World Health Organization

Population-based age-stratified seroprevalence investigation template protocol for respiratory pathogens with pandemic potential

Unity Studies
Respiratory Investigations & Studies

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Design by Lushomo

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Abbreviations

CONSISE	Consortium for the Standardization of Influenza Seroepidemiology
COVID-19	Coronavirus disease 2019
DHS	Demographic and Health Surveys
ELISA	Enzyme linked immunosorbent assay
HLIP	High-level Implementation Plan
Ig	Immunoglobulin
IHR	International Health Regulations
ILI	Influenza-Like Illness
LFIA	Lateral Flow Immunoassay
MICS	Multiple Indicator Cluster Surveys
PHIA	Population-based HIV impact assessment
PHSM	Public Health and Social Measures
PIP	Pandemic Influenza Preparedness
PPE	Personal Protective Equipment
RDT	Rapid Diagnostic Tests
SARI	Severe Acute Respiratory Infection
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
WHE	Health Emergencies Programme
WHO	World Health Organization

Summary

The detection and spread of a novel or re-emerging pathogen with pandemic potential is accompanied by uncertainty over the transmission patterns, severity, clinical features and risk factors for infection. Well-reported seroprevalence studies help inform the understanding of the extent of infection, as determined by antibody seroprevalence in the general population, the proportion of the population who remain susceptible to infection, and to refine estimates of infection severity and transmission.

This document sets out the methods on how to conduct a population-based age-stratified seroprevalence investigation for respiratory pathogens with pandemic potential, as part of the Respiratory Investigations and Studies, Unity Studies initiative. CLICK TO VIEW **For the purposes of this protocol, the conceptual respiratory pathogen in question will be referred to as pathogen X, which causes disease X. Pathogen X might be a novel pathogen (e.g., SARS-CoV-2 in late 2019) or a re-emerging existing pathogen (e.g., a newly emerging strain of influenza).** The findings of this investigation can be used to inform public health responses to novel or re-emerging respiratory pathogens of pandemic potential.

The World Health Organization (WHO), in collaboration with technical partners, has developed a series of enhanced surveillance protocols that are harmonized to help provide detailed insight into the epidemiological characteristics of respiratory pathogens with pandemic potential. They build on previous protocols developed by the global Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) *(1)*, and WHO's Influenza Pandemic Special Investigations and Studies, as well as those developed by WHO as part of the Unity Studies for COVID-19 *(2)*.

All WHO Respiratory Investigations and Studies protocols are available on the WHO website. CLICK TO VIEW

Please contact unity@who.int for any questions.

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.

Full title of study	Population-based age-stratified seroprevalence investigation template protocol for respiratory pathogens with pandemic potential
Assumption	Valid and reliable serological testing for pathogen X is available, whether by point of care assays, local laboratories, or reference laboratories.
Study population	Age-stratified sample from general population
Potential output and analysis	Estimate or inform estimates of: • Seroprevalence of anti-pathogen X antibodies • Cumulative incidence of infection • Infection attack rates • Fraction of asymptomatic infection • Case fatality ratio
Study design	Population-based sample from the general population, stratified by age There are three possibilities for how this study can be implemented: 1) One-time cross-sectional investigation 2) Repeated cross-sectional investigation in the same geographic area but not necessarily sampling the same individuals each time 3) Longitudinal cohort investigation
Study duration	The study can be conducted as a one-time cross-sectional investigation, or can include serial sampling either with repeated cross-sections or as a cohort study.
Minimum information and specimens to be obtained from participants	Data collection: Epidemiological data including basic demographics, clinical symptoms, and vaccination status (if/when a vaccine is available). Specimens: Serum samples to inform seroprevalence inferences.

1. Background

1.1 Background to Pandemic Investigations and Studies

The detection and spread of a novel or re-emerging respiratory pathogen with pandemic potential are accompanied by scientific uncertainty relating to their epidemiological and serologic characteristics, transmissibility (i.e. ability to spread in a population), and virulence (i.e. case-severity). This was the case for the SARS-CoV-2 virus, first reported in Wuhan city, China in December 2019, where advances in seroepidemiological surveillance and operational research were critical to understanding population infection rates and infection severity *(3)*. Looking ahead, enhancing existing surveillance approaches and pre-preparing standardized investigation protocols and exercising them will be key to prepare for future potential pan-respiratory pathogen threats *(4) (5)*.

Based on the learning of the COVID-19 response, a series of standardized template protocols have been developed for both disease specific investigations such as influenza as well as for any novel respiratory pathogen of pandemic potential for the implementation of standardized and quality investigations and studies to ensure readiness in advance of a future pandemic. These protocols have been developed as part of the WHO Investigations and Studies, Unity Studies initiative. CLICK TO VIEW The initiative aims to provide an 'at the ready' international framework for preparedness and response to future pandemics, providing a suite of enhanced surveillance and operational research activities that are standardized to help provide detailed insight into the epidemiological characteristics of novel or re-emerging respiratory pathogens of pandemic potential. They are included in both the Global Influenza Strategy *(6)* (pillar 6: "Number of sites (or geographical coverage) primed to conduct at least one of the WHO Pandemic Influenza Special Investigations in case of a pandemic with target for 2023: "At least 2 operationally ready sites in each WHO region (so 12 sites)" and the third version of the High-Level Implementation Plan (HLIP III) for pandemic preparedness (PIP) *(7)* for the 2024-2030 period under Output Indicator 2.11 "Number of sites participating in the WHO Investigations and studies network (Unity Studies)".

Investigations and Studies are also included in the Mosaic Respiratory Surveillance Framework *(8)*. The Mosaic framework presents respiratory virus surveillance systems in a collaborative context where they are each focused on the objectives to which they are best suited. The major routine surveillance systems [sentinel SARI (severe acute respiratory infection), ILI (influenza-like illness), event-based surveillance, etc] can maintain their efficiency if they are well-coordinated with other systems that have different objectives. Discrete studies and early investigations such as the Unity Studies can address certain public health objectives that are not efficiently met by existing systems such as to rapidly assess transmissibility, estimate population susceptibility/immunity and infection severity, aid identification of population groups in need to target interventions, and estimate burden of disease and vaccine effectiveness *(8)*.

