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Evaluating Detection of SARS-CoV-2 antibodies using home test kits (EDSAB-HOME study)

Protocol 30.05.2020 Version 04.03

RAPID EVALUATION PROTOCOL

General information

Protocol title

Evaluating Detection of SARS-CoV-2 antibodies using home test kits (EDSAB-HOME)

Rationale & background information

After SARS-CoV-2 infection, individuals mount antibody responses against the virus, details of which are in the Appendix. In view of this, the UK Government is currently planning two major initiatives under Workstream 3 (WS3) to detect antibodies against SARS-CoV-2, which, subject to confirmation of assay performance, will be made available to key workers and to the general public.

In both initiatives, the tests will use blood for samples taken at home.

These initiatives are for the delivery of:

i) A laboratory based enzyme immunoassay (EIA) testing system
ii) A testing system similar to a pregnancy testing kit which is readable at home ('home test kits' (HTK))

The aim of the mass testing programme(s) will be to provide information to individuals on whether they have been previously exposed to the virus.

The implementation of the programme is being led by the Department of Health and Social Care (DHSC), with PHE contribution on assay performance and the prioritisation of populations who might benefit from the testing programmes; currently the top priority group for testing comprises key workers.

PHE, in collaboration with the HPRU in Behavioural Science and Evaluation, aims to lead on the rapid evaluation of the HTKs used in this programme, through evaluating the HTKs' performance in detecting anti-SARS-CoV-2 antibody. In addition, it will explore the acceptability and feasibility of the programme. The first phase of the programme will also allow integration across both laboratory test, home bleed programs and HTKs programs of work within WS3, and will facilitate the rapid validation of additional EIA assays developed by industry consortia.

The current evaluation proposal for HTKs will involve a three-staged implementation. This design is driven in part by several unknowns in this process, including:

- when the HTKs will become available;
- how accurate the HTKs are in detecting anti-SARS-CoV-2 antibody;
- whether the HTKs as read by the general population agree with those as read by a trained expert;
- whether all cases of SARS-CoV-2 infection lead to an antibody response;
- whether previous infection or antibody presence as measured by the HTKs will translate into protection in the field.

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This rapid evaluation will aim to understand the potential limitations of the mass HTK testing programme and provide a framework to maximise its value going forward. The proposed evaluation will also help to inform what advice people who test negative and positive on the HTK should be given, including whether there is a need for confirmatory antibody testing using laboratory based assays.

The first HTKs to be used will be supplied by the UK Rapid Test Consortium (UK-RTC), which comprises of the University of Oxford, Abingdon Health, Omega Diagnostics, BBI Solutions and CIGA Healthcare. Of note, other manufacturers including MoLogic are producing similar products, and these may be tested if available.

Study goals and objectives

The goal is to address the key uncertainties around the delivery of community based mass antibody testing for previous SARS-CoV-2 infection, to allow rapid programme development. The project will evaluate the "first purchase" HTKs which the national programme will be using, while providing a route to rapid validation & verification of alternatives which may be available later in 2020, including validation of industry consortia EIA kits.

Objective: To evaluate the population testing programme including;

1. To describe the accuracy of the HTKs in detecting anti-SARS-CoV-2 antibody, and in detecting previous SARS-CoV-2 infection, when read by a trained professional;
2. To describe the agreement between reading of the HTK results (i) by users of the service, (ii) by a trained professional examining a photograph, (iii) by a trained professional in a community setting - and subsequently the accuracy of user-reading in detection of anti-SARS-CoV-2 antibody
3. To assess whether home photographic recording of HTK is feasible and enhances accuracy of recording relative to user reading;
4. To determine the acceptability and usability of the tests in the population;
5. To inform the instructions and other advice to be provided to people undertaking the tests and to those who have a positive and a negative result;
6. To describe the numbers of coronavirus responsive T cells in peripheral blood of subjects using ELISPOT technology, and its relationship to serological tests for SARS-CoV-2, and to clinical risk factors for COVID-19;
7. To provide estimates of the hazard of development of COVID-19 compatible symptoms, or of COVID-19 related hospitalisation, among individuals with a positive vs negative laboratory and HTK antibody test kit results, and its relationship to SARS-CoV-2 responsive T cell numbers;
8. To provide a panel of plasma which can be used for proficiency testing, batch release assays, and the laboratory-based evaluation of future kits.

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Timeframe: The study is anticipated to commence in mid-May, prior to (and in the anticipation of) the HTKs being ready for use.

- We aim to commence recruitment for blood collection in w/c 18th May
- We aim to complete stage one within 2-3 weeks
- We aim to complete laboratory comparison by 15th June, to assess whether the HTK kit has met the Target Product Profile (TPP). We are aware the MHRA is currently consulting on what such TPPs should be. At the time of writing, the current guidance was available at https://www.gov.uk/guidance/guidance-on-coronavirus-covid-19-tests-and-testing-kits.
- If so, we aim to commence stage two in late June, and complete in 1-2 weeks.
- We aim to complete laboratory comparison and write up within 8 weeks of launch.

Expected outcomes:

1. Estimates of the accuracy of a HTK in detecting anti-SARS-CoV-2 antibody when read by a trained professional;
2. Estimates of the accuracy of user-reading of the HTK result, and of reading of the HTK result by a trained professional examining a photograph, in detecting anti-SARS-CoV-2 antibody;
3. An evaluation of whether home photographic recording of HTKs is feasible, yields images of sufficient quality, and enhances accuracy of recording (compared to user reading);
4. Questionnaire responses indicating the acceptability and usability of HTKs in the population;
5. A set of advice for the ongoing development of the programme and recommendations on the information provided for users;
6. A description of the numbers of coronavirus responsive T cells in peripheral blood of subjects using ELISPOT technology, and its relationship to serological tests for SARS-CoV-2, and to clinical risk factors for COVID-19;
7. An estimate of the hazard of development to COVID-19 compatible symptoms, or COVID-19 related hospitalisation, among individuals with a positive vs negative laboratory and HTK anti-coronavirus antibody results, alone and in combination with SARS-CoV-2 responsive T cell numbers;
8. A panel of plasma which can be used to evaluate the accuracy of any next-generation immunochromatographic kits in a 'bridging' validation / verification study, for quality assurance, and lot qualification.

