Population-based age-stratified seroepidemiological investigation protocol for coronavirus 2019 (COVID-19) infection
Reference:
The emergence of a new virus means that understanding transmission patterns, severity, clinical features and risk factors for infection will be limited at the start of an outbreak. To address these unknowns, WHO has provided Four Early sero-epidemiological Investigation Protocols (rebranded the WHO Unity Studies). One additional study to evaluate environmental contamination of COVID-19 is also provided. These protocols are designed to rapidly and systematically collect and share data in a format that facilitates aggregation, tabulation and analysis across different settings globally. Data collected using these investigation protocols will be critical to refine recommendations for case definitions and surveillance, characterize key epidemiological features of COVID-19, help understand spread, severity, spectrum of disease, and impact on the community and to inform guidance for application of countermeasures such as case isolation and contact tracing.


COVID-19 investigations and studies protocols currently available include:

1. The First Few X cases and contacts (FFX) investigation protocol for coronavirus disease 2019 (COVID-19).
5. Surface sampling of COVID-19 virus: a practical “how to” protocol for health care and public health professionals

Please contact earlyinvestigations-2019-nCoV@who.int for any questions.

All WHO protocols for COVID-19 are available on the WHO website together with the technical guidance documents.
Version Control

Main updates to version 2.0 by each section include:

- **Objectives**: More specific objective 1 which is to measure sero-prevalence in the population by age group. Inclusion of a further secondary objective to contribute to an improved understanding of antibody kinetics following COVID-19 infection.
- **Recruitment of population**: Ideal age groupings for which age-specific sero-prevalence should be reported are proposed. Greater detail provided of options for convenience sampling are described and less for general household surveys.
- **Specimen collection**: Deletion of Figure 1.
- **Serological testing**: Inclusion of rapid diagnostic tests (RDTs) as an option for immunoassays to be employed in sero-epidemiological studies.
- **Confirmation of presence of neutralizing antibodies**: Confirmation procedure to include equivocal results. Confirmation procedures can be performed on a sample.
- **Sample size**: Reference to open-source on-line sample size calculator. Sample size estimates are likely to increase for household surveys as a consequence of design effect. For serial sampling investigations, sample size calculations need to be powered to detect differences between the different rounds of the survey.
- **Epidemiological indicators**: Indicators described in Table 1 fully updated and related to specific objectives of a sero-epidemiological investigation.
- **Reporting**: Minimal reporting should also include study design and response rates.
- **Questionnaires Appendices**: Addition of symptoms, update of the laboratory reporting form and also changes to the suggested reporting forms to ensure standardization with other Unity studies protocols.
- **Alignment of structure and format to technically edited other Unity studies protocols**
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### Summary

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</tbody>
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Comments for the user’s consideration are provided in purple text throughout the document as the user may need to modify methods slightly because of the local context in which this study will be carried out.
1. Background

1.1 Introduction

The detection and spread of an emerging respiratory pathogen are accompanied by uncertainty over the key epidemiological and serologic characteristics of the novel pathogen and particularly its transmissibility (i.e. ability to spread in a population) and its virulence (i.e. case-severity). This is the case for the SARS-CoV-2 virus, first detected in Wuhan city, China in December 2019.

To date initial surveillance has focused primarily on patients with symptoms or severe disease, and, as such, the full spectrum of the disease, including the extent and fraction of mild or asymptomatic infections that do not require medical attention are not clear. Estimates of the case fatality ratio, and other epidemiological parameters, will likely be lower than current estimates once the full spectrum of disease is able to be included in the denominator. In addition, the role of pre-symptomatic, asymptomatic or subclinical infections in human-to-human transmission of SARS-CoV-2 virus is not well understood.

With a novel coronavirus, initial seroprevalence in the population is assumed to be negligible due to the virus being novel in origin. Therefore, surveillance of antibody seropositivity in a population can allow inferences to be made about the extent of infection and about the cumulative incidence of infection in the population.

The following protocol has been designed to investigate the extent of infection, as determined by seropositivity in the general population, in any country in which COVID-19 virus infection has been reported. Each country may need to tailor some aspects of this protocol to align with public health, laboratory and clinical systems, according to capacity, availability of resources and cultural appropriateness. However, using a standardized protocol such as this one below, epidemiological exposure data and biological samples can be systematically collected and shared rapidly in a format that can be easily aggregated, tabulated and analyzed across many different settings globally for timely estimates of COVID-19 virus infection, severity and attack rates, as well as to inform public health responses and policy decisions. This is particularly important in the context of a novel respiratory pathogen, such as COVID-19s.