1.2 Introduction to seroprevalence investigations and their approach

Seroprevalence studies aim to measure the presence and amount of antibodies against a particular pathogen, acquired by either natural infection or vaccination, in a sample of humans from a population with the intention to extrapolate information from that sample and provide and estimated profile of humoral immunity within a population.

With a novel respiratory pathogen, initial seroprevalence in the population is assumed to be negligible due to the pathogen being novel in origin, although this could be verified using either banked samples or samples collected as early as possible in a new outbreak. It is important for pandemic risk assessment to determine whether some individuals might have cross-reactive antibodies due to historical infections (or perhaps vaccination) against different but related strains. As a specific example of this, in the 2009 influenza pandemic some older adults had pre-existing antibodies which cross-reacted to H1N1pdm09 because of the similarity of pre-1950 H1N1 strains to H1N1pdm09. Nevertheless in most circumstances it is anticipated that initial seroprevalence will be very low, and surveillance of changes in antibody seroprevalence in a population can then allow straightforward inferences to be made about the extent of infection and about the cumulative incidence of infection in the population, beyond what is accessible by routine surveillance.

Well-reported seroprevalence investigations help inform the understanding of the proportion of the population who remain susceptible to infection, especially vulnerable populations such as the elderly, and in turn, guide public health decision-making *(9)*. They can be used to refine estimates of infection severity and transmission.

As a supplement to other data from routine surveillance systems, in populations reported with high vaccine coverage, seroprevalence investigations provide a supplement to vaccine coverage data and are an important tool for the evaluation of vaccination programs. Seroprevalence data is especially important to lead targeted vaccination approaches, such as in geographic areas of low vaccine coverage due to poor vaccine uptake or access to health services.

The following protocol is an adaptation of generic protocols developed by several countries before and during the 2009 influenza pandemic. These were subsequently modified and further developed by the CONSISE group *(1)* and then as part of the Unity Studies initiative during the COVID-19 pandemic *(2)*.

The following protocol has been primarily designed to investigate the extent of infection, as determined by seropositivity in the general population. The results generated can also potentially supplement vaccine coverage data and support the evaluation of vaccination programs, in any country in which a novel or re-emerging respiratory pathogen with pandemic potential 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.

Using a standardized protocol such as the below, epidemiological exposure data and biological samples can be systematically collected in a format that can be easily compared over time and across many different settings globally for estimates of viral 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 SARS-CoV-2.

Due to the nature of seroprevalence studies, it is recommended that a multi-disciplinary team is involved in their planning and implementation. As a minimum, both laboratory specialists and epidemiologists should be involved in study design, implementation, analysis and interpretation of results. Statistical analysis support may be required for sample size calculations and analysis. It is also strongly recommended to include a social scientist, such as a risk communication and community engagement specialist, to ensure the study procedure is clearly explained for informed consent (see sections 2.4 CLICK TO VIEW and 2.8.1 CLICK TO VIEW), to prevent loss to follow-up (for longitudinal studies), and to manage participants' expectations e.g. regarding individual result timing and interpretation. Communication specialists are also important for explaining the study results to the community and to decision makers (see section 4 CLICK TO VIEW).

For the purposes of this protocol, the conceptual respiratory pathogen in question will be referred to as pathogen X, which causes disease X. Pathogen X might be a novel pathogen (e.g., SARS-CoV-2 in late 2019) or a re-emerging existing pathogen (e.g., circulating strains of influenza). The findings of this investigation can be used to inform public health responses to novel or re-emerging respiratory pathogens of pandemic potential. Specifically, it can provide estimates of the otherwise unrecognized infection in the population, as well as the likely susceptibility of the population to further epidemic peaks of infection.

It is important to note that the investigator's approach to designing a study according to this protocol will differ depending on if pathogen X is a novel pathogen (like SARS-CoV-2 in 2019) or a re-emerging existing pathogen, like circulating strains of influenza. This protocol will provide options for both immune-naive populations and immune-challenged populations (i.e., post-vaccination or a re-emerging existing virus).

Toolkit item

The timely and standardized implementation of this protocol should be supported by a toolkit developed by WHO and implementing partners. The toolkit will comprise components to support different elements of the protocol. These will be highlighted throughout the protocol and discussed in Section 5. CLICK TO VIEW Toolkit components will be available on the WHO website. CLICK TO VIEW

Toolkit items to support this section may include:

- Checklist for Unity Studies alignment
- Terms of Reference for Unity Studies network sites

See additional 'Toolkit item' boxes throughout this document.

1.3 Objectives

There are **two primary objectives** for this seroprevalence investigation:

1. To measure the **seroprevalence of antibodies against pathogen X in the general population by sex, age group and vaccination status;** and
2. To estimate the **fraction of asymptomatic or subclinical infections in the population and by sex and age group.**

Seroprevalence investigations provide the opportunity to inform or evaluate **secondary objectives**, such as, but not limited to:

3. Determine **risk factors for infection** by comparing the exposures of infected and non-infected individuals;
4. Contribute to estimations of the **infection severity profile such as the proportion of infections which are fatal in different age groups**;
5. Contribute to an improved understanding of **antibody kinetics and humoral immunity at the level of populations** following pathogen X infection, re-infection or vaccination;
6. Assessing cross-reactivity and cross-immunity for respiratory pathogens;
7. Estimate uptake of vaccination against pathogen X in the population by sex, age and priority target groups and developing vaccination strategies, and;
8. Explore relationships between population seroprevalence and behavioural and social drivers for vaccination and Public Health and Social Measure (PHSM) in the population by sex and age.

Comment: This protocol assumes reliable serological tests are widely available for such pathogens, or become rapidly available following the emergence of an unknown pathogen. See section 2.7.2. CLICK TO VIEW

Comment: Depending on the nature of pathogen X, asymptomatic infected persons may clear the virus at a different pace than symptomatic patients. Antibody titers in the asymptomatic persons may be lower, if they seroconvert at all, than among infected patients exhibiting symptoms. These are considerations for the interpretation of sero-epidemiological investigations.

1.4 Glossary of terminologies

The following glossary provides definitions for key epidemiological terms used in this protocol.

Box 1. Key epidemiologic and laboratory definitions

Asymptomatic fraction:
The proportion of infected individuals who do not develop or perceive signs or symptoms of infection with pathogen X.

Infection-fatality ratio:
The proportion of persons with a laboratory confirmed pathogen X infection who die as a consequence of their infection.

