections in 0 – 24 year olds, and variation in prevalence in time, age, ethnicity and geography (cross-sectional sero-epidemiological study) • To determine the kinetics of antibodies specific to SARS-CoV-2 following infection in a paediatric population (serial blood sampling in population sub-group) • To determine relationship between serum and salivary antibodies against SARS-CoV-2	
Exploratory	• Comparison between recruitment strategies between groups • The presence of SARS-CoV-2 virus in saliva in a sub-set of study participants • To characterise T cell responses against SARS-CoV-2 in antibody seropositive and seronegative participants.	• Representativeness of participants sampled, in terms of the local population's ethnicity, community identity, migrant population and socioeconomic background • Differences in immunological read outs • PCR for SARS-CoV-2 on saliva samples stored and processed at the end of the study.

	• Humoral and cellular immunity against non SARS-CoV-2 coronaviruses	• T cell responses to SARS-CoV-2 antigens including, but not limited to S, M and N proteins, as measured by techniques including, but not limited to o ELISpot o ICS o Proliferation assay • Antigen specific IgG and T cells against non-SARS-CoV-2 coronaviruses (e.g. NL62 and 229E)

4. ABBREVIATIONS

BAME	Black, Asian and Minority ethnic
CI	Chief Investigator
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
CDM	Clinical Data Management
CRF	Case Report Form
CHIS	Child Health Immunisation Service
COVID-19	Coronavirus Disease 2019 (also known as 2019-nCoV)
DNA	Deoxyribonucleic Acid
CTRG	Clinical Trials & Research Governance, University of Oxford
EDC	Electronic Data Capture
ELISpot	Enzyme-linked immunosorbent spot
ESEN	European Sero-epidemiology Network
ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and Nutrition
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GMT	Geometric mean titre
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
ICS	Intracellular cytokine staining
IMD	Index of Multiple Deprivation
IRAS	Integrated Research Application
NIHR	National Institute for Health Research
NHS	National Health Service
PHE	Public Health England
RES	Research Ethics Service
PBMC	Peripheral blood mononuclear cell

PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
RNA	Ribonucleic acid
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SARS-COV-2	Severe acute respiratory syndrome coronavirus 2 which causes COVID-19 disease
SBA	Serum bactericidal activity
SOP	Standard Operating Procedure
WHO	World Health Organisation
VEU	Vaccine Evaluation Unit

5. BACKGROUND AND RATIONALE

Public Health England has an ongoing sero-prevalence programme to assess population level immunity using residual serum samples from participating laboratories. However, these samples may not be representative of the general population particularly in younger age groups. National sero-epidemiological surveys have successfully taken place in the Netherlands, which consist of a prospective collection of serum samples from a representative cross section of society to assess population level immunity. This type of approach would address the limitations of using residual serum samples, and would potentially allow assessment of a number of diseases.

We are therefore conducting a pilot study to assess the feasibility of establishing a national sero-epidemiological survey in England in individuals aged 0 – 24 years, focussing initially on COVID-19, diphtheria and Group C invasive meningococcal disease. Specifically, we wish to assess the population level immunity to diphtheria following the pre-school booster vaccine in children aged between 3 and 14 years, and the immunity to Men C in individuals aged 12 – 24 years who may have received either the adolescent Men C or Men ACWY vaccine, or did not receive either of these vaccines.

Furthermore, in response to the outbreak of COVID-19 these samples will be used to look for changes in seropositivity to SARS-CoV-2 in England children +/- young adults through 2020/2021 (and beyond if necessary) as a marker of infection with this novel coronavirus. This is to support Public Health England in its SARS-CoV-2 seroprevalence work, with a goal of providing blood samples from 400 participants per month through 2020 as a repeat cross-sectional seroprevalence study. While the majority of participants will provide a single blood sample, a subset of participants will be enrolled into a longitudinal sampling cohort, providing up to a total of 4 blood samples (and 3 saliva samples) allowing analysis of the kinetics of rise and fall of SARS-CoV-2 specific antibody concentrations following SARS-CoV-2 infection, and the relationship between serum and saliva antibodies. This will in turn inform the interpretation of data from the repeat cross-sectional seroprevalence study. The 'repeat' blood samples collected as part of this study will be counted towards the study target of blood samples from a sub-set of participants. The longitudinal sampling cohort will comprise of both seropositive and seronegative participants.

The longitudinal cohort also provides an opportunity to address the knowledge gap regarding T cell responses to SARS-CoV-2 infections. At present, it is not clear how many people who are infected do not mount an antibody response and whether they have a detectable T cell response only. Commentary in the literature suggests that measuring antibody levels alone may underestimate population level immunity against COVID-19 (Sekine et al., 2020). The longitudinal cohort provides an opportunity to examine both T cell responses and the kinetics of this response.

There will be enhanced recruitment in Black, Asian and Minority ethnic (BAME) populations to develop an understanding on whether higher rates of disease in the BAME community are a result of

- Greater exposure to COVID-19 contacts
- A higher likelihood of being infected once exposed
- Greater risk of disease once infection occurs

Previous seroprevalence studies

Seroprevalence studies using residual sera

PHE has been utilising residual serum samples from participating laboratories across the country as part of its ongoing seroprevalence programme for many years. These samples are used as a serum bank for

investigating population immunity to a range of infections including vaccine preventable infections. These samples have been previously used to assess the population immunity for diphtheria with the last study undertaken using samples collected in 2009 ((Wagner et al.; Wagner et al., 2012), Box 1), whilst a number of seroprevalence studies have been undertaken at different times to study Men C population immunity. The most recent sampling was undertaken in 2014 (Box 2) when the teenage MCC vaccination programme was newly introduced.

Box 1. Wagner et al (2012) Immunity to tetanus and diphtheria in the UK in 2009

In this study, 150 residual sera were tested in each age group, in order to estimate the proportion of the population protected to within ± 8% with 95% confidence. It found that:

- 75% of the UK population had antitoxin levels ≥0.01IU/mL correlating to basic diphtheria protection
- 41% had antitoxin levels ≥0. 1IU/mL correlating to full diphtheria protection
- Between ages 1 and 9 years, the proportion with antitoxin levels correlating to full protection remained stable (65%-71%)
- Thereafter, the proportion with antitoxin levels correlating to full protection declined to a low of 44% amongst those aged 10-11 years.
- The proportion with antitoxin levels ≥0.01IY/mL increased again for teenagers and young adults, before declining in older adults.
- The highest number of susceptibles with antitoxin levels < 0.01IU/mL were observed in the age groups <1 year (37%), 35-44 years (27%), 45-69 years (41%) and 70+ years (33%).

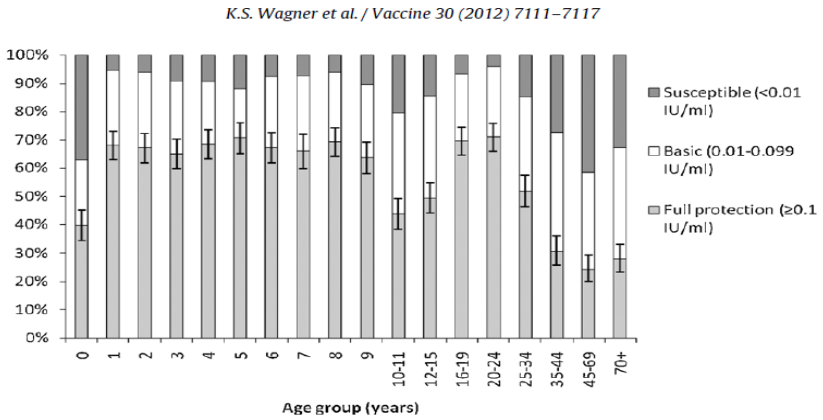


Figure 1: Diphtheria antitoxin distribution by age group in England, 2009. Error bars indicate 95% confidence intervals for full protection (Fig 5 from Wagner et al. (2012))