Study Design

Methods: Test evaluation, including a prospective cohort study of high risk (healthcare workers) and lower risk (e.g. police) populations for COVID-19.

Population: The test evaluation population will reflect the population projected to use the home testing service in the initial stages of HM Government test rollout, which is likely to be front line workers such as healthcare staff and police/fire/rescue officers. A representative sample from a group of organisations will be recruited if possible.

Subsequently, testing may be extended to the general public, once the first two target groups have been studied.

In addition, a selection of individuals with previous positive SARS-CoV-2 tests ("known positives") from OLS-WS-2 (Antigen testing) (at least 2 weeks after initial infection) may be included into the population.

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Inclusion criteria:

- Currently working at their place of work;
- Aged 18 years or older;
- Able to read and English, so as to understand the protocol;
- Has an in-use personal email address and mobile phone.

Exclusion criteria:

- Is currently experiencing COVID-19 compatible symptoms.
- Has experienced COVID-19 compatible symptoms in the last seven days;
- Meets definition of "exceptionally vulnerable" on medical grounds, including immunosuppression, previous oncological treatments – these people should not be at work anyway;
- Unable to read normal sized print with glasses;
- Taking part in vaccine studies.

Methodology

Recruitment:

PHE will aim to recruit initial users of the HTK program, with the overall aim of obtaining a representative sample of initial intended users. This will comprise of three key cohorts, with different expected prevalence, allowing some exploration of whether sensitivity may vary by severity:

- Healthcare workers (1,500 persons). All individuals working on site will be eligible to enrol.
- Police workers (1,000 persons) [or other cohort of non-healthcare workers]. All individuals working on site will be eligible to enrol.
- General population, the recruitment of which will be considered at a later date once the first two groups have been studied.

Additionally, a fourth cohort will be approached. These are individuals who have previous tested positive as part of health care worker screening, and whose details are known to the NHS Business Services Authority (NHSBSA) . The DHSC has undertaken legal review, and has confirmed to us that their processing of the personal details of these individuals for this purpose is lawful, and that they are prepared to send a communication to them on our behalf. We will attempt to recruit up to 500 such workers.

Recruitment strategy: Workplace based recruitment

Senior leadership (CEO or equivalent) from selected workplaces will be sent a letter signed by senior leadership in DHSC inviting their institution to take part in the study and their specific ask for taking part. They will have the opportunity to have a phone call with senior leadership in DHSC to discuss the proposed processes and answer any questions they may have.

If they agree to take part, they will be sent a template email to send out to their employees. This will include information on the criteria for taking part, the participant information sheet, and links to the consent form, online questionnaire, and appointment booking system. They will also be sent a template poster which can be put up in their place of work. This will emphasise to their staff that the study is voluntary, and they have no obligation to take part. Depending on recruitment rates, we may restrict recruitment to a convenience sample of the first 100-150 people to book an appointment per institution. Additional, walk in slots may be available, depending on logistical capacity.

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Recruitment strategy: Workplace based recruitment

DHSC, in collaboration with the NHSBSA, will send a closely related template email to that sent out by NHS Trusts to individuals who have previously tested positive.

Three stages:

The study will take place in three stages (Figure 1):

1) The anticipated initial users of the service (including up to 500 "known positives") are recruited (2,500 persons) prior to the HTKs becoming available, and invited to attend a community testing centre on specific day(s). At the centres, metadata will be collected on all individuals (in the form of an electronic questionnaire) and a venous blood sample taken.

   In a small number (n ~ 100) of individuals, a capillary blood sample and dried blood spot (DBS) will be taken. This will allow assay optimisation for enzyme immunoassays which may in future use capillary blood samples.

   The blood samples will be tested with one or more commercial EIAs. These include products by
   EuroImmun (anti-Spike)
   Roche (anti-N)
   Abbott (anti-N).

   Emerging data indicates that anti-N assays have very good performance, with enhanced sensitivity relative to anti-S assays. For all the assays, sensitivity, particularly early in disease and in cases of asymptomatic infection, is less well characterised.

   We will use a single assay with the highest published performance available, which we will refer to as the High Performance Assay (HPA). We envisage this will be one of the two available anti-N EIAs. Subject to availability, we will perform both an anti-S and anti-N assay on all samples as well, but the initial analysis of the HTK will be against the HPA. Other experimental assays under development by the DHSC project or PHE may also be used. A plasma bank will be set up, ready to be tested with the HTK in the laboratory once available. The clinical performance of the HTK will be estimated in two separate analyses (1) assuming the HPA is 100% sensitive and specific (which may, however, be incorrect); and (2) Bayesian evidence synthesis methods incorporating other data sources (see below). (Objective 1, 6, 7).

   The capillary blood samples will be used to compare EIA signals between samples derived from DBS, venous plasma and capillary plasma samples as part of a proof-of-concept, methods development approach. The 100 sample size is based on the need for at least 10 positives, and is based on an assumed 10% seroprevalence. We will modify the number of capillary samples taken to ensure sufficient positive samples are available based on seroprevalence data.

2) Once the HTKs become available for use and pass the TPP requirements, individuals will be invited to participate in stage two based on a combination of stratified and random sampling, if necessary, based on geography, age, symptom history and EIA result. During this stage, an additional blood sample will be taken. Individuals who complete the HTK will do so independently, and will also take a photograph of their HTK result. Comparative accuracy of reading of HTK results by trained professionals (in the community, and by photograph) will also be explored. Data from this stage will