Protective effectiveness:
The reduction of disease occurrence (or other outcome, i.e. disease severity, hospitalization, etc.) for those with some kind of immunity against a disease from vaccination, prior infection, or a combination of both compared to those who were either not vaccinated, have not yet been infected, or have had fewer immunological events.

Seropositivity:
A serum sample with the presence of pathogen X specific antibodies, or, if appropriate for pathogen X, presence of pathogen X specific antibodies above a certain threshold detected using serological testing. An appropriate threshold indicating a positive test would ideally be established by the manufacturers of the serologic test or by reference laboratories.

Seroprevalence:
The proportion of seropositive individuals in a sampled population at a given timepoint.

Vaccine effectiveness:
The reduction of disease occurrence (or other outcome, i.e. disease severity, hospitalization, etc.) for those vaccinated against a disease compared to those who were not vaccinated against a disease, or other comparison group (i.e., differing courses of booster doses, hybrid immunity, etc.) in real-world conditions; estimated from observational (non-randomized) studies.

Vaccine uptake:
The proportion of the population who has received a vaccine.

1.5 Overview of methodology

Section 2 CLICK TO VIEW of this protocol will describe in detail the methodology for this investigation including the study design, population of interest, recruitment of population, eligibility criteria, data collection, laboratory testing and ethical considerations.

The timely implementation of the investigation requires the coordination of the following roles and responsibilities:

Table 1: Coordination matrix of roles and responsibilities in [Country Y]

What?	Who?
Overall coordination of the investigation	[Cite institution/body/person(s)]
Identification of study population informed	[Cite institution/body/person(s)]
Recruitment, informed consent, enrolment	[Cite institution/body/person(s)]
Data and sample collection from enrolled participants	[Cite institution/body/person(s)]
Laboratory testing and storage of samples	[Cite institution/body/person(s)]
Analysis of data and reporting	[Cite institution/body/person(s)]
Data management	[Cite institution/body/person(s)]
IT management	[Cite institution/body/person(s)]
Informing participants of their individual results and communication of overall findings of investigation	[Cite institution/body/person(s)]
[add more roles, as per country context]	[Cite institution/body/person(s)]

The **seroprevalence investigation** will be maintained centrally by [cite institution/body/person(s)]. Centralized coordination will require development of a "command and control" plan, to allow for prioritization of investigations.

It is noted that undertaking this kind of investigation will require resources and this needs to be fully planned for.

2. Methods

2.1 Study design

This seroprevalence investigation for pathogen X infection is a **population-based, age-stratified study**. It is intended to provide key epidemiological and serologic characteristics of pathogen X in the general population. Key steps of a population-based age-stratified seroprevalence investigation are listed in Table 2. In determining the study design, robust methodology should be utilised in order to minimise the risk of bias *(10)*.

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

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).

Table 2: Steps of Population-based age-stratified seroprevalence investigation

A. Preparation phase

- Adapt the protocol for the national/regional context: include a detailed description of the study objectives, timing of the study, the sampling strategy chosen and describe the different steps of sampling in sufficient detail (target population, study population, sample frame, sampling method, primary and final sampling units, sample size calculation, recruitment method)
- Establish a plan of analysis, including dummy tables, in advance of starting the investigation
- Obtain permissions from the relevant public health authorities, submit adapted protocol to institutional review board, according to national and local regulations

B. Initial steps

Step	Lead	Activity	Timeline
1. Training of study team	Investigation team	• Undertake training of the study team(s)	Start of the investigation
2. Recruitment of individuals	Investigation team	• Explain investigation to individuals (and parent/ guardian), obtain informed consent/assent	Day 1
3. Data and sample collection	Investigation team	• Complete data collection form • Collect and store blood sample	Day 1: Form 1

C. Follow-up steps (optional – depends on design of investigation)			
4. Repeated data and sample collection	Investigation team	• Repeat completion data collection form • Repeat collection and storage of blood sample	Intervals should be determined based on the expected infection and transmission dynamics of pathogen X
D. Final steps			
5. Laboratory evaluations	Investigation team – laboratory	• Analyse collected samples	End of the investigation
6. Data analysis	Investigation team – data analysis	• Analyse data • Interpret data	End of the investigation
7. Feedback to community	Coordination level	• Report overall findings to community, including implications of findings • Study team to decide on feedback of results to participants	End of the investigation
8. Feedback to public health authorities	Coordination level	• Provide a final report, including implications of findings for public health response	End of the investigation

2.2 Population of interest

Study population: General population, any age group. Age stratification is **not** required but all attempts should be made to include participants from a range of ages (see section 2.4 CLICK TO VIEW on sampling), since age-stratified analyses are likely to provide valuable additional insights on epidemiology and transmission dynamics.

Geographic scope: The geographic scope of the investigation should be defined. This may be national (the most preferred option, if resources allow, as it provides the most representative sample of the general population) or limited to a **local** or **regional** investigation, ideally representative of overall burden of infection of the general population (i.e. include both high and low incidence areas informed by the latest information on pathogen X circulation). One other consideration in selecting local areas might be the availability of other relevant surveillance data at a higher quality such as data on incidence of severe cases.

2.3 Timing of the study

The **timing of the study** will depend on the specific public health questions that need to be addressed and for each of the three study designs for seroprevalence studies, the following issues should be considered:

- ***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[1] (repeated cross-sectional surveys) or the same (longitudinal cohort studies) individuals. It is best to initiate these investigations as early as possible after the emergence of pathogen X. Serial sampling can then be conducted for as long as necessary, based on the characteristics of pathogen X and the availability of capacity and resources.
  - o Intervals between each round of collecting specimens should be determined based on the expected dynamics of pathogen X. For some respiratory viruses, such as those with a short incubation period and rapid transmission (e.g. SARS-CoV-2, influenza virus, human metapneumovirus), it may be necessary to have short intervals between sampling rounds to capture the dynamics of the pathogen accurately depending on the factors mentioned above and time to seroconversion.
  - o For longitudinal cohort investigations, the epidemic curve generated from surveillance data (daily number of new confirmed cases) can be used to adjust the frequency with which samples are collected. This approach allows for real-time estimates of seropositivity in the general population. If pathogen X has a shorter time to outcome (e.g., faster development of symptoms or shorter duration of infection), it may be necessary to collect samples more frequently to capture the seropositivity rates accurately.
  - o Longitudinal cohort studies could be used as a study platform to estimate vaccine effectiveness although this would require a review of study objectives, supplementary virological testing, larger sample sizes and appropriate ethical approvals.
  - o Longitudinal cohort studies could also be used to estimate outcomes such as antibody waning, seroconversion, and sero-reversion over time.