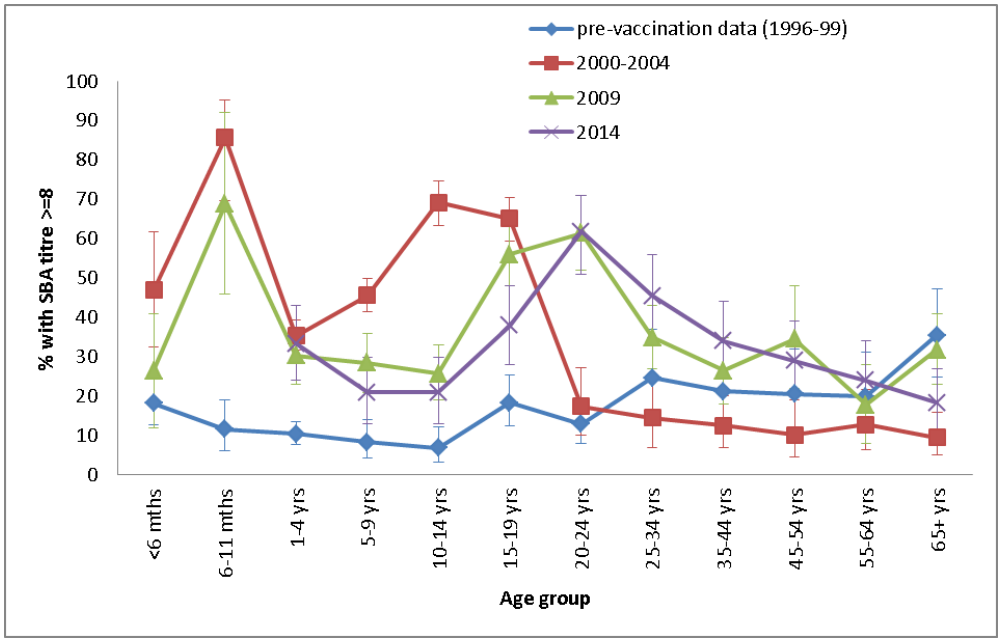


Figure 2. Seroprotection against serogroup C meningococci measured by proportions with serum bactericidal antibody (SBA) titres of ≥8. Comparison of levels in 2014 with the previous surveys conducted prior to MCC vaccine introduction in 1996-1999 and following introduction in 2000-2004 and 2009 (Findlow et al., 2019)

Disadvantages of residual seroprevalence studies

1. Samples may not be representative of the whole population. Previous review of the source of these samples have shown considerable variation by age e.g. paediatric samples from immunocompromised children and samples for adults sourced from those attending Genito-Urinary Medicine clinics.

2. Generally, the number of samples obtained from children is low and thus unlikely to be sufficient for stratification by individual age bands and region, which are particularly relevant to evaluate childhood vaccine programme for specific antigens.

3. Individual vaccination histories are not available but are derived from vaccination programmes and known coverage at a population level.

National seroprevalence study in the Netherlands

In 2006 / 2007, a large serum bank was established in the Netherlands by means of a cross-sectional population based study (Klis et al., 2009) . A similar serum bank was collected in 1995 / 1996 (De Melker and Conyn-Van Spaendonck, 1998; Melker and Spaendonck, 1998).

Dutch inhabitants (aged 0 – 79 years), identified from the national population register, were invited to participate from 40 municipalities throughout the country. Oversampling took place in areas with low vaccine coverage and migrant populations.

Over 17 000 individuals were invited to participate. Individuals received a letter of invitation together with a brochure containing information on the study, a questionnaire, an informed consent form, and a prescheduled appointment form for blood donation at a local clinic. Participants were offered a gift voucher.

Overall, a 32% response rate was achieved (6386 serum samples). The highest response rate was in women aged 10–49 and aged 50–79 with a response rate of 38%. A response rate of 27% and 26% was seen in male and female children aged 0-9 respectively.

In the first study in 1995 / 1996 – an overall response rate of 50% was achieved. It was suggested that the response rate fell in the second study because the distance to travel to the clinic was much further compared to the first study.

Use of seroprevalence studies in pandemics

In a worldwide pandemic, such as the current SARS-COV-2 outbreak, residual samples are used to help determine the true number of infections by detecting asymptomatic and mild infections. This will enable us to more accurately describe the severity of infection across the population. The timing of this study allows us to use the serological library alongside the residual sample method which is already being used in the SARS-CoV-2 response. This is important as the study will gather samples from healthy children who are not well represented in the residual sample model and this information will be used to evaluate the public health benefit of running a sero-epidemiological programme. This study design is being conducted in accordance with the World Health Organisation 'Populations-based age stratified seroepidemiological investigation protocol for COVID-19 virus infection', available at: https://www.who.int/publications-detail/population-based-age-stratified-seroepidemiological-investigation-protocol-for-covid-19-virus-infection

6. **OBJECTIVES AND OUTCOME MEASURES**

	Objectives	Outcome Measures
Primary	• To evaluate the feasibility and added public health benefit of an England, population based sero - epidemiological programme in 0 to 24 year olds	• Representativeness of participants sampled, in terms of the local population's ethnicity, community identity, migrant population and socioeconomic background • Comparison with serological markers of immunity for vaccine preventable diseases as measured in an age matched cohort in current residual sera programme
Secondary	• To evaluate the effectiveness of recruitment methods employed • To assess, in relevant age groups, antibody concentrations against infections and vaccine preventable diseases including, but not limited to diphtheria, group C meningococcus and novel coronavirus (COVID-19) • To develop a store of sera from a representative section of 0 to 24 year olds available for future testing of immunity against other infectious diseases of relevance to UK immunisation schedule and public health. • To determine the prevalence of SARS-CoV-2 infections in 0 – 24 year olds, and variation in prevalence in time, age, ethnicity and geography (cross-sectional sero-epidemiological study)	• Recruitment rate per month, recruitment rates as percentage of potential participants contacted, • cost per sample obtained • disease specific correlates of protection/markers of immunity, e.g. : o Anti-Diphtheria Toxoid IgG concentrations o Capsular Group C meningococcal Serum bactericidal activity (SBA) titres o Serum IgG to SARS-CoV-2 antigens, including spike protein and/or nucleocapsid (as measured by ELISA and/or neutralising assay) • A collection of anonymised sera from participants with appropriate consent and known demographic details and immunisation history' • Serum IgG to SARS-CoV-2 antigens, including spike protein and/ or nucleocapsid (as measured by ELISA and/or neutralising assay)

	• To determine the kinetics of antibodies specific to SARS-CoV-2 following infection in a paediatric population (serial blood sampling in population sub-group) • To determine relationship between serum and salivary antibodies against SARS-CoV-2	• Serum IgG to SARS-CoV-2 antigens, including spike protein and/or nucleocapsid (as measured by ELISA and/or neutralising assay) • Salivary IgG to SARS-CoV-2 antigens, including spike protein and/or nucleocapsid (as measured by ELISA)
Exploratory	• Comparison between recruitment strategies in group 1 and group 2. • The presence of SARS-CoV-2 virus in saliva in a sub-set of study participants • To characterise T cell responses against SARS-CoV-2 in antibody seropositive and seronegative participants. • Humoral and cellular immunity against non SARS-CoV-2 coronaviruses	• Representativeness of participants sampled, in terms of the local population's ethnicity, community identity, migrant population and socioeconomic background • Differences in immunological read outs • PCR for SARS-CoV-2 on saliva samples stored and processed at the end of the study. • T cell responses to SARS-CoV-2 antigens including, but not limited to S, M and N proteins, as measured by techniques including, but not limited to o ELISpot o ICS o Proliferation assay Antigen specific IgG and T cells against non-SARS-CoV-2 coronaviruses (e.g. NL62 and 229E)

7. STUDY DESIGN

7.1. Study design

The study will be a repeat cross sectional sero-epidmiology study, with an embedded longitudinal study cohort.

The goals of this study are to:

- Provide blood +/- saliva samples from 2300 children and young adults, representative of multiple geographic regions across England to evaluate the concentrations of antibodies against vaccine preventable diseases ('Group 1'). These need to be equally distributed such that there will be 100 in each of 22 age groups (1 year cohorts from 0 to 20 years) and 200 participants in the young adult cohort from 21 to 24 years). To help ensure these are representative of the study region recruitment will be from postcodes designated by PHE.
- To provide additional blood +/- saliva samples to assess changes in the proportion of children/adolescents with antibodies against SARS-CoV-2 during the COVID-19 pandemic. This will be achieved by:
    - Recruitment of additional participants (Group 2 and 3) that will not be included in the final vaccine sero-epidemiology analysis.
    - Group 2 participants will be recruited at all 'Group 1' sites, and at least 2 additional sites, and will be equally distributed across 4 age bands (0 – 4, 5 to 9, 10 to 14 and 15 to 19 years)
    - Group 3 participants, recruited at a sub-set of sites depending on capacity and the demographic profile of the local population
    - Taking up to 3 repeat blood samples (i.e. a total of 4 blood samples) at a minimal interval of 2 months apart from a sub-set of participants at all sites who consent for this (longitudinal sampling cohort, maximum number of participants enrolled defined in the clinical study plan). Participants in this cohort will also have a saliva sample collected at each follow-on visit.
- Evaluate T cell responses to SARS-CoV-2 infection. Within the longitudinal cohort selected sites who are able to provide blood samples to CCVTM for same-day processing for separation of peripheral blood mononuclear cells (PBMCs) will provide samples from participants who fit into the following groups:
    - *Have had no exposure, and are seronegative to SARS-CoV-2 at V1*
    - *Have had a household exposure, but are seronegative at V1*
    - *Have had symptoms of COVID-19, but are seronegative at V1*
    - *Are seropositive at V1*

A total of up to 150 participants will be tested. Each participant will fit into one of the groups above. From these 150 participants some will provide only a single sample for T cell responses, others will provide up to a maximum of three samples.