1.2 Objectives

There are two primary objectives for this sero-epidemiological investigation:

1. To measure the seroprevalence of antibodies to COVID-19 in the general population by sex and age group, in order to ascertain the cumulative population immunity; and
2. To estimate the fraction of asymptomatic, pre-symptomatic or subclinical infections in the population and by sex and age group.

Sero-epidemiological investigations provide the opportunity to inform or evaluate secondary objectives, such as, but not limited to:

3. To determine risk factors for infection by comparing the exposures of infected and non-infected individuals;
4. To contribute to determine the case fatality ratio; and
5. To contribute to an improved understanding of antibody kinetics following COVID-19 infection.
COMMENT: Little is currently known about COVID-19 virus antibody kinetics. Asymptomatic infected persons may clear the virus more quickly than do symptomatic patients. Antibody titers in the asymptomatic persons are likely to be lower, if they seroconvert at all, than in infected patients exhibiting symptoms. These are considerations for the interpretation of any COVID-19 virus sero-epidemiological investigation.

2 Methods

2.1 Design

The sero-epidemiological investigation for COVID-19 virus infection is a population-based, age-stratified prospective study. It is intended to provide key epidemiological and serologic characteristics of SARS-CoV-2 virus.

There are three study designs that can be used:

1) One-time cross-sectional investigation
2) Repeated cross-sectional investigation in the same geographic area (but not sampling the same individuals)
3) Longitudinal cohort investigation with serial sampling of the same individuals each time

COMMENT: The first option will likely be the easiest for countries to implement, while the third provides the most comprehensive information on attack rates, as described below. The choice as to how this study will be implemented should be determined by the objectives, feasibility and available capacity (e.g. capital, financial, and personnel).

The timing of the study will depend on the specific public health questions that need to be addressed.

- One-time cross-sectional investigations: there may be an interest in completing the investigation after the first or subsequent peaks of transmission of the epidemic waves. However, a cross-sectional investigation, conducted at any time of the epidemic, will provide important information that can be used to inform public health responses.
- Repeated cross-sectional and longitudinal cohort investigations both entail serial sampling, either from different or the same individuals. It is best to initiate these investigations as early as possible. Serial sampling can then be conducted as long as possible, as determined by capacity and resources. Intervals between each round of collecting specimens should be of a period of greater than 21 days. For longitudinal cohort investigations with serial sampling, the epidemic curve from surveillance (daily number of new confirmed cases) can be used to adjust the frequency with which samples are collected to provide real-time estimates of seropositivity in the general population.

2.2 Population

The geographic scope of the investigation should be defined. This may be limited to a local or regional investigation, or may be conducted as a national investigation. Ideally, the geographic scope of the investigation should be representative of the overall burden of infection (i.e. include both high and low incidence areas) and this selection can be informed by the latest information on SARS-CoV2 virus circulation, available on the WHO website.
2.3 Recruitment of population

The method to recruit the investigation population will depend on the objectives, the feasibility and the resources available to conduct the study.

Whichever method is used to identify and recruit the investigation population, all attempts should be made to include participants over a range of ages in order to determine and compare age-specific sero-prevalence. Crude age-specific estimates will need to be adjusted for age structures in the population. Ideally, investigations should ensure that the following 10 age groups can be reported: 1-4, 5-9, 10-14, 15-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70+.

COMMENT: If age-groups detailed above are not feasible, investigators should ensure that age categories employed are coherent with these. Reporting age-specific indicators for the younger ages by 5-year age bands (i.e. 1-4, 5-9, 10-14, and 15-19) will better inform plans for loosening lock down policies (i.e. the opening of schools). The younger groups can be collapsed into 10-year age bands (i.e. 1-9 and 10-19) if this is more feasible.

Populations can be recruited by using existing studies (e.g. general health surveys, research population, or patient cohorts, etc.) or by establishing new studies.

To recruit participants, sampling can be either by:

- **Convenience Sampling:** Individuals attending medical services (e.g. blood donors, pregnant mothers, primary care attendees, etc.) can be approached to participate in the study. The advantage of working with blood donors is that they are usually forthcoming to be contacted for future follow-up and you may be able to track long-term antibody dynamics. For COVID-19, the age-specific attack rates in blood donors are likely to be similar to that in the general population except for those with substantial comorbidities or elevated exposure (e.g. healthcare workers). However, some age-groups will not be captured. Convenience samples can also be constructed using residual sera taken from patients for other investigations. Investigations using residual sera can be easier to implement and can reflect the exposure in the general population, but the information collected can be limited (e.g. location, age and sex).