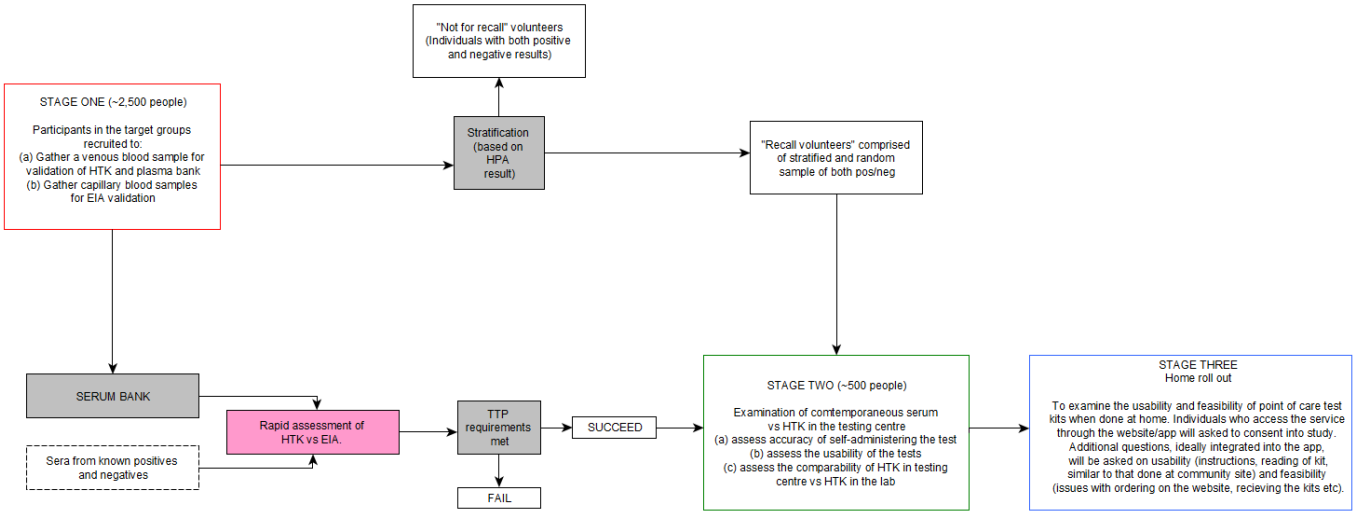
allow us to assess agreement between user reading, reading of test results by a trained professional in the community and using a photograph, and reading in a laboratory setting. This will allow us to determine whether accuracy estimates from stage one are applicable to these other types of reading. (Objective 2, 3, 5, 6).

All participants will be followed up with weekly symptom questionnaires and linked to routine laboratory and hospital record statistics, to identify progression to severe COVID-19 disease (and possibly mild COVID-19 disease, confirmed microbiologically, depending on changes to future testing strategy) (Objective 4).

In the event that antibody kits are available earlier than expected, and at scale, stage one and two may be combined with all subjects in stage one (above) additionally doing POC tests. In addition, depending on operational capacity, stage two and stage three may also run in parallel.

3) The service evaluation will be extended when the programme expands to home use; however, the evaluation will focus on assessing the usability and accessibility of the programme and to identify any logistical/operational limitations associated with the home delivery system, and where improvements may be required. In addition, the three-stage service evaluation may be extended at a later date to the general population, contingent on sufficient clinical performance of the HTK within the healthcare workers and police officers. (Objective 5)

Figure 1: Overview of the three-stage evaluation study methodology



Recruitment sites and study areas will reside within the UK, and will be chosen based on the epidemiology and operational capacity available during the study launch.

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Study workflow (Stage one, Community testing centre):

1. The study will be advertised in target workplaces, through senior leadership at the workplace.
2. Patient information leaflets are distributed to interested persons, who are invited to a clinic.
3. Informed consent is obtained. The subject is given the option to receive results from validated assays, if these are available.
4. The subject completes a questionnaire, including information on their demographics, and questions (such as prior symptoms, prior symptoms in family, health care worker/school contact, and area of residence) relevant to the risk they have been exposed/infected by COVID-19. Additional questions on their current behaviour will also be included.
5. Up to 20ml venous blood sample will be obtained by a trained on-site phlebotomist. We will take 1x 6ml EDTA anticoagulated samples and 1 x 6ml or 10ml lithium heparin anticoagulated tube. This may occur in a specially convened clinic; alternatively, if before the visit, the NHS decided to start staff antibody screening, we will do our best to coordinate and make sure that any blood samples needed are all taken in one sitting.
6. In a proportion of subjects, the subject may also be asked to provide a capillary blood sample and/or dried blood spot. They will be assisted in doing so.
7. The subject is asked whether they would like to be a volunteer who may be called back for further testing.
8. The subject is thanked, given a leaflet or email explaining that we may contact them again to take part in stage two, and what that would involve.
9. The EDTA venous blood sample is sent to a PHE Sero Epidemiology Unit (SEU) Laboratory in Manchester, and/or another laboratory as necessary. Plasma will be separated by centrifugation.
10. If the subject has specifically consented, waste material not used for serological analysis (including blood cells and DNA) is banked for future studies. Recent experiments have shown the immune response can be analysed by analysing the DNA rearranged during T cell and B cell receptor formation (see: Fowler A et al 2020 BMC Genomics 20202 21:176). Such studies may be the basis of prognostic tests in the future, so banking this is relevant to the COVID-19 containment campaign.
11. Plasma is banked.
12. The lithium heparin anticoagulated sample is sent to Oxford Immunotec Ltd, Milton Park, Oxford for T-SPOT SARS-CoV-2 testing. This is a Research Use only (RUO) ELISPOT based kit produced by Oxford Immunotec which is due to be launched on 15 May 2020. It measures the number of CD4 and CD8 cells specifically producing interferon-γ in response to a proprietary mixture of proteins and/or peptides derived from SARS-CoV-2 proteins. The tube sent to Oxford Immunotec will be identified by a study number only. Oxford Immunotec return the results to PHE.
13. Banked plasma is analysed for anti-SARS-CoV-2 antibodies using the HPA EIA assay, and other assays as they are developed. Additional samples (capillary blood and/or dried blood spot) will also be banked and despatched to laboratories as directed by the DHSC program evaluating team.
14. Banked plasma is made available for laboratory evaluation of HTKs in a laboratory setting, when such kits become available.
15. If the subject has requested the result of their serological results, we will provide them when
    - The assays used have been deployed, or are scheduled for deployment, by the NHS at the time of reporting, and so are considered to have a sufficient level of validation.
    - We have sufficient confidence in the technical processes.

    Substantial uncertainty remains about the clinical interpretation of these tests (for example – are the individuals protected if seropositive), and data is accruing all the time. The NHS is currently drawing up interpretation guidelines. We will provide the results with an explanation congruent with those issued by the NHS when these complete. We will test these materials with the HRA's Patient and

Public Involvement group before we use them, and provide a report on this activity to the PHE REC before going ahead.

In all cases we will state that, in the event that clinical decisions need to be made based on antibody levels, the subjects should have a test performed by an NHS Laboratory at the relevant time. This is because antibody levels may change over time.