Consideration also needs to be given about the **timing of seroprevalence investigations within the pandemic periods**. Early seroprevalence investigations can be conducted in the "emergence or introduction" pandemic period and provide invaluable early information, depending on human and financial resources as well as availability of serological test kits (especially well performing kits). Most investigations are therefore likely to be conducted during periods of sustained and disseminated community transmission.

1 For repeated cross-sectional surveys, participants that participated in previous rounds should ideally be excluded in further rounds.

When transmission is widespread, seroprevalence studies should focus on studying population differences (e.g., age, sex, geography, race, etc.) and high risk and vulnerable populations (e.g., those with comorbidities, refugee populations) to identify susceptible sub-populations and monitor them over time to inform priorities for vaccination coverage and prevention and control measures. For interpretation of results during different pandemic periods, see also section 3.3. CLICK TO VIEW

2.4 Sampling and recruitment of population

The method to sample and recruit the study population will depend on the objectives, the feasibility and the resources available to conduct the investigation. The recruitment of the study population should be based on a robust and validated sampling frame of the study population. Protocols where participants are recruited without a sampling frame (e.g. through advertising or volunteers at a venue) are not recommended due to both the very high risk of introducing bias and the inability to assess the representativeness of the study population.

Whichever method is used to sample 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 seroprevalence. Crude age-specific estimates will need to be adjusted for age structures in the population. Information on the age structure of the population will also need to be collected from local/national health authorities to enable such adjustments. Where this information is not available, the age and sex structures of proxy populations can be used for standardization purposes, but investigators should carefully consider the possible biases of doing so and the impact on the accuracy of the estimates. Ideally, investigations should ensure that the following 10 age group categories can be reported: 1-4, 5-9, 10-14, 15-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70+ years.

Comment: If the age group categories 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 Public Health and Social Measures (PHSM). The younger groups can be collapsed into 10-year age bands (i.e. 1-9 and 10-19) if this is more feasible.

For further details on the sample size calculation, see section 3. CLICK TO VIEW

Sampling: Both random (probability) sampling or convenience sampling can be used. Random sampling is preferable because the results would be less subject to bias, provided that a high proportion of randomly selected individuals agree to participate in the study.

- ***Random (probability) Sampling:*** Random sampling is when all individuals within a population (i.e. sampling frame) will have the same probability of being approached to participate in the study *(11)*. The recruitment of study participants by random sampling requires a comprehensive sampling frame (e.g. population list) and is considered the gold standard. However, obtaining such a sampling frame can be difficult and the method can be costly and difficult to implement. Individuals may be selected from the sampling frame (simple random sampling) or by stratified or multi-stage sampling in which the population is grouped into homogenous strata (e.g. by age, region, general practice). Strata are randomly selected first, and then individuals are selected randomly from within each of those strata. This may have logistical advantages while retaining the advantages of a random sample.
- ***Cluster Sampling:*** Cluster sampling is a form of multi-stage probability sampling in which a population is divided into groups (e.g. households) and all members are recruited from randomly chosen groups *(11)*. Cluster sampling can be more efficient than random sampling, especially where a study takes place over a wide geographical region. Disadvantages include individuals within a cluster, such as a household, are more likely to be homogenous for a key characteristic (e.g. sero-positivity) and, thus, there is an increased sampling error and the need to increase sample size using a design effect (see section 3 CLICK TO VIEW). The statistical analysis plan must be explicit on how clustering will be taken into account in the analysis. Furthermore, if the chosen clusters are not representative of the population, there is an increased risk of bias, although this risk can be minimized by drawing participants from a larger number of smaller clusters rather than a smaller number of larger clusters.

  Seroprevalence studies may use probability sampling to select households or community members. Individuals can be approached to participate in investigations using a variety of techniques such as random digit dialing *(12)* 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 *(13)*. It usually excludes residential institutions, such as boarding schools, dormitories, hostels or prisons. If survey weights are available, for example from general household surveys, these survey weights can be applied to correct seroprevalence estimates for differences between the characteristics of survey participants and the population.

- ***Convenience Sampling:*** Where a robust sampling frame can be described that approximates to a wider population, convenience samples can be considered. For example, 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 being contacted for future follow-up and you may be able to track long-term antibody dynamics. However, there are a number of limitations of blood donors, for example in some locations individuals with certain medical conditions or certain behaviors can

be prohibited from donating blood (e.g. men who have sex with men), and data on antibody levels in children would have to be obtained through other sources.

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) and also subject to biases depending on the source of the samples.

As this is a population-based seroprevalence investigation, sampling of specific (e.g. healthcare workers) or closed (e.g. residents of institutions such as boarding schools or prisons) populations are not recommended due to the higher risk of exposure and the bias introduced. Parallel studies in closed settings can be useful but the results cannot be extrapolated to the general population.

Comment: A patient population for a prevalent disease may be considered as a proxy for the general population depending on likely representativeness of the patient population to the general population based on local context/epidemiology.

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.

Comment: If retrospectively collected blood donations or residual sera are used as a study population, the findings of the investigation will be limited. Such samples will have limited individual data (e.g. demographic, clinical), impeding the presentation of results by key demographic (e.g. residence, social class) or clinical (e.g. comorbidities, prior treatments) variables. Furthermore, it may be difficult to check for duplicate samples being provided by the same individual and there will be no possibility to contact individuals to inform them of their results. Investigators will need to clarify whether the informed consent provided with these samples includes the use of these samples in other investigations.

Comment: While sampling techniques should prioritize feasibility and inclusiveness, please note that if household sampling techniques are used, sampling more than one or two individuals per household may affect the precision of seroprevalence estimates due to increased likelihood of transmission in close proximities. Post-hoc statistical adjustment techniques such as adjusting for clustering may be used if this is the case.

For recruitment methods that involve the prospective identification of eligible participants, a trained member of the investigation team will need to be able to explain the purpose and procedures of the investigation to eligible individuals who are willing to participate in the investigation and gather informed consent/assent from eligible participants.