- **Random Sampling:** Individuals can be approached to participate in investigations using a variety of probability sampling techniques such as random digit dialing and general household surveys (e.g. in low middle income countries Demographic and Health Surveys (DHS), Multiple Indicator Cluster Surveys (MICS), population-based HIV impact assessment (PHIA). Households are often defined as a group of people (2 or more) living in the same residence, but in practice, the technical definition may vary due to social, political and cultural practices. It usually excludes residential institutions, such as boarding schools, dormitories, hostels or prisons.

COMMENT: Depending on which method of study recruitment is chosen, the group implementing the study may choose either to conduct home visits to collect data and specimens or to centralize data and specimen collection at one location, asking participants to travel to the location to participate in the study. Decisions as to how to implement the study should be determined by feasibility and resources (including personnel) availability.

---

2.4 Eligibility criteria

**Inclusion criteria**: All individuals identified for recruitment into the investigation, irrespective of age, irrespective of acute or prior COVID-19 infection.

**Exclusion criteria**: Refusal to give informed consent, or contraindication to venipuncture.

**COMMENT**: Suspected or confirmed acute or prior COVID-19 infection should not be excluded. Doing so would underestimate the extent of infection in the population. For individuals currently receiving medical care for COVID-19 infection, a family member or proxy may be used to complete the questionnaire on his/her behalf.

2.5 Data collection

Each participant recruited and sampled for the investigation should be asked to complete a **questionnaire** which covers demographic, clinical and exposure information. An example of an investigation questionnaire which may be used can be found in the Appendix: *Form 1 “Reporting form for each participant”*. This questionnaire is not exhaustive and may need to be adapted to the local setting and outbreak characteristics, but it provides an outline as to the data to be collected in order to calculate the epidemiological parameters (see 3.2 Epidemiological indicators).

The data collection includes also the reported COVID-19 laboratory testing by investigation subjects. An example of a **laboratory investigation reporting form** which may be used can be found in the Appendix: *Form 2 “Laboratory results reporting form”*. This table will need to be completed for every serum sample collected, as determined by the chosen specimen collection schedule and design of the study.

2.6 Laboratory evaluations

Laboratory and biosafety guidance for COVID-19 can be found on the WHO website.

Serologic assays specific to COVID-19 are currently under development / in the process of evaluation. The protocols or Standard Operating Procedures (SOPs) will be published on the WHO website once they become available. Cross reactivity to other coronaviruses may be an issue and should be considered in the interpretation of data. Multiple assays may be required to confirm a seropositive for COVID-19 virus.

Laboratory procedures involving sample manipulation must be carried out in a biosafety cabinet (BSC).

2.6.1 Specimen collection

A serum sample needs to be collected from each participant upon recruitment into the investigation. All those involved in the collection and transportation of specimens should be trained in safe handling practices and spill decontamination procedures. For details regarding the transport of samples collected and infection control advice, please refer to case management algorithm and laboratory guidance in the country or WHO laboratory guidance, available on the WHO website.
2.6.2 Specimen transport

For each biological sample collected, the time of collection, the conditions for transportation and the time of arrival at the study laboratory will be recorded. Specimens should reach the laboratory as soon as possible after collection. Serum should be separated from whole blood and can be shipped at 4°C or frozen to -20°C or lower (at -80°C) and shipped on dry ice. If the specimen is not likely to reach the laboratory within 72 hours, specimens should be frozen, preferably at -80°C, and shipped on dry ice.

It is, however, important to avoid repeated freezing and thawing of specimens. It is recommended to aliquot samples prior to freezing, to minimize freeze thaw cycles.

Transport of specimens within national borders should comply with applicable national regulations. International transport of specimens should follow applicable international regulations as described in the WHO Guidance on Regulations for the Transport of Infectious Substances 2019-2020.

COMMENT: Other specimens (e.g. nasopharyngeal) may be collected to determine acute COVID-19 infection, as determined by the objectives of the investigation and the available resources and capacity.