16. The subject is followed up by PHE actively, with weekly symptom and activity questionnaires, and passively, with weekly database (SGSS, a microbiology database containing COVID-19 results, and other appropriate queries) searches for all evaluated subjects, allowing quantification of hospitalisation with positive SARS-CoV-2 tests, a proxy for severe disease.

Study workflow (Stage two, Community testing centre):

1. The subject is asked to give consent to testing a HTK.
2. The subject is asked to complete short questions on their current behaviour over the prior weeks.
3. Up to 20ml venous blood sample will be obtained by a trained on-site phlebotomist exactly as in Stage 1.
4. The subject does a HTK without assistance, using the packaging and training proposed for home use.
5. The subject may optionally be asked to read the result not of their own test, but of another person, following a randomisation procedure to which the subject is blinded. The necessity for this step is discussed in Objective 2, below.
6. The subject takes a photo of the result on their mobile phone (perhaps with the NHSX or another appropriate app), and/or uploads the result to the NHSX designed app, or via another appropriate tested mechanism agreed with MHRA.
7. The result will also be independently read by a trained professional and a second independent reviewer and/or algorithm (e.g. in-app test reader).
8. The subject completes an additional questionnaire, including questions on ease of use and reading of the kits, acceptability of the kits etc. In some cases, structured, semi-structured interviews may occur.
9. The subject is thanked, given a leaflet or email providing contact details if they have any further questions.
10. The blood sample is sent to PHE Sero Epidemiology Unit (SEU) Laboratory in Manchester, and/or another laboratory as necessary, and analysed for anti-SARS-CoV-2 antibodies using the HPA EIA assay, and other assays as they are developed. Other experimental assays may also be used.
11. The lithium heparin anticoagulated sample is sent to Oxford Immunotec Ltd and processed exactly as in Phase 1.
12. The HTK is repeated in the laboratory on the blood sample taken, to determine whether results are the same as from the HTK when done in the community testing centre.
13. The subject continues to be followed up by PHE actively, and passively, as outlined previously.

As above, in the event that antibody kits are available earlier than expected, and at scale, stage one and two may be combined with all subjects in stage one (above) additionally using HTKs (steps 4-7 of Stage 2) after step 4 of stage one.

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Study workflow (Stage three, Extension to home setting):

1. The subject is identified through the NHSX HTK service and is asked to give consent for participation
2. The subject is sent a questionnaire to complete, including information on their demographics, questions relevant to the risk of infection by COVID-19, and questions on their current behaviour. We anticipate this will either be an online system, e.g. SelectSurvey or Snap Survey, or integrated directly into the NHSX app.
3. The subject is asked to complete their HTK, following the instructions provided to them.
4. The subject records their result and photographs their kit in the NHSX system, or another tested alternative system.
5. The subject completes further questions around the acceptability and usability of the kits, including specific questions around the ordering of the kit through the online system.
6. The subject is thanked for their participation.
7. The subject is followed up by PHE actively, with weekly symptom and activity questionnaires, and passively, with weekly database (SGSS, a microbiology database containing COVID-19 results, and other appropriate queries) searches for all evaluated subjects, allowing quantification of hospitalisation with positive coronavirus tests, a proxy for severe disease.

Follow-Up:

Some participants from stage one may be invited back to participate in stage two. Participants will be actively followed up through weekly questionnaires, and passively followed up through data linkage with routine databases.

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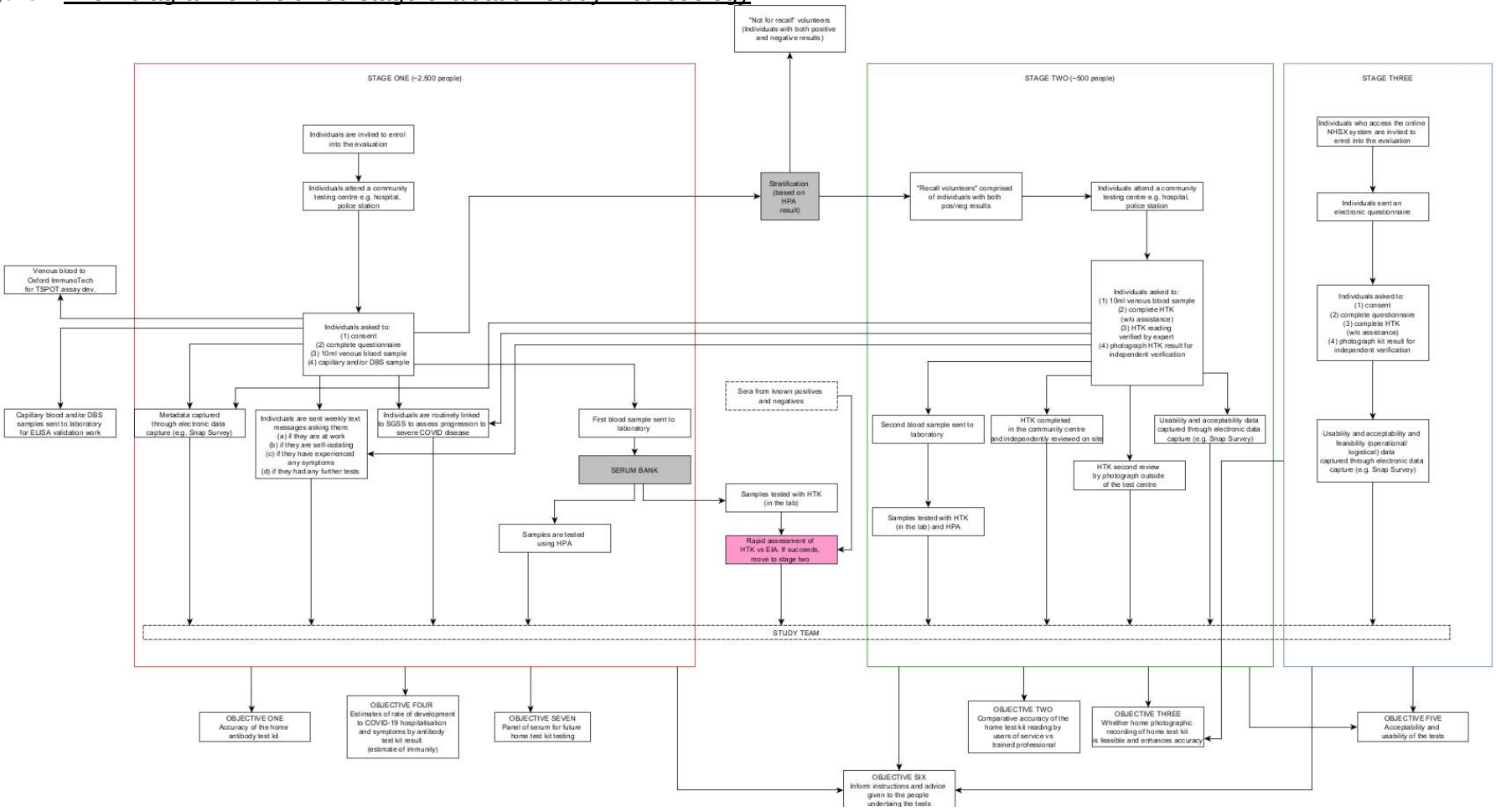
Figure 2: Flow diagram of the three-stage evaluation study methodology