Recruitment to this study will be managed to achieve these goals, while also ensuring equal distribution for age group and geographic region across time. For more detailed guidelines for how to recruit on a month by month basis please see the clinical study plan.

7.2. **Study Sites**

The study sites will be:

- University of Oxford
- Sheffield Children's Hospital NHS trust
- Bradford Teaching Hospitals NHS Foundation Trust
- Leeds teaching Hospitals NHS trust
- University Hospitals Bristol NHS Foundation Trust
- University of Southampton NHS Foundation trust
- Royal Manchester Children's Hospital – Manchester University NHS trust
- St George's University Hospitals NHS Trust
- University of Nottingham Health Service
- University Hospitals Plymouth NHS Trust
- The Newcastle Upon Tyne Hospitals NHS Foundation Trust
- Imperial College Healthcare NHS Trust
- West Suffolk NHS Foundation Trust

Note that the Sheffield, Leeds and Bradford sites are collectively considered the 'Yorkshire and Humber region' for purposes of recruitment numbers. Nottingham and Plymouth will not be recruiting to Group 1.

GP practices and Pharmacies located in the same regions as the participating sites can be added as Participant Identification Centres in order to facilitate with recruitment.

7.3. **Identification of individuals**

Refer to section 9.1

7.4. **Sample Size**

2800 to 3800 participants, to provide blood +/- saliva samples as per section 7.1. This will include up to 300 participants specifically recruited to enhance representation of participants from Black, Asian and Minority ethnic groups with at least 100 from the Black/ African/ Caribbean/Black British community.

Additional samples can be received from other ethically approved research projects with the appropriate contractual arrangements in place provided that the participants have consented for the use of their samples outside of the study.

7.5. **Collection of samples**

Blood (and, if applicable, saliva) samples will be collected in specially designated clinics or home visits. This approach may be adapted to suit different age groups depending on local needs. For example, home visits may increase the response rate for preschool children, whilst specially designated clinics run during school holiday periods or weekends may be appropriate for school aged children. Collection of samples will take into account infection control measures in place in response to the COVID-19 outbreak, as outlined in the Clinical Study Plan.

7.6. **Questionnaire & Vaccination History**

Basic demographic characteristics will be collected by questionnaire and/or case report form (CRF) and will include: gender, GP details, ethnic group, association with communities of special interest (e.g. faith communities) household income, vaccination history and personal and household history of recent respiratory or coronavirus infections.

Vaccination history will be verified during serum sample collection using the Red Book or other vaccination records, or checking with the general practitioner or the Child Health Information Service (CHIS) database. Where possible this will include batch information for diphtheria pre-school booster to determine which specific product was received.

Contemporaneous vaccination history can also be obtained from CHIS for children aged 0 –5 years, and historical vaccination history can be obtained via GP records in participants aged 2 – 24 years. It is however acknowledged that the quality and completeness of vaccination history in GP records varies from practice to practice, particularly in older age groups that may have been vaccinated at a different practice or at school.

7.7. Compensation

Participants will be offered a £20 voucher per visit as reimbursement for travelling to the study clinic. If they are seen at home there will be no reimbursement. Based on the average EU income levels, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) suggest that an incentive of up to the value of 30 Euros is considered acceptable for children and adolescents, and may be offered as cash, vouchers or gifts or toys (Mis et al., 2018).(Mis et al., 2018) Other studies in adolescent age groups in the UK have had approval to use £10 book vouchers, and incentives such as these, or age appropriate gifts or toys would be preferable to cash for recruiting adolescents and younger children.

7.8. Comparison with Residual Serum Samples

Data collected in this study will be compared with that obtained in the anonymised residual serum samples collected in 2019 from individuals aged 0 – 24 years by the PHE seroepidemiology unit from participating laboratories across England. These will be used as the basis of comparisons between immune responses measured through residual sample testing and through the active sample collection outlined in this protocol. The age, sex and year of collection will be known via a unique identity number: immunisation status will not be known.

In 2017, 3290 samples were collected in individuals aged 0 – 24. It is envisaged that a similar sample size will be collected in 2019/20, and that testing of the residual sera can take place in 2021.

8. PARTICIPANT IDENTIFICATION

8.1. Study Participants

Individuals living in England aged 0– 24 years.

8.2. Inclusion Criteria

Participants MUST FULFILL each of the below criterion:

- Parents/legal guardians or adult participant* is willing and able to give informed consent for participation in the study.
- Male or Female, aged 0 - 24 years inclusive (Group 1) and 0 – 19 inclusive (Group 2 and 3).
- Parents/legal guardians or adult participants are willing to allow their General Practitioner or relevant NHS databases to be contacted for a full immunisation history

* For the purposes of this study an adult will be defined as all those 16 years of age or over.

8.3. Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- **Group 1 only** If participants do not live in the postcode districts selected by PHE
- **Group 3 only** if participants are not from the BAME population
- Participants who have a member of their household already enrolled in the study where their ages are less than 5 years apart.
- Any significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the research study, or may influence the result of the research study, or the participant's ability to participate in the research study. Examples of disorders or diseases which would be excluded include
    - Medically diagnosed bleeding disorder
    - Medically diagnosed platelet disorder
    - Anticoagulant medication
    - Pregnancy

Temporary exclusion criteria

The participant may not enter the study if they or any member of their household is under temporary isolation measures for suspected SARS-CoV-2 infection.

9. PROTOCOL PROCEDURES

9.1. Recruitment Group 1

The recruitment plan below aims to recruit a representative sample of the region. Potential participants will be contacted by mailing out invitation letters with study information booklets to the parents/legal guardians of age appropriate children via the NHS England Databases, the Child Health Information Service or through the Clinical Research Network. There may also be other promotion such as website based advertising, social media, radio, printed publications, contacting families registered with a study site research database and poster advertisements. Potential participants can also be identified by the local study team through their visits for other research studies. Dissemination of the study information, including through GP practices and with health visitors may also be employed. The participant information will be available on websites if available and recruitment material can direct potential participants to this. Participants interested in taking part will contact sites to arrange visits. In the first stage recruitment will be capped (e.g. at 10 per age group per region) to allow for corrections if the initial sample is not representative of the region.

We are aiming to ensure that the sample is broadly representative of the region according to IMD (Index of multiple deprivation scores). Other details collected in the questionnaire such as ethnicity, community identity and FASiii are for later analysis.

We are mailing out using NHS England Databases with the expectation that 5-10% of those contacted will respond.

<u>Recruitment plan</u>

1. PHE will be generating a list of all postcodes in recruiting regions and determining the quintiles of IMD (index of multiple deprivation scores) within that region.

2. All sites to email us postcodes in their catchment areas (i.e. areas from which they can recruit). They will also indicate whether they are rural or urban areas. There is a recognition that there is no formal definition of these terms, and many post codes will be mixed, but this is an area where local knowledge could be applied.

3. For each region (i.e. Bristol, Yorkshire and Humber, Southampton etc.) PHE will randomly select 5-7 postcode districts stratified to match the ratio of rural to urban postcode districts to the region's urban/rural population distribution. A postcode district is the first two letters and number e.g.

OX	3	7	LE
Area	District	Sector	Unit

4. Mail outs will be conducted either through NHS England Databases (coordinated by OVG) or through local Child Health Information Services (CHIS).

5. In order to maintain even recruitment across time, age and postcode, recruitment numbers will be managed as per section 7.1 (study design).

6. It will be made clear in the participant information that participants (and/or their families) may not hear from us immediately, as the aim is to achieve a representative sample for that region. Therefore, every participant that contacts us from the first mail out can go into a study database containing basic contact details and age. This can be searched to see if any of the participants are from quintiles that are under-represented in the sample.