2.6.3 Serological testing

Serum samples should be screened for the presence of COVID-19 virus specific antibodies using serological testing. Tests for IgG, IgM, IgA or total antibodies are commercially available. For the purpose of the study and based on current evidence on performance, detection of total antibodies or IgG should be preferred. Serological testing should be carried out using enzyme linked immunosorbent assay (ELISA), immunofluorescence (IFA) or, in case of limited lab capacity, Rapid Diagnostic Tests (RDT). Other in house tests may be used if validated with a comprehensive panel of antibody-positive and negative samples. The Foundation for Innovative New Diagnostic (FIND) is evaluating immunoassays (further details available https://www.finddx.org/covid-19/sarscov2-eval-immuno/).

ELISA testing should be carried out in a facility with at least biosafety level 2 (BSL-2) capacity.

In countries with limited laboratory capacity, a carefully chosen RDT can be used, and serum specimen collected for confirmatory testing with a highly specific and sensitive ELISA. Of note, compared to RT-PCR results, RDTs and some ELISA will have higher sensitivities 1-2 weeks after symptoms onset.

2.6.4 Confirmation of the presence of neutralizing antibodies

If a sample is positive or equivocal for either IgM, IgA or IgG using RDT or ELISA, a neutralizing assay (eg microneutralization or Plaque Reduction Neutralization Test) should ideally be performed.

Neutralizing assays with wild type virus should be carried out in a facility with at least BSL-3 capacity, as they require handling of live virus. Whenever possible, these assays should be used to validate neutralizing assays that use pseudo or surrogate virus, as the latter are faster and easier to perform, and can be done outside BSL3 facilities. Promising pseudo/surrogate viral neutralizing assays are currently under development and may become commercially available soon.

If laboratories have limited capacity, they could send positives (or select a representative subset of samples (e.g. different times since symptoms onset, titers, severity of infection etc) for testing or
shipping to an international reference laboratory for confirmation using neutralizing assays (e.g. microneutralization test or Plaque Reduction Neutralization Test). List on those laboratories can be found on WHO website.

2.6.5 Sample storage

In the case that serum samples cannot be processed immediately, they should be stored at -80°C (see section on specimen collection and transport above for more details). It is recommended to aliquot samples prior to freezing, to minimize freeze thaw cycles. The storage of serum specimens in domestic frost-free freezers should be avoided, owing to their wide temperature fluctuations.

COMMENT: These recommendations are subject to changes as new, reliable serological assays become available.

COMMENT: If serological testing is not available in the country in which serum samples are collected, they may be stored or shipped to an international reference laboratory. WHO is able to facilitate communication with international referral laboratories in order for samples to be shipped for further testing. Information can be found on WHO website

2.6 Ethical considerations

Ethical requirements will vary by country. In some countries, this investigation may fall under public health surveillance (emergency response) acts and may not require ethical approval from an Institutional Review Board.

2.6.1 Informed consent

The purpose of the investigation will be explained to all individuals identified for recruitment into the investigation. Informed consent will be obtained from all individuals willing to participate in the investigation before any procedure is performed as part of the investigation by a trained member of the investigation team. Consent for children under the legal age of consent will be obtained from a parent or legal guardian. Each participant must be informed that participation in the investigation is voluntary and that s/he is free to withdraw, without justification, from the investigation at any time without consequences and without affecting professional responsibilities.

COMMENT: The age of consent may vary by country. Check the requirements of local, regional or national authorities.

Informed consent will seek approval to collect blood and epidemiological data for the intended purpose of this investigation, that samples may be shipped outside of the country for additional testing and that samples may be used for future research purposes.

COMMENT: For study designs in which data will be collected from named individuals (e.g. longitudinal cohort), investigators will need to decide whether to inform participants of any results, an important consideration for which will be the diagnostic performance of assays employed.

2.6.2 Risks and benefits for subjects
This investigation poses minimal risk to participants, involving the collection of a small amount of blood. The primary benefit of the study is indirect in that data collected will help improve and guide efforts to understand extent of COVID-19 virus infection and may prevent further transmission of the virus.

2.6.3 Confidentiality

Participant confidentiality will be maintained throughout the investigation. All subjects who participate in the investigation will be assigned a study identification number by the investigation team for the labelling of questionnaires and specimens. The link of this identification number to individuals will be maintained by the investigation team and the Ministry of Health (or equivalent) and will not be disclosed elsewhere.

If the data is shared by the implementing organization to WHO or any agency or institution providing support for data analysis, data shared will include only the study identification number and not any personably identifiable information.