Those responsible for recruiting individuals will be required to put in place effective informed consent processes and these will include a requirement to ensure that potential participants are not asked for consent/assent in circumstances in which it would be difficult for them to say no e.g. they should not be invited to participate by someone who is in a relationship of authority to them. Equally, there should be no professional or health impact if any eligible individual refuses to participate in the investigation. For further details, see Ethical Consideration section (section 2.8 CLICK TO VIEW).

2.5 Eligibility criteria

Inclusion criteria: All consenting individuals identified for recruitment into the investigation, irrespective of age, irrespective of acute or prior infection with pathogen X should be considered for participation in the investigation.

Exclusion criteria: Refusal to give informed consent, or contraindication to venipuncture. Individuals with substantial comorbidities or elevated exposure should be avoided in general population studies.

Comment: All eligible individuals, regardless of whether or not they are well or unwell, or receiving medical care for pathogen X, should be considered for participation in the investigation. The participation in another study should also not preclude inclusion in this investigation. Suspected or confirmed acute or prior infection with pathogen X should not be excluded (with an exception for excluding active cases for research staff safety reasons or local isolation and public health protocols). Doing so would underestimate the extent of infection in the population. For individuals currently receiving medical care for pathogen X, a family member or proxy may be used to complete the questionnaire on his/her behalf. However, some sites may decide to exclude those with severe disease who are unable to complete questionnaire. In either case, the exclusion criteria need to be clearly stated in the adapted protocol, and in the reporting of the results.

2.6 Data collection

Each participant who provides informed consent/assent and who is recruited into the investigation will be asked to complete a **questionnaire** which covers demographic, vaccination status as well as clinical and exposure information each time a sample is taken. An example of an investigation questionnaire which may be used can be found in the Appendix: *Form 1 "Reporting form for each participant"*. CLICK TO VIEW 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 Table 3 Epidemiological indicators CLICK TO VIEW).

The data collection includes also the reported 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"* . CLICK TO VIEW 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.

Toolkit item

Toolkit items to support this section may include:

- Data dictionaries
- Data quality checklist
- Study team training template

Toolkit items will be available on the WHO website. CLICK TO VIEW

2.7 Sample collection and transport

Laboratory guidance for pathogen X will be developed and posted on the WHO website.

Serologic assays specific to pathogen X antibodies will likely be available, whether they are already developed for an existing re-emerging pathogen or newly developed for a novel pathogen. Cross reactivity to other pathogens may be an issue and should be considered in the interpretation of data. Multiple assays may be required to confirm a seropositivity.

Comment: This protocol assumes valid and reliable serological tests are widely available for such pathogens, or become rapidly available following the emergence of an unknown pathogen. Parallel data on the diagnostic performance of the serological test (sensitivity/specificity) in recovered individuals would be valuable, with the caveat that antibody responses might vary by infection severity, with antibody response being weaker and more difficult to detect in mild infections. Evidence on rates of sero-reversion would also be valuable when interpreting seroprevalence data.

Comment: Serologic testing cannot typically determine acute infection and this is not the purpose of serological studies. Other specimens (e.g. nasopharyngeal) may be collected to determine acute pathogen X infection. This is beyond the scope of this investigation and the objectives and procedures of the investigation would need to be adapted accordingly.

2.7.1 Specimen collection, storage and transport

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 of infectious substances and infectious spill decontamination procedures. For details regarding the collection and transport of samples, please refer to case management algorithm and laboratory guidance in the country and to WHO laboratory guidance *(14)*.

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. If the specimen is not likely to reach the laboratory within 48 hours, specimens should be frozen, preferably at -80°C, and shipped on dry ice as per applicable WHO guidance *(14)*. 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. The storage of serum specimens in domestic frost-free freezers should be avoided, owing to their wide temperature fluctuations.

Serum should be separated from whole blood and can be shipped at 4°C for one week and shipped at 4°C or frozen to -20°C or lower (at -80°C) and shipped on dry ice.

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 2021-2022 *(15)*.

Comment: These recommendations are subject to changes as new, reliable serological assays become available. For example, some studies of SARS-CoV-2 seroprevalence used dried blood spots.

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 *(15)*.

Toolkit item

Toolkit items to support this section may include:

- Standard operating procedure for sample collection

Toolkit items will be available on the WHO website. CLICK TO VIEW

2.7.2 Serological testing

Serum samples should be screened for the presence of pathogen X specific antibodies using serological testing. Tests for **IgG, IgM, IgA or total antibodies may be commercially available**. Evidence on performance (i.e., sensitivity and specificity) and viral immune response should be consulted in determining a hierarchy for preferred isotype detection. Independent evaluation of commercial test performance on local serum samples are recommended, where circumstances permit. Serological testing may be carried out using qualitative **enzyme linked immunosorbent assay (ELISA), immunofluorescence (IFA), chemiluminescence immunoassay (CLIA) or neutralizing assay**. Other in-house tests may be used if validated with a comprehensive panel of antibody-positive and negative samples. The derived seroprevalence from testing results should be adjusted for the test's performance metrics. To improve precision, a combination of screening and confirmation tests could be employed, while the overall sensitivity and specificity of the combination should be assessed.

A testing algorithm predicated on a different vaccine and infection induced antibody responses to vaccine or infection may be used to distinguish individuals vaccinated or infected with pathogen X, depending on the nature of the virus. For example, with SARS-CoV-2, many vaccines that were deployed elicited an antibody response against the spike (S) protein of SARS-CoV-2 whilst individuals infected by wild virus would have antibodies to both spike and nucleocapsid (N) proteins *(16)*. Algorithms for these kinds of antibody detection approaches and inferences need to be based on current evidence for pathogen X, fully developed, and validated before being applied.

Serological testing should be carried out in a facility with appropriate biosafety level capacity according to the pathogen X designation and threat level.

Comment: If pathogen X is a re-emerging existing virus, or the study is being conducted in a later-stage outbreak of a novel pathogen or post-vaccination campaign, quantitative serological testing should be prioritized if possible - i.e. assays which allow the detection of the amount of antibodies and measurement of antibody kinetics/wane. In such scenarios, qualitative tests which only indicate whether an individual is sero-positive or negative (Lateral Flow Immunoassays (LFIA), Rapid Diagnostics Tests (RDT), etc) may not produce as useful data when a large proportion of the population likely already has a baseline level of immunity, whether that be from natural infection, re-infection, vaccination, or a combination of the latter. With such scenarios, each immunological challenge can be considered as a 'booster' - therefore measuring the quantity and amplitude of antibody response will allow more detailed inference on protection against infection and immune response post-infection. In addition, if pathogen X is evolving, analysis of antibody levels against older and newer strains may provide additional insights.