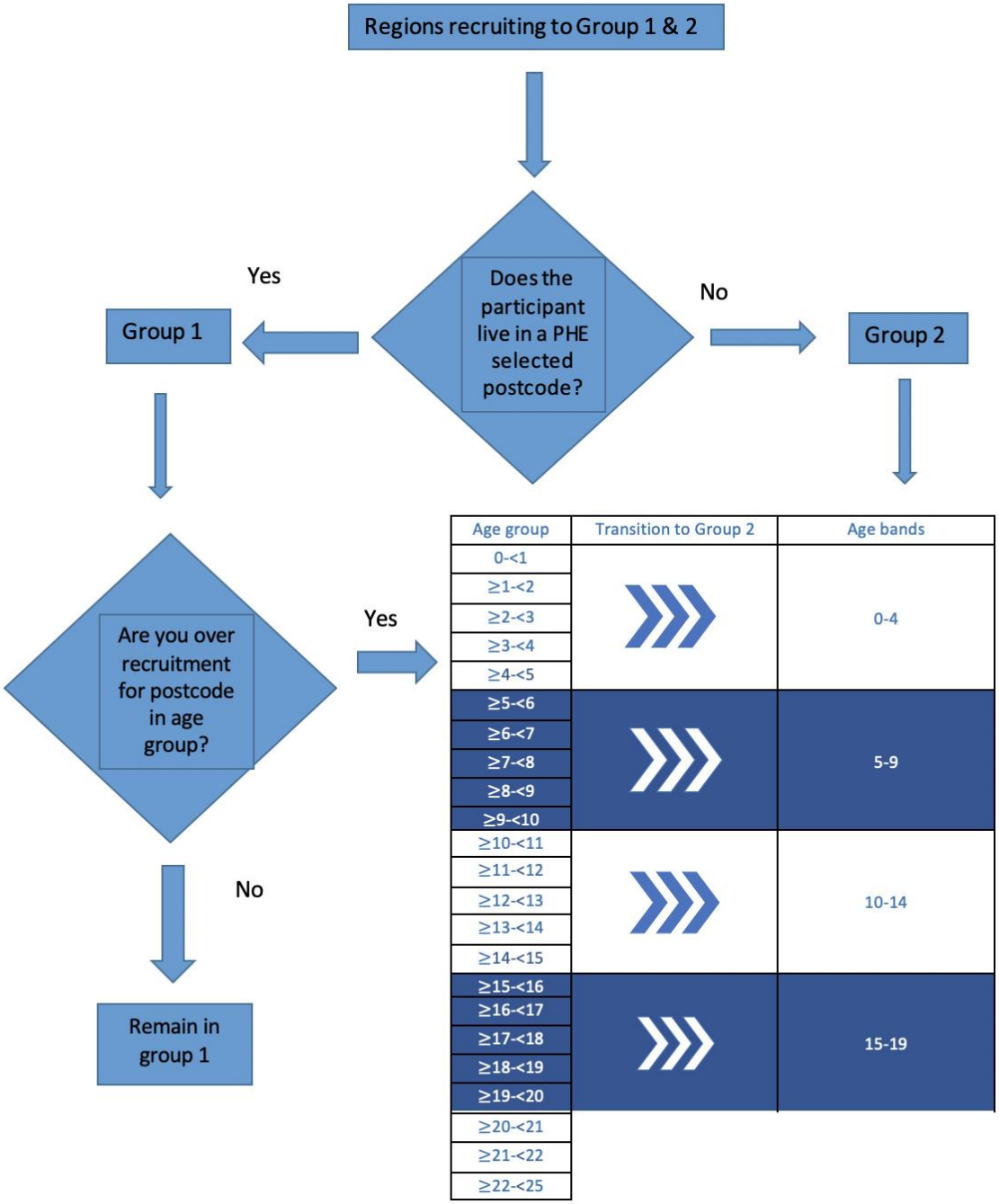
Timings of mail outs will be adjusted for sites dependent on their own local recruitment rates.

Those parents/legal guardians that indicate that they do not want to take part in the study and/or receive further communication about the study will not be included in any subsequent contact lists.

For non-responders, postcode and therefore deprivation level (according to Index of Multiple Deprivation) could potentially be estimated. Alternatively, a non-responder questionnaire, with basic demographic details could be administered by mail as per the Dutch study, enabling an assessment of potential bias due to non-response.

9.2. Recruitment Group 2

For the sites recruiting to Group 1, enrolment to group 2 will primarily be by re-allocation of participants from Group 1, as shown in figure 3.



Recruitment in sites only recruiting to group 2 will be carried out using various recruiting procedures, such as generalised mail outs, press releases, radio, social media adverts, schools or community clinics, dissemination of the study information through GP practices and identification of potential participants by the local study team, staff communication channels and inpatients or outpatients clinics as long as potential participants are not patients.

9.3. Group 3

Recruitment will be by multiple approaches, including mail outs and advertising in community (e.g. community centres, religious establishments,) or GP practices and Pharmacies where we have ethics approval for them to act as PICs. These can vary according to each site's experience and their contacts within their local community on how is best to approach the BAME community. Individuals will be directed

to the study website (https://whatsthestory.web.ox.ac.uk/) where they can find details about the study available in ten different languages as well as online registration form and contact details of the local study team. Potential participants can also be identified by the local study team through their visits for other research studies, staff communication channels and inpatients or outpatients clinics as long as potential participants are not patients. The reason for not approaching patients is that this would potentially skew the sample making it less representative of the paediatric population.

9.4. Screening and Eligibility Assessment

Given the low risk nature of the study there is no formal requirement for screening prior to the first study visit. However sites may choose to arrange contact with potential participants to discuss the study and a arrange clinic appointment or home visit. This is where exclusion criteria can be checked prior to arranging a visit.

Responses received from postcode districts not selected by PHE will not be eligible for group 1. Each postcode district has been selected based on sampling all quintiles of the IMD and to ensure representative data of the region.

In all groups, in order to ensure that there is a representative cohort from each age group multiple eligible participants from one household will only be eligible to take part if there are 5 years or greater between their ages.

During the study visit, the participant's eligibility will be assessed by a member of the study team. A brief medical history will be taken where participants will be asked about current health issues and medications as well as taking a vaccine history and personal and household history of respiratory/coronavirus infections from February 2020 onwards. Parents/legal guardians must have given written informed consent prior to an eligibility check being performed if the participant cannot consent for themselves. In these instances where the child is older than 11 years old there will be an assent form to complete.

9.5. Informed Consent

The parent/legal guardian of the participant or the participant themselves if able to consent will personally sign and date the latest approved version of the Informed Consent form.

A written version and verbal explanation of the Study Information leaflet and Informed Consent will be presented to the participant/parent/legal guardian of the participant detailing:

- the exact nature of the study
- what it will involve for the participant
- the implications and constraints of the protocol
- the known side effects and any risks involved in taking part
- sample handling – participants will be informed that anonymised samples taken during the course of study may be shared with study collaborators.
- Individual results will not be shared with participants – the study aim is to not actively enrol individuals with known or suspected COVID-19, but instead provide a series of snapshots of the general population to describe sero-prevalence to SARS-COV-2 at a population level.

- It will be clearly stated that the participant is free to withdraw from the research study at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal. Data up until that point will be kept unless the participant states they wish this data to be withdrawn.

If for any reason a participant gives consent but either blood is not obtained, or the sample is less than the minimum sample required (as defined by the clinical study plan) then they are **not** considered a withdrawal. Instead they will be considered as a failed enrolment and will not count towards the recruitment numbers, i.e. they will be replaced by another participant. These participants will still be given a voucher if they have travelled to a clinic.

The parent/legal guardian of the participant or adult participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written informed consent will then be obtained by means of the adult participant or the parent/legal guardian of the participant dated signature, and dated signature of the person who presented and obtained the Informed consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator and listed on the delegation log. A copy of the signed informed consent will be given to the participant or parent/legal guardian of the participant. The original signed form will be retained at the research study site.

The option of videoconferencing (e.g. facetime) can be made available in case of omissions or correction of errors in order to avoid unnecessary contact between the study team and participants during the COVID-19 pandemic. These corrections can be made by the parent or guardian if the participant is less than 16 years of age and the participant themselves if 16 years and over.

For the purposes of this study it will be assumed that participants over the age of 16 years are able to self-consent, but as with all participants will only be enrolled if the staff member taking consent is confident that the potential participant understands the study and is therefore able to give informed consent.