Article 45 of the IHR (2005) describes the “treatment of personal data”. Person identifiable data collected under the IHR should be kept confidential and processed anonymously, as required by national law. However, such data may be disclosed for assessments and management of public health risks, provided the data are processed fairly and lawfully.

2.6.4 Prevention of COVID-19 virus infection in investigation personnel

All personnel involved in the investigation need to be trained in infection prevention and control procedures (standard contact and droplet precautions, as determined by national or local guidelines). These procedures should include proper hand hygiene and the correct use of surgical masks, if necessary, not only to minimize their own risk of infection when in close contact with individuals with COVID-19 infection, but also to minimize the risk of spread among other participants in the investigation.

WHO technical guidance on infection prevention and control specific to COVID-19 can be found on the WHO website.

2.7 Financing

Resources incurred in data collection, sample collection and laboratory testing would be funded by [national or international funding source identified by the country].

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2 World Health Organization. *International Health Regulations (2005)*
3 Statistical analyses

3.1 Sample size

The sample size will be determined by the chosen study design, study population and the specific objectives to be responded to by the study.

The figure below provides estimates of margin of error as a function of seroprevalence for 100, 200 and 300 samples per age group. For a given seroprevalence rate \( p \) and sample size \( N \), the expected margin of error corresponds to the expected width of the 95% confidence interval associated with the point estimate of \( p \) obtained using binomial likelihood.

**Figure 1**: Expected margins of errors at different seroprevalences for investigations of 100, 200 or 300 sample sizes.

Sample sizes can be calculated using **statistical formulas or tools** available online (e.g. [http://www.openepi.com/Menu/OE_Menu.htm](http://www.openepi.com/Menu/OE_Menu.htm)) or in standard statistical packages. Note that for household surveys, the design effect is likely to increase the required sample size of the study. For the serial sampling investigations (i.e. repeated cross-sectional or longitudinal cohort investigations) or risk factor studies, investigators should perform sample size calculations to ensure that their investigations are adequately powered.
3.2 Epidemiological indicators

The table below provides an overview of the epidemiological parameters that can be measured as part of this investigation.

Table 1: Indicators to inform investigation objectives

<table>
<thead>
<tr>
<th>Objective</th>
<th>Parameter</th>
<th>Definition (in bracket: “simplified” expression of it)</th>
<th>Data source to calculate the parameters concerned</th>
<th>Comments, limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Measure the seroprevalence of antibodies to COVID-19 in the general population by age group in order to ascertain the cumulative population immunity</td>
<td>Seroprevalence (population and age-specific)</td>
<td>The proportion of individuals per age strata who show seropositivity for COVID-19 virus infection</td>
<td>Seropositivity Age group</td>
<td>Population sero-prevalence to be calculated using direct standardization methods, so that the proportion is adjusted for any difference in the age stratification of the participants and the overall population. Age-specific sero-prevalence is same as Age-specific attack rate and cumulative incidence. If data is collected, sero-prevalence by different groups (e.g. geography, profession, residence) will be an important sub-analysis.</td>
</tr>
<tr>
<td>2. Estimate the fraction of asymptomatic or pre-symptomatic/subclinical infections in the population and by age group.</td>
<td>Asymptomatic fraction (proportion of cases that are asymptomatic)</td>
<td>The proportion of individuals who reported no symptoms of COVID-19 infection of individuals seropositive for COVID-19</td>
<td>Seropositivity Reported symptoms</td>
<td>The numerator is the number of individuals reporting no symptoms and the denominator is the total number of individuals seropositive for COVID-19. This parameter will be difficult to calculate if investigations collect limited data (e.g. using residual sera).</td>
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<tr>
<td></td>
<td>Fraction severe disease</td>
<td>The number individuals with severe infection</td>
<td>Seropositivity Reported symptoms Age group</td>
<td>Severe disease to be defined (e.g. hospitalization). The number individuals with severe infection divided by the number with COVID-19 infection as determined by sero-positivity.</td>
</tr>
<tr>
<td>Objective</td>
<td>Parameter</td>
<td>Definition</td>
<td>Data source to calculate the parameters concerned</td>
<td>Comments, limitations</td>
</tr>
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<tr>
<td>3. Determine risk factors for infection by comparing the exposures of infected and non-infected individuals</td>
<td>Population groups most at risk</td>
<td>The identification of groups who are most vulnerable to COVID-19 virus infection (e.g. age groups, gender, occupation)</td>
<td>Seropositivity Reported symptoms Exposure of interest (e.g. age group)</td>
<td>May only be an early signal, a nested case-control study could be conducted to evaluate risk factors for infection</td>
</tr>
<tr>
<td>4. Contribute to determine the case fatality ratio</td>
<td>Case fatality ratio</td>
<td>The proportion of individuals with fatal outcome for COVID-19 infection</td>
<td>Seropositivity Mortality Age group</td>
<td>Indicator best measured using longitudinal cohort investigations although sample size to record sufficient events (i.e. deaths) will need to be very large. May require extended follow-up to determine outcome of those with COVID-19 infection</td>
</tr>
<tr>
<td>5. To contribute to an improved understanding of antibody kinetics following COVID-19 infection.</td>
<td>Serological response to infection</td>
<td>The change in serum level of specific antibodies to COVID-19 virus (increase in titer)</td>
<td>Antibody titer</td>
<td>Changes in titers should be calculated using geometric mean titers (GMTs)</td>
</tr>
</tbody>
</table>
4 Reporting of findings