2.7.3 Test performance and validation

Serological tests should be internally and externally validated to ensure accurate and reliable results. Internal validation focuses on assessing the test's analytical performance parameters, such as sensitivity, specificity, precision, and linearity. It involves testing known positive and negative samples to determine the test's ability to correctly identify the presence or absence of specific antibodies. External validation, on the other hand, involves evaluating the test's performance in a larger population with varying disease prevalence. This helps determine the test's reliability and generalizability in real-world scenarios. External validation often involves comparing the test results with those obtained from a gold standard reference method.

Comment: Investigators should also consider target sensitivity and specificity levels for serological tests in the detection of pathogen X. For example, for qualitative SARS-CoV-2 serological tests, the WHO recommended assays have minimum 90%+ sensitivity and 97% specificity. Guidance on acceptable performance will vary depending on the specific nature of the pathogen in question, so investigators are advised to monitor and refer to guidance from WHO when available.

2.7.4 Confirmation of the presence of neutralizing antibodies

If a sample is positive or equivocal for either Immunoglobulin M (IgM), IgA or IgG using enzyme linked immunosorbent assay (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 **appropriate capacity (e.g., Biosafety level (BSL)-3, BSL-4)**, 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.

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.

2.7.5 Future use of samples

The investigators may decide on potential future use of specimens and the time-frame for destruction of specimens and seek appropriate approvals for this. If this is the case, the investigators will need to provide more specific information on potential future use of specimens and the time-frame for destruction of specimens, including in the information for the participant and the informed consent/assent form. Additional consent forms may need to be developed by the country, to comply with national laws and regulations.

2.8 Ethical considerations

National and local ethical requirements must be followed. Ethical approval should be sought as per individual country requirements as 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. It will be upon national authorities to advise.

It is important, wherever possible, ethics pre-approval is sought in advance of a pandemic to reduce time to activation of Respiratory Investigations and Studies in accordance with local, regional and national authorities. This will ensure that investigations are able to be implemented when necessary and with minimal delay.

For further information on the ethical considerations of importance to public health surveillance can be found in key WHO guidance:

- WHO guidelines on ethical issues in public health surveillance *(17)* CLICK TO VIEW
- Guidance for managing ethical issues in infectious disease outbreaks *(18)* CLICK TO VIEW

Toolkit item

Toolkit items to support this section may include:

- Ethical exemption/clearance letter templates
- Links to key WHO guidance on ethical considerations of importance to public health surveillance
- Template consent and assent forms

Toolkit items will be available on the WHO website. CLICK TO VIEW

2.8.1 Informed consent

The purpose of the investigation will be explained to all individuals willing to participate, before the start of the investigation. For all investigation activities not included in routine public health management, informed consent and assent may be required. This will depend on the country's national ethical requirements. Informed consent will seek relevant approvals for the collection of all data and specimens for the purposes of the investigation as determined by Country Y.

- **Consent** for:
  - Adults; and
  - children under the legal age of consent (usually 18 years, but will vary from country to country) from a parent or legal guardian.
- **Assent** from:
  - children and adolescents under the legal age of consent, but who can understand the implications of informed consent and go through the necessary procedures. This is usually children over the age of 12 to 13 years, but this will vary from country to country. A consent form from a parent or legal guardian will also be collected.

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

Comment: If older adults are being included, assessment of cognitive function (affecting ability to consent) could be included, and consent of legal guardians might be required for adults with cognitive decline if these individuals are to be considered for inclusion.

Templates of informed consent form as well as assent forms will be included in the supporting investigation toolkit.

All eligible individuals, regardless of whether or not they are well or unwell, or receiving medical care for pathogen X, should be considered for participation in the investigation. For individuals who lack the decisional capacity to consent at the time of the investigation, consent/assent by proxy (parent/ guardian/ spouse/ family member) may be considered so as to not unduly exclude individuals from participating in the investigation.

The processes related to withdrawal of a participant need to be described both in the protocol and in the information for the participant given to the participant at the time of enrolment. In this description it must be made clear that a participant can withdraw from the investigation, without justification, at any time by informing one of the members of the investigation team. The contact details of one of the members of the investigation need to be provided in the information for the participant. If any participant decides to withdraw during the investigation, the samples collected and data should be discarded, except if the participant indicates that these can be kept for the purpose of conducting the investigation, or for future studies of other infectious pathogens (See Section 2.7.5. Future use of samples CLICK TO VIEW).

Informed consent will seek approval to collect blood and epidemiological data for the intended purpose of this investigation. It may also seek approval that samples may be shipped outside of the country for additional testing and that samples may be used for future public health needs, in accordance with national laws and regulations. Additional detail in the consent/assent forms may be needed, according to national laws and regulations, if the investigation calls for storage and future use of samples. See further details in Section 2.7.5. CLICK TO VIEW

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.

2.8.2 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 the extent of infection and humoral immunity and may prevent further transmission of the virus.

2.8.3 Reporting of serious adverse events, including death of a participant

Any serious adverse event, including death, of a participant during the investigation period, needs to be immediately (within 24 hours) reported to the Principal Investigator and the institution responsible for the investigation. The contact details for reporting serious adverse events needs to be provided to each member of the investigation team.

In accordance with national regulations, any serious adverse event may also have to be reported to the local ethical review committee, if the adapted protocol was not deemed exempt from the local ethical review committee.

2.8.4 Confidentiality

Participant confidentiality will be maintained throughout the investigation. All subjects who participate in the investigation will be assigned a unique identification number by the investigation team for the labeling 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.

Data and specimens will be securely stored nationally. If the data are shared by the implementing organization with WHO or any agency or institution providing support for data analysis, data shared will include only the investigation identification number and not any personally identifiable information. Data sharing outside [Country Y] will be managed according to national laws and regulations, as appropriate.

Comment: The investigators will need to describe how data and specimens will be securely stored, the duration of storage and the destruction of data and specimens at the end of the duration of storage, in accordance with national laws and regulations.

Article 45 of the International Health Regulations (IHR) (2005) describes the "treatment of personal data" *(19)*. 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.