In addition to the informed consent for the provision of serum and the first visit of this study, participants (or their parents/guardians) will be given the opportunity to provide (optional) consent for:

1. Donation of the blood clot left after centrifugation of whole blood to the biobank at the Oxford Vaccine Group. This would allow for extraction of DNA to interrogate the influence of donor's genotype on vaccine/infection induced immunity, and other aspects related to the interdependence between genetics and immunity against infectious diseases (see section 9.5)
2. Participation in the longitudinal cohort, in which additional blood samples are taken along with a saliva sample and repeat administrations of the COVID-19 questionnaire

Note that participants enrolled under previous versions of this protocol will not have been asked regarding the possibility of additional visits at the time of their consent, but will have given consent to be re-approached regarding further research projects. Accordingly, these participants (or their parents/legal guardians) will be contacted by the study site team to determine if they would be willing to undertake the additional visits outlined in point 2 above. All participants in the longitudinal cohort will be reconsented, with those as sites contributing to the evaluation of T cell responses giving specific consent for this and storage of human tissues.

9.6. Study visits

9.6.1 Visit 1

The study visit will be conducted by research study staff either at the participant's home, or at convenient and suitable venues.

- Provide explanation of the study to participant or parents/legal guardians.
- Obtain written informed consent from the participant or parents/legal guardians of the participant
- Appropriately trained staff will perform a thorough check of inclusion and exclusion criteria using recall of relevant medical history and record findings, including:
    - Medical history of relevance to the inclusion/exclusion criteria
    - Details and indications of any prescription medications and vaccines
- If all inclusion and exclusion criteria are met the participant will be considered enrolled into the study.
- Ask participant or parent/ guardian (if participant below 16 years of age) to fill in questionnaire which will demographic data.
- Study staff to complete paper source or electronic CRF which will include the participants' immunisation history.

Blood sampling (+/- saliva sampling) will be carried out in line with local SOPs. A local anaesthetic cream or spray will be offered to child participants prior to venepuncture but will be made available to all age groups if required. When a visit has been booked and will take place in the participants home or at a suitable convenient venue, the anaesthetic cream will be sent by post with written instructions for application. If the cream is sent by post, parents will be asked to apply the cream an hour before the appointment. If participants are coming to a clinic then anaesthetic cream will be applied after verbal consent. Formal consent processes, medical/vaccine history and the questionnaire can be filled in while the cream is taking effect. If anaesthetic spray is used, this is applied immediately before the procedure to numb the skin. If the initial attempt at venepuncture is not successful (see Clinical Study Plan for minimum recommended volumes) verbal consent will be sought for a further attempt at that visit. No more than two attempts will be made in one visit. An additional visit may be rescheduled for another day if no blood is obtained at all. Maximum blood volumes based on 0.8ml/kg in line with guidance given by the European Commission of public health are tabled below in table 1. The weights for each age group are based on the 0.4th centile on the female UK-WHO growth chart.

A participant is only considered enrolled when a blood sample has been taken.

Table 1

Age	Maximum (target) volume of blood (mls)
<2 months	2ml
2-6 months	3ml
6-12 months	5ml
1-2 years	6ml
3-7 years	10ml
8-11 years	15ml
12-14 years	20ml
15 -24 years	30mls

9.6.2 Subsequent visits (longitudinal sampling cohort)

A subset of participants, distributed equally across 0-19 age groups, will be recruited into a longitudinal sampling sub-study.

If the participant provides consent for subsequent visits, then up to 3 additional visits will be undertaken with a minimum 2 months between visits. These visits will be conducted as for Visit 1, except that the questionnaire will be limited to COVID-19 symptoms in the participant or household members and a sample of saliva will be collected by the participant or their parents/legal guardians, as per the Clinical Study Plan.

Sites undertaking PBMC processing in the longitudinal cohort will select individuals where a whole blood sample will be taken. Total blood volume will remain the same, with whole blood subsequently separated into plasma and PBMC's.

9.7. Laboratory methods

The blood samples obtained will be centrifuged, separated and frozen at local sites at -80 degrees Celsius. Ideally this will happen within 24 hours but there is a window of up to 72 hours. Shipping of sera to the laboratories of PHE will be as outlined in the Laboratory Analysis Plan. Residual sera not sent to PHE will be shipped to the Oxford Vaccine Group for storage and/or further analysis. For participants where consent is obtained for DNA extraction and storage in the Oxford Vaccine Centre biobank residual blood clots will be shipped to the Oxford Vaccine Group for this purpose.

Saliva samples with be processed in line with local SOPs.

Diphtheria

A multiplexed fluorescent bead assay will be used to quantify IgG antibodies to diphtheria toxoid, based upon previously published methodology (Pickering et al., 2002). For diphtheria, anti-toxin levels < 0.01IU/mL denote susceptibility, antitoxin levels 0.01 – 0.099 IU/mL provide basic protection, and antitoxin levels ≥ 0.1IU/mL are fully protective, as per the international standard (2009).

Group C Meningococcus

Serum bactericidal antibody (SBA) assays will be performed against the serogroup C target strain, C11 (phenotype C:16:P1.7-1,1) as previously described (Maslanka et al., 1997). The complement source that will be used in the SBA is pooled serum from 3-4 week old rabbits (Pel Freez Biologicals, WI USA). Titres will be expressed as the reciprocal serum dilutions yielding ≥ 50% killing after 60 min. The lower limit of detection will be a titre of 4. Titres of <4 will be assigned a value of two for geometric mean titre (GMT) analysis. Titres of ≥8 will be considered protective against MenC disease (Borrow et al., 2005).

Novel Coronavirus (SARS-COV-2)

Assays are currently under development by Public Health England and University of Oxford to measure:

- Concentrations of serum and saliva IgG specific to SARS-COV-2 spike proteins and/or nucleocapsid by ELISA
- SARS COV-2 Virion/pseudovirion neutralising activity.

T cell responses

T cell responses will be determined by measures including, but not limited to, ELISpot, Intracellular cytokine staining (ICS) and proliferations assays.

A pool of viral peptides such as S1, S2, M, N, ORF3, ORF1 will be used in ELISpot assays to measure T cell stimulation.

DNA storage

The DNA samples obtained in the course of this study will be added to the Oxford Vaccine Centre's existing 'Biobank' of stored biological samples to facilitate further research on immunisation, immunity and infectious diseases.

One area of particular interest is the role for host genetics in dictating immune responses, hence the benefit of storing genetic material from study participants. Elucidating the genetic determinants of vaccine or infection induced responses may expand our understanding of vaccine/microbe-host interactions and anticipate an era of 'predictive vaccinology'.

Previous studies investigating genetic determinates of vaccine/microbe responses have explored a limited number of candidate genes and have not been able to account for the degree of heritability inferred by twin studies. Many genes are likely to play a small but significant part in determining responses to vaccination/infection. We intend to use contemporary genotyping techniques to help elucidate these complex vaccine/microbe-host interactions.

9.8. Discontinuation/Withdrawal of participants from research study

The participants have the right to withdraw from the research study at any time. In addition, the Investigator may discontinue a participant from the research study at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (e.g. if this becomes apparent during the study visit)
- Significant protocol deviation
- Withdrawal of Consent after a blood sample is taken.

Data from participants will continue to be analysed for the study unless the parents/legal guardians or adult participant request this to be withdrawn. Systems are in place to recover stored samples if the participants wish to withdraw their sample.

The reason for withdrawal will be recorded in the CRF.

If for any reason a participant gives consent but either blood is not obtained, or the sample is less than the minimum sample required (as defined by the clinical study plan) then they are **not** considered a withdrawal. Instead they will be considered as a failed enrolment and will be replaced.

Participants who consent to taking part in the longitudinal cohort will have the option of taking part in up to three additional visits. At each longitudinal visit they will be asked if they would like to opt for another appointment, if they do not wish to do so the CRF will be marked as complete.

9.9. Definition of the end of research study

The end of study will be defined as the completion of data collection.

10. SAFETY REPORTING

10.1. Reporting Procedures for All Adverse Events

Blood and saliva samples are all that is required of participants. No medicinal products will be administered. Given this fact, it is not intended to report non-serious adverse events.

10.2. Reporting Procedures for Serious Adverse Events

Blood and saliva samples and completion of questionnaire is all that is required of participants. No medicinal products will be administered. Given this fact and the scale of the study we will only report serious adverse events that are the result of study procedures.