Any investigation of this nature should include reporting on the following information:

1. the study design;
2. the number of households and/or individuals approached and the number included in the investigation;
3. the age and sex of all individuals included;
4. the time in the outbreak of sample collection and the antibody titre levels of each specimen collected;
5. the number of individuals with serologic evidence of COVID-19 virus infection. If sample size permits, these numbers should be stratified by age; and
6. the number of individuals with serologic evidence of COVID-19 virus infection who have reported symptoms.

It is also important to fully document the study design, how individuals were recruited, and the serological assay and methods used to ensure that data can be pooled to increase power in estimating epidemiological parameters.

Ideally, information would be collected in a standardized format according to the questionnaires and tools in this generic protocol to assist with data harmonization and comparison of results (see reporting forms in Appendix).

To enable results to be aggregated across study sites and across county sites and by extension, strengthen the statistical power of the results, it will be valuable if individual de-identified data can be shared with WHO.

5 References

WHO Situation reports
https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/

The Unity studies: early investigations protocols

Surveillance, rapid response team and case definitions

Laboratory

Clinical care
Infection prevention and control / WASH

Risk communications and community engagement

Examples of sero-epidemiological studies
https://www.medrxiv.org/content/10.1101/2020.04.19.20071563v1
https://www.medrxiv.org/content/10.1101/2020.04.13.20060467v1
https://www.medrxiv.org/content/10.1101/2020.04.14.20062463v1

WHO Scientific brief "Immunity passports" in the context of COVID-19

Online courses
There are training resources for COVID-19 available on the WHO online learning platform (https://openwho.org/, accessed 12 February 2020).

6 Acknowledgments

This generic protocol was adapted from the protocol entitled “Prospective longitudinal cohort study of influenza infection during epidemic periods” by the Consortium for the Standardisation for Influenza Seroepidemiology (CONSISE). CONSISE is a global partnership aiming to develop influenza investigation protocols and standardise seroepidemiology to inform public health policy for pandemic, zoonotic and seasonal influenza. This international partnership was created out of a need, identified during the 2009 H1N1 pandemic, for better (standardised, validated) seroepidemiological data to estimate infection attack rates and severity of the pandemic virus and to inform policy decisions.

This generic protocol built also on experience and the protocols developed through the WHO Pandemic Influenza Special Investigations and Studies work.

This document was developed by: Maria Van Kerkhove, Isabel Bergeri, Rebecca Grant, Lorenzo Subissi (from the Health Emergencies Program, WHE, World Health Organization), Joseph Wu and Malik Pereis (Hong Kong University), John Watson (US CDC), Marta Valenciano, Anthony Nardone (from Epicontect).
Appendix: Questionnaires

Population-based age-stratified seroepidemiological investigation protocol for COVID-19 infection

Form 1: Reporting form for each participant

<table>
<thead>
<tr>
<th>Unique ID</th>
</tr>
</thead>
</table>

**Current status**

- [ ] Alive
- [ ] Dead
- [ ] Unknown/lost to follow-up

1. Data Collector Information

<table>
<thead>
<tr>
<th>Name of data collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data collector institution</td>
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<tr>
<td>Data collector telephone number</td>
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<tr>
<td>Mobile number</td>
</tr>
<tr>
<td>Email</td>
</tr>
<tr>
<td>Form completion date (dd/mm/yyyy)</td>
</tr>
<tr>
<td>Date of interview with informant (dd/mm/yyyy)</td>
</tr>
</tbody>
</table>