2.8.5 Prevention of infection

Participants

As part of the recruitment process, all eligible participants should be provided information as to how the respiratory pathogen spreads and what measures can be taken to avoid infection. This should include information as to where to seek medical advice related to

the investigation, the symptoms associated with infection and what to do if symptoms develop during the investigation.

Investigation personnel

All personnel involved in the investigation need to be trained in infection prevention and control procedures (as determined by national or local guidelines) *(20)*. These procedures should include proper hand hygiene and the correct use of personal protective equipment, as per national or local guidelines, provided to members of the investigation team, not only to minimize their own risk of infection when in close contact with individuals with pathogen X, but also to minimize the risk of spread among other participants in the investigation.

If public health and social measures restrict movement of individuals, investigation personnel may consider, if feasible, administering the questionnaire over the phone. Investigation members collecting blood samples should follow all infection prevention and control measures, as per national and local guidelines.

Mitigation of stigmatization of participants

There is a possibility of stigmatization of those involved in the investigation, through participation in the investigation and potentially the results of the investigation, if, for example, particular ethnic minorities, or perhaps those of lower socioeconomic status, are found to have higher rates of infection. The investigators will need to provide specific information on how the risks of stigmatization will be mitigated as part of the implementation of the investigation and the communication of the findings.

2.9 Financing

The investigators will need to detail how the resource costs incurred in data collection, sample collection and laboratory testing will be financed.

Toolkit item

Toolkit items to support this section may include:

- Funding guidance
- How to access support
- Project management/budgeting guidance

Toolkit items will be available on the WHO website. CLICK TO VIEW

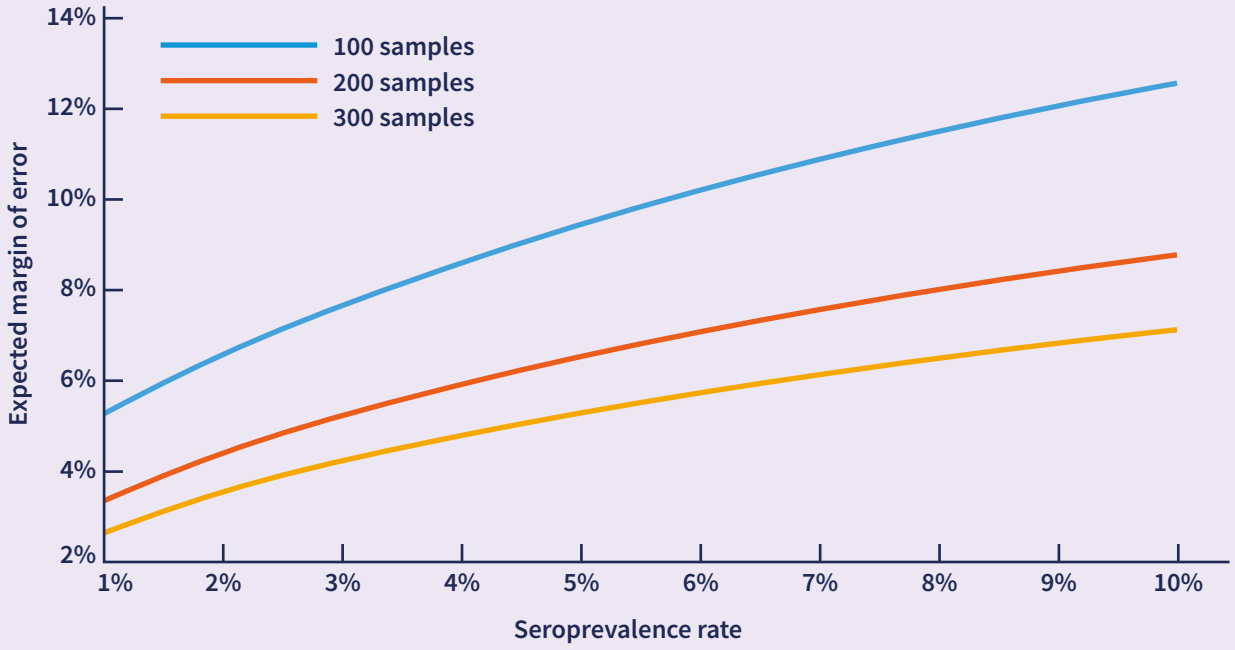
3. Statistical analyses

3.1 Sample size

The sample size of Country Y 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. 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.



Note, this figure does not account for the sensitivity and specificity characteristics of the serologic test.

Sample sizes can be calculated using **statistical formulas or tools** available online (e.g. http://www.openepi.com/Menu/OE_Menu.htm CLICK TO VIEW) or in standard statistical packages. Note that:

- Sample sizes are calculated for each stratum of analysis (e.g. age, sex or vaccination status);
- For cluster surveys, the design effect will increase the required sample size of the study;
- For 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.

For further details please refer to the Toolkit item, Data Analysis Plan, available on the WHO website. CLICK TO VIEW

3.2 Plan of analysis

The combination of epidemiological, virological (genomic, antigenic) and serological data can provide unparalleled early situational awareness of the pandemic, which will promote a proportionate and targeted public health response.

Prior to commencing the investigation, an analysis plan should be developed incorporating the study objectives, definitions and planned analysis to address each objective. Pre-specifying the analysis plan will help to ensure that all relevant data are being collected. Please refer to the WHO Data Analysis Plan as part of the supporting Toolkit.

Toolkit item

Toolkit items to support this section may include:

- Template data analysis plans
- Data analysis scripts
- SOP for interpretation
- Reporting guidelines for outcomes