All SAEs must be reported on the Oxford Vaccine Group SAE reporting form to the Chief Investigator or delegate within 24 hours of the Site Study Team becoming aware of the event. The CI or delegate will perform an initial check of the report, request any additional information, and ensure it is forwarded to the Medical Monitor on a weekly basis. It will also be reviewed at the next Research Study Safety Group meeting. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and emailed to CTRG.

The principal Investigator's opinion will be used to determine if the event was 'related' (resulted from administration of any of the research procedures) and/or 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs will be submitted to the ethics committee within 15 working days of the Principal Investigator becoming aware of the event, using the NRES report of serious adverse event form (see IRAS/NRES website).

10.3. Criteria for the Termination of the Study

The study does not involve the administration of any medications to participants. It is therefore unlikely that any safety issues would lead to termination of the study.

The investigator has the right to discontinue this study at any time. Recruitment will stop immediately if the study is prematurely terminated.

11. STATISTICS AND ANALYSIS

11.1. Description of the Statistical Methods

A detailed statistical analysis plan will be produced prior to receipt of the data by the Statistician.

Data analysis will include:

<u>Primary Objectives and Outcomes</u>

- Representativeness assessment by comparison to census or other population data on sex, ethnicity, community membership, migrant population, socioeconomic background, vaccination uptake (from PHE Cover data). At a site level representativeness will be to local census data and/or according to characteristics of those who respond to those who do not. Overall representativeness will be compared to national data. This will be done descriptively, and differences assessed by

Chi-squared or Fisher's exact test and multivariable methods as appropriate to adjust for the age distribution.

- A comparison of the proportions protected against diphtheria and Men C using residual and prospectively collected samples. This will be done overall and within broad age groups (see sample size section) with adjustment in a logistic regression analysis for confounding factors such as finer age groups and, if necessary sex and region.

Secondary Objectives and Outcomes

- Description of recruitment rates (participants recruited per month) and cost per sample obtained. This will be done overall, by site and by method of survey.
- Description of response rates (proportion invited that respond and the proportion that respond that are recruited and bled). This will be overall and by site and survey method as well as by age group. Proportions will be calculated with 95% confidence intervals. A more detailed analysis of response rates will be done by post-code and other demographics collected from the non-response survey.
- For prospectively collected samples where vaccination history is known in children aged between 3 and 14 years, the proportion of individuals within each age band that have antibodies to diphtheria antitoxin ≥0.01IU/mL and antibodies to diphtheria antitoxin ≥0. 1IU/mL, and whether levels of diphtheria anti-toxin are associated with the brand of diphtheria pre-school booster administered. Trends by age and differences by vaccination status will be assessed by Chi-squared of Fisher's exact test as well as by using logistic regression. Comparisons by geometric mean titres will also be done by t-tests or Kruskal Wallis as appropriate and by plotting geometric means with 95% confidence intervals by age groups.
- For prospectively collected samples where vaccination history is known, in individuals aged 12 - 24 years, the proportion of individuals within each age band that have serum bactericidal titres of ≥8 to group C meningococcal disease, and whether SBA titres are associated with adolescent Men ACWY or MCC vaccine or not having received vaccine. Trends by age and differences by vaccination status will be assessed by Chi-squared of Fisher's exact test as well as by using logistic regression. Comparisons by geometric mean titres will also be done by t-tests or Kruskal Wallis as appropriate and by plotting geometric means with 95% confidence intervals by age groups.
- Across the whole age range (0-24 years) a comparison of proportions protected with previous seroprevalence studies carried out in 2009 and 1996 for diphtheria and 1996-99, 2002-4, 2009 and 2014 for Men C. This will be done by using all the samples and calculating prevalence within individual age bands with 95% confidence intervals and inferring differences based on non-overlapping 95% CIs which is conservative to allow for multiple testing when assessing many age bands.
- For all participants, a repeat cross-sectional analysis of the geometric mean titres (with 95% CI) of antibodies against SARS-CoV-2, and proportion (with 95% CI) of participants with anti SARS-CoV-2 antibodies above a (yet to be determined) threshold of 'positivity' throughout the study period
- For participants providing multiple serum samples, analysis of the proportion of participants who were initially seronegative who subsequently developed antibodies against SARS -CoV-2
- For participants providing multiple serum samples, analysis of the kinetics of the rise and fall of antibodies against SARS -CoV-2
- Calculation of the sensitivity and specificity of salivary antibody testing compared to serum.

Exploratory analyses

- Whether ethnicity, household income or other factors collected on the questionnaire are associated with antibodies against diphtheria and / or Men C and/or SARS-CoV-2. This will be done by multivariable logistic regression.
- Comparisons between groups 1 and 2 will be performed as detailed in study statistical analysis plan in terms of:
    - representativeness of ethnicity, community identity, migrant population and socioeconomic background of local communities
    - immunological end-points
- Comparisons in the immunological end points will be performed as detailed in study statistical analysis plan between samples collected in this study and those obtained from testing of residual samples through the existing PHE seroepidemiology unit

11.2. Sample Size Determination

11.2.1: Group 1

The sample size for Group 1 is determined based on the primary objectives as well as the secondary objective to have a store of sera for future testing of immunity.

Historically serosurveys such as those done as part of the European Sero-epidemiology Network (ESEN) have had target sample sizes per age band of interest of 100-200. Age bands of interest have usually been one year bands until adult ages. For the purpose of this calculation this is taken as one year age bands to age 21 then a single band for 22-24 year olds. For the secondary objective 100 per age band will allow the precision of estimates of seroprevalence to be as given in table 2.

Table 2: Precision of seroprevalence estimates

Prevalence	95% CI Observed around the estimate with 100 per age band (2300 total)
10%	4.9-17.6
20%	12.7-29.2
30%	21.2-40.0
40%	30.3-50.3
50%	39.8-60.2
60%	49.7-69.7
70%	60.0-78.8
80%	70.8-87.3
90%	82.4-95.1

Precision will be improved when combining age bands for specific questions or when assessing trends by age for other secondary objectives. For example for diphtheria age bands of interest could be divided into <4,4-8,9-13,14-24 and for Men C into 0-10,11-12,13-15,16-19,20-24.

Focussing on the primary objectives

Representativeness of participants sampled

Within a study site representativeness is assessed by comparison to local population demographics. Each study region will have up to approximately 383 individuals allocated into Group 1. Overall all 2300 samples will be available for analysis in Group 1. With these numbers the precision of estimates of characteristics in the population and differences that would be significant to population data are given in Table 3. So, for example, if a specific ethnic group was 20% of the survey sample then in a region this would have a 95% CI of 16.1-24.4% and would allow a proportion in the population of <14.3% or >26.6% to be detectable as different.

We will also compare our sample demographics with the population demographics of the corresponding NHS region. This will increase the sample size in some regions are there are multiple sites within that region.

Table 3: Assessment of representativeness, precision and detectable differences

	Region (N=383)		**Overall (N=2300)**	
Prevalence of characteristic in sample	95% confidence interval	Prevalence below, above this in population that would significantly differ (80% power, 5% significance, assuming population much larger than sample (>=10 fold))	95% confidence interval	Prevalence below, above this in population that would significantly differ (80% power, 5% significance, assuming population much larger than sample (>=10 fold))
5%	3.0-7.7	2.3,9.1	4.1-6.0	3.8,6.5
10%	7.2-13.5	6.0,15.3	8.8-11.3	8.2,12.0
15%	11.6-19.0	10.0,21.0	13.6-16.5	12.9,17.3
20%	16.1-24.4	14.3,26.6	18.4-21.7	17.6,22.5
25%	20.7-29.7	18.8,32.0	23.2-26.8	22.4,27.7
30%	25.5-34.9	23.3,37.3	28.1-31.9	27.2,32.9

Comparison with residual samples

For comparison with the residual samples this is considered within age strata and is based on the 2017 data on residual samples for the numbers of residual strata by age. Due to the smaller number of residual samples in younger age groups (one of the key reasons for undertaking a community based seroprevelance survey), detectable differences are relatively large in those <4 years. In older age groups relatively, small differences can be detected in >4 year age bands. The example given below is for when the observed prevalence is 50% is the survey which is conservative as this gives the largest detectable differences.