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Data management and analysis

Data Management and Quality Assurance

Data will be entered in Snap Survey or another suitable technical platform and stored on a secure PHE server using a relational database management system. Only those who are conducting data management, cleaning or analysis will have access to the data. Any data shared outside of PHE will have a suitable data sharing agreement in place, and only pseudo-anonymised datasets will be shared.

We aim to maintain the volunteer bank, which contains identifiable information, for up to 2 years, whereby volunteers may be recalled to test new antibody devices as they become available. One year following the conclusion of the volunteer bank all personal identifiable information will be removed.

Data management will be performed in R and statistical analysis using a combination of R and Bayesian statistical software, WinBUGS. Laboratory methods will follow the requirements of ISO15189.

Data management will follow PHE Information Governance and GDPR requirements.

Statistical Analysis

An initial descriptive analysis will describe the cohort by age, gender, ethnic group, occupation, recent symptoms and previous exposure to SARS-CoV-2 assessed using symptoms, and enzyme immunoassay and T-SPOT coronavirus assays.

The EDSAB-HOME project is intended as part of a programme delivering high quality information to support rapid licensure of HTKs. These may come from different sources, with different amounts of in vitro data comparing HTK vs. other standards, prior to EDSAB-HOME study investigators become involved. Essentially, some of the objectives described in Objective 1 may already have been met to a standard acceptable to regulators for some products to which the EDSAB-HOME protocol is applied. The decision as to whether the activities described in Objective 1 will be conducted, and on what scale, will made by the DHSC who are in charge of the project, in conjunction with the MHRA. The EDSAB-HOME investigators will provide professional advice and assist DHSC in delivering the overall project goals.

Objective 1

To describe the accuracy of the HTKs in detecting anti-SARS-CoV-2 antibody, and in detecting previous SARS-CoV-2 infection, when read by a trained professional;

For each sub-population – and also aggregated across all individuals - results (positive / negative) on the HTK will be tabulated against results on the HPA (dichotomised at a pre-specified level), of format in Table 1, where N is the number of samples analysed:

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Table 1

	Laboratory-based assay positive	Laboratory-based assay negative	Total
Home test kit positive	a	b	a + b
Home test kit negative	c	d	c + d
Total	n+ = a + c	n- = b + d	N = a + b + c + d

We will produce estimates, with 95% credible intervals (Cr-Is), of the test sensitivity and specificity of the HTK, and of the implied Positive Predictive Value (PPV) and Negative Predictive Value (NPV), across a range of values of prevalence or pre-test probability.

We will produce similar analyses for T-SPOT coronavirus results.

We will assess evidence for whether test accuracy varies by sub-population and by key variables from the questionnaire such as reporting of previous COVID-19 like symptoms.

Analysis 1a

In Analysis 1 we will assume that the laboratory based assay, HPA, dichotomised at a pre-specified threshold, is a "gold standard" reference test, i.e. has 100% sensitivity and 100% specificity in detecting anti-SARS-CoV2 antibody.

Sensitivity and specificity of the HTK can then simply be estimated by the proportion of HPA positive samples that are positive on the HTK (i.e. Table 1: a/n+); and the proportion of HPA negative samples that are negative on the HTK (i.e. Table 1: d/n-), respectively. 95% Cr-Is around estimates will be estimated through fitting binomial likelihoods in WinBUGS. The PPV and NPV will be calculated by application of Bayes' rule within the WinBUGS model, such as to produce Cr-Is that account for sampling uncertainty in both sensitivity and specificity.

Estimates of each of these quantities will be provided both 'overall' and also stratified by sub-population or and/or other key variables from the questionnaire.

We will produce similar analyses using T-SPOT coronavirus results.

Sample Size for Analysis 1 of Objective 1

Sample size calculations for this study are challenging because of the lack of a gold standard test, and the fact that prevalence in the study population is both unknown and increasing over time. The following calculations assume that the HPA laboratory-based test is 100% sensitive and 100% specific, which is known not to be the case. The calculations are therefore no more than illustrative. We assumed that the true sensitivity and specificity of the HTK are both 98%. These are the minimum values currently considered acceptable by the MHRA (18/04/2020). The performance metric of the most interest is the PPV, defined as the probability that a person who tests positive does in fact have antibodies. Table 2 shows the expected 95% confidence intervals for sensitivity, specificity and PPV which would be obtained for a sample size of 1000 or 2500 participants, under various assumed values of prevalence in the study population. If we were to consider 90% PPV acceptable, and prevalence in the study sample was 20%, we would require 2,500 participants to obtain a 95% CI which was wholly above 90% PPV.

Table 2 The expected 95% confidence intervals (CIs) around estimated sensitivity, specificity and PPV under potential sample sizes of n = 1000 and n = 2500, assuming true sensitivity and specificity are both 98%.