2. Identifier information

<table>
<thead>
<tr>
<th>First name</th>
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<tbody>
<tr>
<td>Family name</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Date of birth (dd/mm/yyyy)</td>
</tr>
<tr>
<td>Age (years, months)</td>
</tr>
<tr>
<td>Telephone (mobile) number</td>
</tr>
<tr>
<td>Email</td>
</tr>
<tr>
<td>Country of residence</td>
</tr>
<tr>
<td>Nationality</td>
</tr>
<tr>
<td>Ethnicity (optional)</td>
</tr>
<tr>
<td>Occupation</td>
</tr>
<tr>
<td>Have you had contact with a anyone with suspected or confirmed COVID-19 virus infection?</td>
</tr>
<tr>
<td>If Yes, dates of last contact (DD/MM/YYYY):</td>
</tr>
</tbody>
</table>

3. Symptom history

In the past (X) months, have you had any of the following:

**COMMENT:** (X) period to cover time since emergence of COVID-19 virus to date of data collection. If possible, date of system onset should be recorded as well as for some symptoms an indication of severity. The list of possible symptoms of interest will need to be reviewed and extended as more is understood of CV-19 infections.

<p>| Fever (≥38 °C) or history of fever | [ ] Yes [ ] No [ ] Unknown |
| Sore throat | [ ] Yes [ ] No [ ] Unknown |
| Runny nose (rhinorrea) | [ ] Yes [ ] No [ ] Unknown |</p>
<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortness of breath (dyspnea)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Other respiratory symptoms</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Chills</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rash</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Conjunctivitis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Muscle aches</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint ache (myalgia)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Loss of appetite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of smell (anosmia)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of taste (ageusia)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nose bleed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Altered consciousness</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Other neurological signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Patient symptoms: complications

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did any of these symptoms require you to seek medical attention?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did any of these symptoms require you to miss work or school?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalization: Did any of these symptoms require you to be hospitalized?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Population-based age-stratified seroepidemiological investigation protocol for COVID-19 infection

Form 2: Laboratory results reporting form

This table will need to be completed for every serum sample collected, as determined by the chosen specimen collection schedule and design of the study.

<table>
<thead>
<tr>
<th>Laboratory identification number</th>
<th>Date sample collected (dd/mm/yyyy)</th>
<th>Date sample received (dd/mm/yyyy)</th>
<th>Type of sample</th>
<th>Type of test</th>
<th>Result (COVID-19 antibody titres)</th>
<th>Result date (dd/mm/yyyy)</th>
<th>Specimens shipped to other laboratory for confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>/</strong>/___</td>
<td><strong>/</strong>/___</td>
<td>□ Serum</td>
<td>Specify type (ELISA/IFA/RDT, IgM/IgG/IGA/total Ab, microneutralization, PRNT), etc.):</td>
<td>□ POSITIVE If positive, titre:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Other, specify:</td>
<td>□ NEGATIVE □ INCONCLUSIVE</td>
<td><strong><strong>/</strong></strong></td>
<td>□ Yes If Yes, specify date <strong><strong>/</strong></strong>/____ If Yes, name of the laboratory: ____ □ No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Other, specify:</td>
<td>□ NEGATIVE □ INCONCLUSIVE</td>
<td><strong><strong>/</strong></strong></td>
<td>□ Yes If Yes, specify date <strong><strong>/</strong></strong>/____ If Yes, name of the laboratory: ____ □ No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Other, specify:</td>
<td>□ NEGATIVE □ INCONCLUSIVE</td>
<td><strong><strong>/</strong></strong></td>
<td>□ Yes If Yes, specify date <strong><strong>/</strong></strong>/____ If Yes, name of the laboratory: ____ □ No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Other, specify:</td>
<td>□ NEGATIVE □ INCONCLUSIVE</td>
<td><strong><strong>/</strong></strong></td>
<td>□ Yes If Yes, specify date <strong><strong>/</strong></strong>/____ If Yes, name of the laboratory: ____ □ No</td>
<td></td>
</tr>
</tbody>
</table>

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WHO reference number: [WHO/2019-nCoV/Seroepidemiology/2020.2](https://www.who.int)