Table 4: Comparison to residual samples by age strata with a total sample size of 2300

Age	**Detectable difference from 50% (80% power, 5% significance)**
<4	10.7
4 to 8	8.7
9 to 13	8.1
14 to 24	5.7
All Age	3.9

11.2.2: Group 1 and 2 combined

Sample size for SARS-COV-2 incidence based on change in prevalence

Across group 1 and 2 combined there will be over 3500 blood samples from 2800 to 3500 participants in total, at least 3200 of which will be from participants 19 years of age or younger. The intent of this is to obtain blood samples across 4 age bands (i.e. 100 in each of 0 -4 years, 5-9 years, 10-14 years, 15-19 years)to the end of the study. This will include sample taken from the longitudinal cohort, with seroprevalence calculations taking into account over-sampling of participants with positive SARS-Cov_2 antibodies at V1.

The table below shows the precision (95% CI) of estimates of change in prevalence (incidence) with 100 samples at each of two time points. For example with a prevalence of 10% at baseline and 30% at the next time point the incidence is 20% with 95% CI 9.3-30.7. The aim is to achieve 100 in each of 4 age groups at each time point.

Table 5: Precision of estimates of change in prevalence with 100 samples at two time points.

	baseline prevalence		
Prevalence At next time point	**0%**	**10%**	**20%**
10%	10 (4.1, 15.9)		
20%	20 (12.2, 27.8)	10 (0.2, 19.8)	
30%	30 (21.0, 39.0)	20 (9.3, 30.7)	10 (0.0, 21.9)
40%	40 (30.4, 49.6)	30 (18.7, 41.3)	20 (7.6, 32.4)
50%	50 (40.2, 59.8)	40 (28.6, 51.4)	30 (17.4, 42.6)
60%	60 (50.4, 69.6)	50 (38.7, 61.3)	40 (27.6, 52.4)
70%	70 (61.0, 79.0)	60 (49.3, 70.7)	50 (38.1, 61.9)

11.3. Analysis populations

The analysis population for recruitment rates will be all individuals invited, for representativeness will be all individuals providing a sample and for seroprevalence will all individuals providing a sample for which a laboratory result is obtained.

11.4. Group 3

Following discussions with Public Health England the suggested sample size would be 300 participants, with at least 100 from the Black/ African/ Caribbean/Black British community. This is based on preliminary data suggesting rates of seropositivity in adult males identifying as BAME are double that of the white community, and in those identifying as Black/ African/ Caribbean/Black British are 3 fold higher. Based on these proportions, and an estimate of 4% seropositivity in the white community, the above numbers would provide 80% power to detect similar differences in the paediatric populations.

11.5. The Level of Statistical Significance

5% and 95% confidence intervals will be reported. For comparison across individual age bands for seroprevalence differences will be inferred based on non-overlapping 95% confidence intervals.

11.6. Procedure for Accounting for Missing, Unused, and Spurious Data.

The reason for missing data (consent withdrawn or unable to obtain any laboratory results) will be indicated. Missing data will not be imputed.

11.7. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

If there are any changes after finalisation of the analysis plan that would be documented and justified in the analysis plan.

12. DATA MANAGEMENT

12.1. Source Data

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, radiographs, and correspondence.

Information on study participants will either be recorded directly into a web based electronic CRF or onto paper source document and later transferred into a web based electronic CRF (e.g. REDCap database stored on a secure University of Oxford server). REDCap is clinical research study software for electronic data capture (EDC) and clinical data management (CDM), which enables compliance with regulatory guidelines such as 21 CFR Part 11.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

12.3. Data Recording and Record Keeping

CRF data will be recorded directly into an EDC system (e.g. REDCap) or onto a paper source document for later entry into EDC if direct entry is not available. Any additional information that needs recording but is not relevant for the CRF (such as sites for venepuncture, parental availability etc) will be recorded on a separate paper source document. All documents will be stored safely in confidential conditions. The database includes a complete suite of features which are compliant with EU and UK regulations and NHS security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server will both be housed on secure servers operated by the University of Oxford IT Services. The servers are in a physically secure location in Oxford and are backed up in Oxford, with the backups stored in accordance with the IT department schedule of daily, weekly, and monthly tapes retained for 1 month, 3 months, and 6 months, respectively. Weekly backup tapes are stored offsite. The IT servers provide a stable, secure, well-maintained, and high capacity data storage environment, Drupal and MySQL are widely-used, powerful, reliable, well-supported systems. Access to the study's database and the diary will be restricted to the members of the study team by username and password.

All entries made to the research notes should be printed legibly. If any entry error has been made, to correct such an error, a single straight line should be drawn through the incorrect entry and the correct data entered above it. All such changes must be initialled and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, the clarification should be printed above the item,

and this should also be initialled and dated. Information entered into the research notes must be subsequently transferred onto the database by the site collecting the data. The participants will be identified by a unique study specific number in any database. The name and any other identifying detail will NOT be included in any study data file.

The investigator at each investigational site must make arrangements to store the essential study documents, (as defined in Essential Documents for the Conduct of a Clinical Trial (International Conference on Harmonisation (ICH) E6, Guideline for Good Clinical Practice) including the Investigator Site File. Copies of all study documents with participant identifiable information will be retained after the completion or discontinuation of the study for 3 years after the youngest participant turns 18 years. In addition, the investigator is responsible for archiving of all relevant source documents so that the study data can be compared against source data after completion of the study (e.g. in case of inspection from authorities). Storage of anonymised research data will be reviewed every 5 years and files will be confidentially destroyed if storage is no longer required. The investigator is required to ensure the continued storage of the documents, even if the investigator, for example, leaves the clinic/practice or retires before the end of required storage period. Delegation of this transfer of responsibility to their successor must be documented in writing.

The participants will be identified by a unique research study specific number and/or code in any database.

13. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. The study may be inspected by the Clinical Trials and Research Governance Office (CTRG), University of Oxford.

Following a risk based monitoring plan, the monitors will verify that the clinical research study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

14. PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g. consent process or administration of study intervention) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the study master file.

15. SERIOUS BREACHES

A “serious breach” is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the research study subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

16.5. Participant Confidentiality

All documents will be stored securely and only accessible by research study staff and authorised personnel. The study will comply with the General Data Protection Regulations (GDPR) which requires data to be anonymised as soon as it is practical to do so. Any data or samples that relate to participants and that leave the study site will be identified by study number only. All documents will be stored securely and only accessible by study staff and authorised personnel.

16.6. Expenses and Benefits

Participants will not be reimbursed for taking part in the study if we travel to their home. Should participants attend a clinic then they will be reimbursed £20 in the form of a voucher for travel e.g. a book voucher.

The information gained from this study will help to inform any strengths or vulnerabilities in vaccine strategy and may help future vaccine design.

17. FINANCE AND INSURANCE

17.1. Funding

This study is being funded by the National Institute for Health Research Policy Research Programme

17.2. Insurance

The University of Oxford has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

The investigators will co-ordinate dissemination of data from this study. All publications (e.g. manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be reviewed by all investigators prior to submission. Participants will have access to a summary of our study results either by post or an emailed link to our website with an abstract.

19. REFERENCES

2009, The immunolgical basis for immunization series. Module 2: Diphtheria, Update 2009 Geneva, World Health Organization.

Borrow, R., P. Balmer, and E. Miller, 2005, Meningococcal surrogates of protection- serum bactericidal antibody activity: Vaccine, p. 2222-2227.

De Melker, H. E., and M. A. E. Conyn-Van Spaendonck, 1998, Immunosurveillance and the evaluation of national immunization programmes: a population-based approach.

Findlow, H., H. Campbell, J. Lucidarme, N. Andrews, E. Linley, S. Ladhani, and R. Borrow, 2019, Serogroup C Neisseria meningitidis disease epidemiology, seroprevalence, vaccine effectiveness and waning immunity, England, 1998/99 to 2015/16: Eurosurveillance, v. 24, p. 1700818.

Klis, F. v. d., L. Mollema, G. Berbers, H. d. Melker, and R. Coutinho, 2009, Second national serum bank for population-based seroprevalence studies in the Netherlands: The Netherlands Journal of Medicine, p. 301-8.

Maslanka, S., L. Gheesling, D. Libutti, K. Donaldson, H. Harakeh, and J. Dykes, 1997, Standardisation and a multilaboratory comparison of Neisseria meningitidis group A and C serum bactericidal assays.: Clin Diagn Lab Immunol, v. 4, p. 156-67.

Melker, H. D., and M. C.-v. Spaendonck, 1998, Immunosurveillance and the evaluation of national immunization programmes: a population-based approach: Epidemiology and infection, p. 637-43.