	Study size of n = 1000			**Study size of n = 2500**		
Prevalence in study population	Sensitivity 95% CI	Specificity 95% CI	PPV in study population: estimate (95% CI)	Sensitivity 95% CI	Specificity 95% CI	PPV in study population: estimate (95% CI)
5%	0.93,1.00	0.97,0.99	0.72 (0.61,0.82)	0.95,1.00	0.97,0.99	0.72 (0.65,0.79)
10%	0.95,1.00	0.97,0.99	0.85 (0.78,0.91)	0.96,1.00	0.97,0.99	0.85 (0.80,0.89)
15%	0.95,1.00	0.97,0.99	0.90 (0.85,0.94)	0.96,0.99	0.97,0.99	0.90 (0.87,0.92)
20%	0.96,1.00	0.97,0.99	0.93 (0.89,0.96)	0.97,0.99	0.97,0.99	0.92 (0.90,0.95)
25%	0.96,1.00	0.97,0.99	0.94 (0.91,0.97)	0.97,0.99	0.97,0.99	0.94 (0.92,0.96)

Test performance may vary across populations, e.g. due to variation in underlying severity of disease. To allow exploration of this, initially we propose a cohort of 1500 healthcare workers and 1000 police officers, with later possible extension to the general public.

Analysis 1b

We will also report test accuracy in comparison to locally relevant adaptations of the World Health Organisation [20th March, version 6] criteria for confirmed cases (RT-PCR positive), suspect cases (combining symptoms and exposure) and probable cases (suspect cases with inconclusive or no RT-PCR results).

Our adapted definition reflects UK screening and swabbing practice; see Appendix 2. On 5 March, the UK Government confirmed that local COVID-19 transmission was occurring. After this, it changed policy from a 'containment phase' based on active case finding and molecular screening of individuals meeting a definition based on WHO criteria to one of 'delay' in which testing was largely confined to hospitalised cases, based on limitations in testing capacity.

Our definition includes the WHO's recommended confirmed, suspect and probable cases. In addition, we are pre-specifying a category of 'symptoms but tested negative' because molecular testing may be insensitive.

Our approach will otherwise be similar to that in Analysis 1a.

Analysis 2

The key assumption of Analysis 1 (that the laboratory-based assay makes no errors) is known to be untrue. In Analysis 2, we will use the "known positive" status of up to 500 of the study participants, alongside additional data, to estimate the sensitivity and specificity of both the HTK and the laboratory-based assay.

Data from multiple sources, including the present study, will be synthesised together using a Bayesian latent class modelling / multi-parameter evidence synthesis approach.

As described on page 2, we propose additional data collection through two main routes. We will also attempt to identify as many other relevant data sources as possible. Relevant studies will be those involving the HPA assay, HPA and other assays including T-SPOT Coronavirus, HPA and the home test kit, and other assays which have been compared to HPA and the home test kit. Data from studies with each of these designs will improve estimation of the accuracy of both the HTK *and* the HPA assay. Attention will be focused on:

> Other studies using two or more assays on populations with unknown prevalence. Information from studies with a wide range of different (unknown) seroprevalence values can contribute useful information about the sensitivity and specificity of the assays concerned.

The multi-parameter evidence synthesis model, combining data across all identified data sources, will produce estimates of the sensitivity and specificity of the home test kit, the sensitivity and specificity of the HPA assay (and of any other laboratory-based assays included) and implied PPVs and NPVs across pre-test probabilities.

Analyses will account for anticipated positive dependencies between test results on the HTK and other antibody tests (e.g. EuroImmun, Roche) in both antibody positive and antibody negative samples, i.e. 'conditional dependence'.

Objective 2

To describe the agreement between reading of the HTK results (i) by users of the service, (ii) by a trained professional examining a photograph, (iii) by a trained professional in a community setting - and subsequently the accuracy of user-reading in detection of anti-SARS-CoV-2 antibody

Increasingly, antibody results may be available to NHS and other staff participating in the study. This is because both NHS and private test provision is increasingly available, independent of any release by the study of antibody tests generated at Phase 1. If a human is reading the result of the HTK, their knowledge of the expected result (based on EIA results) may represent a bias in their result reading.

To assess accuracy of reading, we need to deal with this bias. There are two effective strategies available:

- The subject does not read their result; it is read by an automated process which does not know the previous status (such the NHSX app under development). This is expected to be the case with the NHSX App being built to read the product being developed by the UK-RTC.
- The subject does not read their result, but rather the result of someone else whose antibody status they do not know. This may be the case if the EDSAB-HOME investigation is applied to a product without a app reader. In this setting, we will ask groups of 2 or more individuals to set up their own tests synchronously, and during the 10-minute incubation we will relabel the tests such that the individuals do not know whose test is being read.

Two analyses of agreement will be produced, one focusing on agreement between self-read and expert-read results, and between photo-read and expert read results. A second analysis will provide estimates of the overall sensitivity and specificity of self-read tests in reflecting antibody status. Both analyses will

assume that the accuracy of self-read tests as assessed against expert-read tests is independent of the true antibody status.

In the first analysis, we will examine the probability of a self-read positive result among expert positive results, and the probability of ("false") self-read positive result among expert negatives, both as functions of reported exposure and illness experience, using logistic regression.

The second analysis will estimate the overall Sensitivity of the self-read HTK as Pr(self-read +ve|Expert +ve) x Sensitivity of the Expert-read POC based on the evidence synthesis results (Objective 1, Analysis 2). Similarly the overall specificity of the self-read POC will be estimated as: Pr(self-read -ve|Expert -ve) x Specificity of the Expert-read HTK based on the evidence synthesis results.

Objective 3

To assess whether home photographic recording of HTK is feasible and enhances accuracy of recording relative to user reading;

If possible, we will also compare to results from an automated pixel analysis of a photograph of the test taken by the person tested, and a visual analysis of a photograph taken under optimal conditions in the field. We will report concordance between the methods of interpreting results, and characteristics of discordant results (including whether they are positive or negative using the HPA test).

Objective 4

To determine the acceptability of the tests to the population;

Data (including both quantitative and free text) obtained from the baseline questionnaire on acceptability and usability of the HTK programme completed by participants will be summarised by counts, descriptive statistics and pseudo-anonymised quotes. In addition, work conducted by user researcher team at NHSX and qualitative interviews conducted prior and during the pilot phase of the programme will be included into the results of the evaluation, where appropriate/necessary.

Objective 5

To inform the instructions and other advice to be provided to people undertaking the tests and to those who have a positive and a negative result;

Results obtained on the sensitivity and specificity of the HTK will inform what advice should be given, including whether confirmatory testing using laboratory based tests will be necessary for any subsets of the population, including healthcare workers.