Mis, N. F., K. Kennedy, M. Fewtrell, C. Campoy, and B. Koletzko, 2018, The Role of Incentives in Long-Term Nutritional and Growth Studies in Children: J Pediatr Gastroenterol Nutr.

Pickering, J., T. Martins, M. Schroder, and H. Hill, 2002, Comparison of a multiplex flow cytometric assay with enzyme linked immunosorbent assay for auantitation of antibodies to tetanus, diphtheria, and Hameophilus influenzae type b. : Clin Diagn Lab Immunol, v. 9, p. 872-6.

Sekine, T., A. Perez-Potti, O. Rivera-Ballesteros, K. Strålin, J.-B. Gorin, A. Olsson, S. Llewellyn-Lacey, H. Kamal, G. Bogdanovic, S. Muschiol, D. J. Wullimann, T. Kammann, J. Emgård, T. Parrot, E. Folkesson, O. Rooyackers, L. I. Eriksson, A. Sönnerborg, T. Allander, J. Albert, M. Nielsen, J. Klingström, S. Gredmark-Russ, N. K. Björkström, J. K. Sandberg, D. A. Price, H.-G. Ljunggren, S. Aleman, and M. Buggert, 2020, Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19: bioRxiv, p. 2020.06.29.174888.

Wagner, K., J. White, N. Andrews, R. Borrow, E. Stanford, E. Newton, and R. Pebody, Immunity to tetanus and diphtheria in the UK in 2009: Vaccine, p. 7111 - 7117.

Wagner, K. S., J. M. White, N. J. Andrews, R. Borrow, E. Stanford, E. Newton, and R. G. Pebody, 2012, Immunity to tetanus and diphtheria in the UK in 2009: Vaccine, v. 30, p. 7111-7.

APPENDIX C: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	V1.1	21-June-2019	Helen Ratcliffe	1. Clarification information storage according to GDPR guidelines 2. Removal of minimum blood volume.
2	V2.0	09-Aug-2019	Helen Ratcliffe	1. Change of PI for Bristol University NHS Trust to Dr Marion Roderick 2. Change of PI for St Georges NHS trust to Dr Eva Galiza 3. Clarification of reimbursement which is for travel and not time taken to participate in study. 4. Addition of anaesthetic spray which can be used instead of anaesthetic cream. 5. Change in exclusion criteria. 6. Clarification of data storage durations in section 13.3
3	V2.1	03-Oct-2019		1. Removal of duplicated section heading and addition of section numbering 2. Removal of sentence from section definition of end of research study 3. Change in table numbering
4	V3.0	21-Feb-2020	Helen Ratcliffe	1. Addition of testing for SARS-COV-2 2. Questionnaire questions regarding respiratory infections introduced
5	V4.0	19-Mar-2020	Helen Ratcliffe	1. Addition of additional 1200 participants aged 0-19 years of age 2. Addition of end-points for comparisons between group 1 and group 2 3. Change to exclusion criteria to specify selected post-code does not apply to the additional 1200 participants 4. Inclusion criteria changed in protocol to reflect the different age groups for Group 1 and 2 5. Recruitment method details for the newly added Group 2, which include generalised mail outs, press releases, social media

				adverts, schools or community clinics. 6. Update of sample size calculation section to include additional 1200 participants 7. Addition of temporary exclusion criteria for suspected COVID-19
6	V5.0	30th April 2020	Matthew Snape	1. Incorporation of additional study sites and Principal investigators 2. Change of sample size from 3500 to '2800 to 3500', to change emphasis to at least 400 samples per month, inclusive of repeat blood samples from longitudinal cohort. 3. Addition of longitudinal sampling cohort, with repeat blood samples, and saliva samples 4. Update of lay summary and background and Rationale to better explain COVID-19 methodology and longitudinal sampling cohort 5. Update of study objectives to a. Substitute 'antibody concentrations' for 'immunity' b. Substitute SARS-CoV-2 where appropriate c. Add in cross sectional sero-epidemiology for SARS-CoV-2 d. Add in assessment of SARS-CoV-2 antibody kinetics for longitudinal cohort e. Add in objectives for saliva sample collection 6. Extensive expansion of section 7.1 'Study design' to: a. Explain the goals of the study, and allocation of participants to Group 1, Group 2 and longitudinal cohort study b. Give guidelines for recruitment in light of the above 7. Deletion of previous section 7.2 (inclusion criteria) as a duplication of latter sections of protocol

				8. Simplification of section 7.4 (sample size) 9. Adding in household contacts for questionnaire (question section 7.6) 10. Clarification that £20 compensation will apply for visit for longitudinal sample 11. Deletion of 'interim analysis' from section 9.1 (inappropriate for cross-sectional sero-prevalence study), instead allocation to Group 1 to be applied retrospectively 12. Amendment to section 9.4 (informed consent), to address optional consent for longitudinal sample cohort 13. New section 9.5.2 to incorporate subsequent visits 14. Paragraph now under laboratory methods ('The blood sample obtained..' moved from study visits 15. Update of statistical analysis plan to incorporate new study objectives
7	V6.0	15th June 2020	Helen Ratcliffe	1. Correction of study end date in section 3.Synopsis 2. Correction of age groups in section 7.1 Study design 3. Correction of collection of samples to December 2020 in section 7.1 Study design 4. Extension of time between longitudinal visits in 7.1 Study design and 9.5.2 Subsequent visits (longitudinal sampling cohort) 5. Clarification on the difference between withdrawal and failed enrolment in sections 9.4 Informed Consent and 9.7 Discontinuation/Withdrawal of participants from research study 6. Simplification of recruitment guidelines (referred to clinical study plan) in 7.1 Study design 7. Correction of Plymouth Hospitals NHS Trust to University Hospitals Plymouth NHS Trust in section 7.2 Study sites

				8. Clarification that study information can be disseminated through GP practices in section 9.1 Recruitment Group1 and 9.2 Recruitment Group 2 9. Change to SARS-CoV-2 IgG testing from spike protein to spike protein and/or nucleocapsid in section 6.Objectives and Outcome Measures and section 9.6 Laboratory methods
8	V7.0	21st July 2020	Helen Ratcliffe	1. Extension of the recruitment period for first study visit to March 2021 in Synopsis 2. Update of exploratory objectives and outcome measures in Synopsis and section 6. Objectives and outcome measure. 3. Addition of taking PBMCs in the longitudinal cohort to allow examination of T cell responses in sections 5. Background and Rational, 7.1 Study Design, 9.5.2 Subsequent visits and 9.6 Laboratory methods 4. Option to add GP practices as PICs in section 7.2 Study Sites 5. Re-consent for all in longitudinal cohort, section 9.4 Informed Consent 6. Sample size for SARS-COV-2 clarification, section 11.2.2. Group 1 and 2 combined 7. Addition of investigator
9	V8.0	02nd December 2020	Helen Ratcliffe	1. Removal of the upper time limit between visits for the longitudinal cohort 2. Addition of the rationale behind adding enhanced recruitment amongst the BAME population. 3. Addition of Group 3 which aims to recruit 300 participants from the BAME population 4. Recruitment methods for group 3 5. Update of statistical analysis section to include sample size justification of group 3 6. Updating the analysis of representativeness of population

				sampled of the local region and NHS region. 7. Addition of investigators and sites 8. Section 9.5: Addition of videoconference consent in case of omissions or errors 9. Section 9.1: Update of recruitment methods with printed publication option 10. Addition of receipt of samples from other studies
10	V8.1	20th January 2021	Iason Vichos	1. Addition of Pharmacies as a way of identifying participants in section 7.2 Study Sites and 9.3 Group 3 2. Correction of eligible age group in section 9.6.2 Subsequent visits (longitudinal sampling cohort)
11	V8.2	26th February 2021	Iason Vichos	1. Extension of the recruitment period for first study visit to May 2021 in Synopsis 2. Addition of recruitment methods in section 9.0: radio, identification of participants by study team, as long as potential participants are not patients.
12	V8.3	21st May 2021	Iason Vichos	1. Synopsis: Extension of the recruitment period for first study visit to June 2021 2. Lay Summary: Removal of outdated text 3. Background and rationale: removal of the requirement of 300-400 blood samples per month 4. Study sites: Removal of Birmingham Heartlands Hospital
13	V9.0	28th June 2021	Iason Vichos	1. Section 3. Synopsis: Extension of Planned Study Period to August 2021 2. Section 9.9: end of study definition amended to include the completion of data collection

List details of all protocol amendments here whenever a new version of the protocol is produced.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee and HRA (where required).