Objective 6

To describe the numbers of coronavirus responsive T cells in peripheral blood of subjects using ELISPOT technology, and its relationship to serological tests for SARS-CoV-2, and to clinical risk factors for COVID-19;

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We will analyse the number of coronavirus responsive T cells, as determined by T-SPOT Coronavirus, as a continuous variable, describing its relationship with age, gender, ethnic group, occupation, recent symptoms and previous exposure to SARS-CoV-2 assessed using symptoms and previous positive tests, as well as with enzyme immunoassay results. Some of these data may be useful as part of a regulatory package applying for regulatory approval of T-SPOT coronavirus tests for clinical use. Discussions as to the appropriate analysis of this data are ongoing between Oxford Immunotec Ltd and MHRA. An appendix pre-specifying these analyses will be added to this protocol when these discussions are complete.

Objective 7

To provide estimates of the hazard of development of COVID-19 compatible symptoms, or of COVID-19 related hospitalisation, among individuals with a positive vs negative laboratory and HTK antibody test kit results, and its relationship to SARS-CoV-2 responsive T cells from the T-SPOT Coronavirus test;

Development of COVID-19 compatible symptoms will be assessed through the weekly follow-up text or email messages. Development to COVID-19 related hospitalisation will be assessed through data linkage to routine laboratory and hospital record systems (SGSS and HES). These will be exploratory end-points, with the acknowledgment that we may be underpowered to detect the rarer, severe endpoints (e.g. hospitalisation) within our current cohort. However, this step aims to assess the feasibility of the process, and may lead to a secondary follow up study (e.g. linkage of the first 50,000 who use this service to laboratory and hospital records) to estimate the rate of development to COVID-19 related hospitalisations, if operationally feasible.

Descriptive statistics, frequency and percentages (life tables) will be used to describe outcomes, split by exposure type. Exposure of primary interest will be considered HTK result (positive or negative), laboratory based EIA result, and SARS-CoV-2 responsive T cell numbers, determined by T-SPOT Coronavirus assays, and outcome will be considered either COVID-19 compatible symptoms and/or progression to severe COVID-19 disease, as defined operationally by hospitalisation.

Secondary exposures of interest will include age, sex, exposure to known confirmed case, occupation, behaviour (i.e. at work or self-isolation), co-morbidities, and other risk factors. We will model the hazard of a COVID-19 related outcome (symptoms, hospitalisation) over time accounting for subject factors as covariates, using generalised linear mixed models and cure-rate type time-to-event models accounting for time-varying covariates.

Objective 8

To provide a panel of plasma which can be used for proficiency testing and batch release assay.

Plasma will be stored in PHE for this purpose. We will work to bank these materials in an NIHR Biorepository.

Other considerations

Safety Considerations

There is little risk to the subjects. A trained phlebotomist or research nurse will collect up to 20ml of blood from individuals, which is a small volume (about 5% of the amount donated at blood donation). Some

people may feel faint when they give blood, but this is transient and is not harmful. Some bruising may occur where the blood is taken, but this is also transient and harmless. All benefits are indirect, and will inform the programme's implementation and subsequent public health advice.

Benefits of taking part

Participants will receive the results from the laboratory-based tests, if they wish to do so. If they take part in stage two, they will have access to HTK results indicating whether they have antibodies against COVID-19. The volunteers contribute to the development and evaluation of the SARS-CoV-2 antibody testing programme.

Dissemination of Results and Publication Policy

We will publish the conclusions as (a) internal PHE communication (b) inter-governmental communication (c) in a peer-reviewed scientific journal and (d) conference presentation (e.g. ESCAIDE or PHE Conference). If the participants wish us to, we will send a copy of the publication(s) to them.

Problems Anticipated

The time frame for the development and implementation of both the programme and the evaluation study is very short, and therefore various logistical and operational challenges may arise during the implementation of the programme and evaluation.

Ethics and Informed Consent Forms

The study will be entirely voluntary. Informed consent will be obtained from all participants through informed consent forms. Ethics will be obtained through the NHS Research Ethics Committee. All data to be shared with colleagues at University of Bristol and other partners (e.g Oxford ImmunoTech) will have a data sharing agreement in place through Office of Data Release (ODR), and through existing agreements through the HPRU.

Name and address of the sponsor/funder.

Public Health England, 61 Colindale Avenue, London.

Health Protection Research Unit on Behavioural Science and Evaluation.

Name and title of the investigator(s) who is (are) responsible for conducting the research

Professor Isabel Oliver, Interim Director of National Infection Service, PHE; Director of Research, Translation and Innovation, PHE; and co-Director for Health Protection Research Unit in Behavioural Science and Evaluation. (Study design, conduct)

Dr David Wyllie, Consultant in Public Health Infection, PHE Field Service, East of England. (Study design, conduct, analysis, writing)

Dr Andre Charlett, Head of the Statistics Unit, PHE, Colindale. (Study design)

Public Health England

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Professor Matthew Hickman, Professor in Public Health and Epidemiology, Head of Population Health Sciences at University of Bristol and co-Director for Health Protection Research Unit in Behavioural Science and Evaluation. (Study design, conduct)

Dr Tim Brooks, Consultant Microbiologist and Head of Rare and Imported Pathogens Laboratory (RIPL), PHE Porton Down. (Study design, conduct, analysis, writing)

Dr Hayley Jones, Senior Lecturer in Medical Statistics, University of Bristol. (Study design, analysis, writing)

Dr Sian Taylor-Philips, Professor in Screening and Test Evaluation, University of Warwick (Study design, writing)

Professor Tony Ades, Professor in Public Health Science, University of Bristol (Study design, analysis, writing)

Ranya Mulchandani, Field Epidemiology Training Programme Fellow, PHE Field Service, West Midlands. (Study design, conduct, analysis, writing)

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APPENDIX ONE

Serological responses to SARS-CoV-2:

Specificity: The specificity of a test refers to the proportion of individuals without the condition who test negative.

Humans make antibodies against coronaviruses following infection, predominantly against the Spike (S) and nucleoproteins (N) (1). Four coronaviruses circulate in the general population, and a high proportion of the population has antibodies against these HKU1, 229E, NL63 and OC43 coronaviruses (2). This is a potential challenge for antibody based tests given homology between coronaviruses; for the application proposed, a highly specific assay (i.e. one which is positive only if individuals are recently exposed to SARS-CoV-2, without false positive tests). Nevertheless, there are indications such specific detection is possible:

- Immune responses against the S1 component of the Spike protein, or the receptor-binding domain (RBD) within it, appear specific to SARS-CoV2, although responses against S2 as more cross reactive predominantly against the Spike (S) and nucleoproteins (N) (1).
- Following SARS-CoV-2 infection, IgG, IgM and IgA responses are generated against the spike protein (3); sensitivity of assays detecting IgA may be slightly higher than that of assays using IgG (2).
- Serological assays against S protein (EIA signal) are strongly correlated with neutralisation assays (2) (4).
- Commercial assays for components of SARS-CoV-2 S protein and N protein are becoming available. One example is a CE marked Enzyme Immunoassay (EIA) from Euroimmun. There are reports of a potential cross reactivity in 2/60 samples from one plasma set analysed using this kit (2), and validation work done in PHE Porton Down[1] found one strong positive and one indeterminate result in 92 samples believed to be negative (Tables 1,2 reproduce data from report cited in footnote). These observations need more investigation – the possible false positive could be investigated using a viral neutralisation assay to determine whether the plasma is in fact a true positive.
- At present, taken together, these data suggest that the EuroImmun assay has specificity in the region of 97 – 99%. Running larger number of known negative samples through this assay is required to obtain a more accurate estimate of specificity.
- Newer anti-N EIAs from Roche and Abbott are also available, and may be deployed in the NHS.

Sensitivity: The sensitivity of a test refers to the proportion of individuals with the condition who test positive. According to our current understanding:

- Sensitivity of detection rises over time. Median IgG seroconversion was 13 days post duration of symptoms in a large Chinese study (5), compatible with smaller studies (4). By 21-days post symptoms, almost all seroconversion had occurred (5).
- Individuals with severe illness vs. milder clinical illness had higher titres early on (before 14 days) but cases with mild disease achieved similar titres to severe disease later (5).
- The sensitivity of detection of historical COVID-19 disease is probably about 80% (5).

[1] PHE "Evaluation data for Euroimmun IgG ELISA for SARS-CoV-2 antibodies" validation report 28-3-2020, Dr Tim Brooks

- Viral mutation is one possible explanation for low sensitivity, especially if very short domains or a small number of peptides are used as antigens (6).
- The sensitivity of diagnosis of SARS-CoV-2 by serological means has not been established in the UK.
- Studies indicate that in people exposed to a household case of COVID-19, a proportion (perhaps 25%) develop antibodies, as detected by anti-S protein EIAs. Whether the other household members were uninfected, or were infected but did not detectable antibody, is unknown (5).

Table 1: Assessment of specificity: sera from historical samples considered low risk for SARS-CoV-2 infection

	EuroImmun assay result			
Test set	**Negative**	**Indeterminate**	**Positive**	**Specificity**
Porton Negative control set (n=92)	90	1	1	99%

Data extracted from PHE "Evaluation data for Euroimmun IgG ELISA for SARS-CoV-2 antibodies" validation report 28-3-2020, Dr Tim Brooks

Table 2: Assessment of sensitivity: sera from known positive cases

	EuroImmun assay result			
Days post onset	**Negative**	**Indeterminate**	**Positive**	**Sensitivity**
0-4	18	0	0	0
5-14	18	3	12	46%
15-28	6	1	19	73%
>28	No data			

Data extracted from PHE "Evaluation data for Euroimmun IgG ELISA for SARS-CoV-2 antibodies" validation report 28-3-2020, Dr Tim Brooks

Clinical utility

The expectation is that individuals who develop effective immunity after infection will have much lower risk of disease given re-exposure, something which might de-risk health care workers and the general public returning to environments where they are likely to be exposed to the virus. This reassurance – akin to the reassurance offered to health care workers successfully vaccinated against Hepatitis B, that they will not develop severe disease if exposed to the pathogen – would be helpful to NHS staff and their occupational health advisers.

Although such protection has not been shown epidemiologically for SARS-CoV-2, there are grounds for thinking this is likely, including:

- Neutralising (i.e. infection blocking) antibodies are made against the SARS-CoV-2 spike protein (a key protein preventing viral entry into human cells) (2, 7), something which is viewed as a valid correlate of protection (1);

- The amount of antibody, as measured by enzyme immunoassay, is highly correlated (r>0.9) with viral neutralisation (2).
- In a pilot study, infusion of plasma from recovered individuals with high anti-viral antibody titres was associated with a rapid, profound reduction in viral detection in blood (8), suggesting that these antibodies may be functionally active *in vivo.*

Lateral "pregnancy test-like" immunochromatographic HTK kits: These pregnancy type kits have the potential to detect anti-SARS-CoV-2 antibody, albeit in a less quantitative way than EIA, and to be administrated at home. Encouraging results have recently been published (9).

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APPENDIX 2

Adaptations of WHO disease categories

LOCALLY MODIFIED DEFINITION BASED ON WHO CRITERIA

WHO Definition	Local adaptation
Confirmed case A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms. See laboratory guidance for details: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance	Answered yes to: Do you think you have had COVID-19 (the disease caused by the SARS-CoV-2 coronavirus)? Yes, and I tested positive.
Suspect case A. A patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath), AND a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset; OR B. A patient with any acute respiratory illness AND having been in contact with a confirmed or probable COVID-19 case (see definition of contact) in the last 14 days prior to symptom onset; OR C. A patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath; AND requiring hospitalization) AND in the absence of an alternative diagnosis that fully explains the clinical presentation.	Answered indicating had COVID-19 compatible symptoms: After 5 March OR Had symptoms and was tested (indicating clinical suspicion; criteria for testing were based on WHO criteria) on or before 5 March, but the test failed. People with negative tests are not included. OR Was hospitalised with symptoms, and no alternative diagnosis reported.

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Probable case A. A suspect case for whom testing for the COVID-19 virus is inconclusive.1 OR B. A suspect case for whom testing could not be performed for any reason	Reported symptoms positive at any time
Uncertain status case (negative laboratory testing)	Reported compatible symptoms and a negative test. We include this because molecular testing for COVID-19 is not necessarily sensitive.

Reference: "Global surveillance for COVID-19 caused by human infection with COVID-19 virus, interim guidance, 20th March 2020" https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov) accessed 28/4/2020, WHO REFERENCE NUMBER: WHO/2019-nCoV/SurveillanceGuidance/2020.6

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