Toolkit items will be available on the WHO website. CLICK TO VIEW

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

Table 3: Indicators to inform investigation objectives

Objective	Parameter	Definition (*in bracket:* "simplified" expression of it)	Data source to calculate the parameters concerned	Comments, limitations
1. **Measure the seroprevalence of pathogen X antibodies in the general population by age group** (and vaccination status) in order to ascertain the cumulative population immunity	Seroprevalence (population and age-specific)	The proportion of individuals per age strata who show seropositivity for pathogen X infection	Seropositivity Age group	Population seroprevalence 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 seroprevalence can be used to estimate the **age-specific attack rate**. If data is collected, seroprevalence by different groups (e.g. geography, profession, residence) will be an important sub-analysis. Overall seroprevalence provides key information on potentially immune population. Seroprevalence of vaccine-induced antibodies can contribute to evaluating vaccination uptake in the population. Seroprevalence of infection-induced antibodies can contribute to evaluating proportion of the population with hybrid immunity.
2. **Estimate the fraction of asymptomatic or subclinical infections in the population and by sex & age group.**	Asymptomatic fraction (proportion of cases that are asymptomatic)	The proportion of individuals who reported no symptoms of infection of individuals seropositive for pathogen X	Seropositivity Reported symptoms	The numerator is the number of individuals reporting no symptoms and the denominator is the total number of individuals seropositive for pathogen X. This parameter will be difficult to calculate if investigations collect limited epidemiological data (e.g. using residual sera). Further, seropositivity post-dates recollection of symptom, so recall bias is likely, hindering a reliable estimate of the true proportion of asymptomatic infections. However, comparison of the proportion of the seropositive population with the number of reported cases can allow inferences as to the proportion of otherwise unrecognized pathogen X infection in the population. This can vary by vaccination status but will give an estimation of the overall burden of symptomatic disease.
	Fraction severe disease	The number individuals with severe infection	Seropositivity Reported symptoms Age group	Severe disease to be defined (e.g. hospitalization, admission to ICU). The number individuals with severe infection divided by the number with antibodies against pathogen X.
3. **Determine risk factors for infection** by comparing the exposures of infected and non-infected individuals	Population groups most at risk	The identification of groups who are most vulnerable to pathogen X infection (e.g. age groups, gender, occupation)	Seropositivity Reported symptoms Exposure of interest (e.g. age group)	May only be an early signal, a nested case-control study could be conducted to evaluate risk factors for infection

Objective	Parameter	Definition (*in bracket:* "simplified" expression of it)	Data source to calculate the parameters concerned	Comments, limitations
4. **Contribute to estimates of the infection fatality ratio**	Infection fatality ratio	The proportion of individuals with fatal outcome for pathogen X infection	Seropositivity Mortality Age group	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 pathogen X infection. Fatality may be different among vaccinated and unvaccinated. It is, therefore, important to collect vaccination status. The CFR may contribute to determining if mortality decreases with increasing vaccination uptake.
5. **Contribute to an improved understanding of antibody kinetics at the level of populations following pathogen X infection, re-infection or vaccination.**	Serological response to infection	The change in serum level of specific antibodies to pathogen X *(Increase in titer)*	Antibody titer Reported symptoms	Changes in titers should be calculated using geometric mean titers (GMTs). Can be reported based on disease severity. For those conducting quantitative testing.
6. **Assessing cross-reactivity and cross-immunity to other pathogens**				
7. **Estimate uptake of vaccination against pathogen X in the population by sex, age and priority target groups and developing vaccination strategies.**	Uptake of vaccination	Reported uptake (either documented or self-reported) among participants eligible for vaccination (e.g. by age, health condition or occupation)	Reported vaccination status	Uptake of vaccination can only be measured subsequent to vaccine roll-out. Inclusion of questions to assess behavioural and social drivers for vaccination and PHSM dependent on local timing and circumstances.
8. **Explore relationships between population seroprevalence and behavioural and social drivers for vaccination and Public Health and Social Measure (PHSM) in the population by sex and age**				Inclusion of questions to explore relationships between population seroprevalence and behavioural and social drivers for vaccination and PHSM dependent on local timing and circumstances.

3.3 Interpretation of results

The following considerations are needed when interpreting the results of this investigation:

- The serologic assay used and the specificity and sensitivity characteristics of the assay itself;
- The population selected and the biases inherent with the selection of the study population;
- The timing of the sample collection with respect to local transmission intensity and the kinetics of antibody development;
- Pre-existing serological cross-reactivity against respiratory viruses with pandemic potential as well as other pathogens in the study location.

Furthermore, the following considerations are relevant for the inference of results:

- Circulation of immune escape variants (e.g. for COVID-19) to aid interpretations of inference of seroprevalence with protection against infection;
- Vaccination availability and coverage.

4. Data sharing and reporting of findings

Data shared from these Respiratory Investigations and Studies may be **pooled and aggregated across multiple sites** by WHO if the data are collected in a consistent manner, to increase analytic power and to improve precision in estimating seroprevalence. If data are shared by the implementing organization or country, with WHO or with any agency or institution providing support for data analysis, data shared will include only the investigation identification number and not any personally identifiable information.

Toolkit item

Toolkit items to support this section may include:

- Data sharing agreement templates
- Platforms for data sharing
- Standardized formats/templates for data sharing

Toolkit items will be available on the WHO website. CLICK TO VIEW

4.1 Reporting of findings to participants

Recruitment method allowing, all participants should be informed of their individual results using the contact information collected as part of the investigation. As scientific understanding of antibody kinetics and how these may correlate to protection against reinfection will be limited, those responsible for informing participants of their individual results need to ensure that those who are seropositive are not falsely reassured into considering themselves as protected from re-infection, rather that they need to remain vigilant in their ongoing adherence to infection prevention and control measures.

Participants should also be made aware that results may be delayed depending on testing procedure and test kit availability (investigators should consider that this may have an effect on recruitment of the same individuals in longitudinal cohort studies). If available and feasible, rapid diagnostic testing could be offered in parallel to specimen collection, to allow participants to obtain a result. Furthermore, if diagnosis for acute infection is available, participants can be provided with information on accessing these services.

The communities in which the investigation is implemented also need to receive a report on the overall findings of the investigation. This 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 (aggregately reported);
(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 pathogen X infection. If sample size permits, these numbers should be stratified by age, and;
(6) the number of individuals with serologic evidence of pathogen X 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).

4.2 Reporting of findings in peer-reviewed scientific reports

Transparent reporting is necessary for the interpretation of seroprevalence studies, as well as to facilitate comparisons in rapid evidence syntheses. Refer to WHO's statement on reporting guidelines for SARS-CoV-2 seroepidemiological studies. *(9)*

Toolkit item

Toolkit items to support this section may include:

- Standard criteria for reporting results
- Templates for presentations and publications
- Training on messaging to different audiences
- Technical briefs

Toolkit items will be available on the WHO website. CLICK TO VIEW

4.3 Science translation for decision makers

Clear and timely reporting and communication of results is necessary to support policy/ decision makers.

Toolkit item

Toolkit items to support this section may include:

- Training on messaging to different audiences
- Policy makers questions that will be answered by study objectives

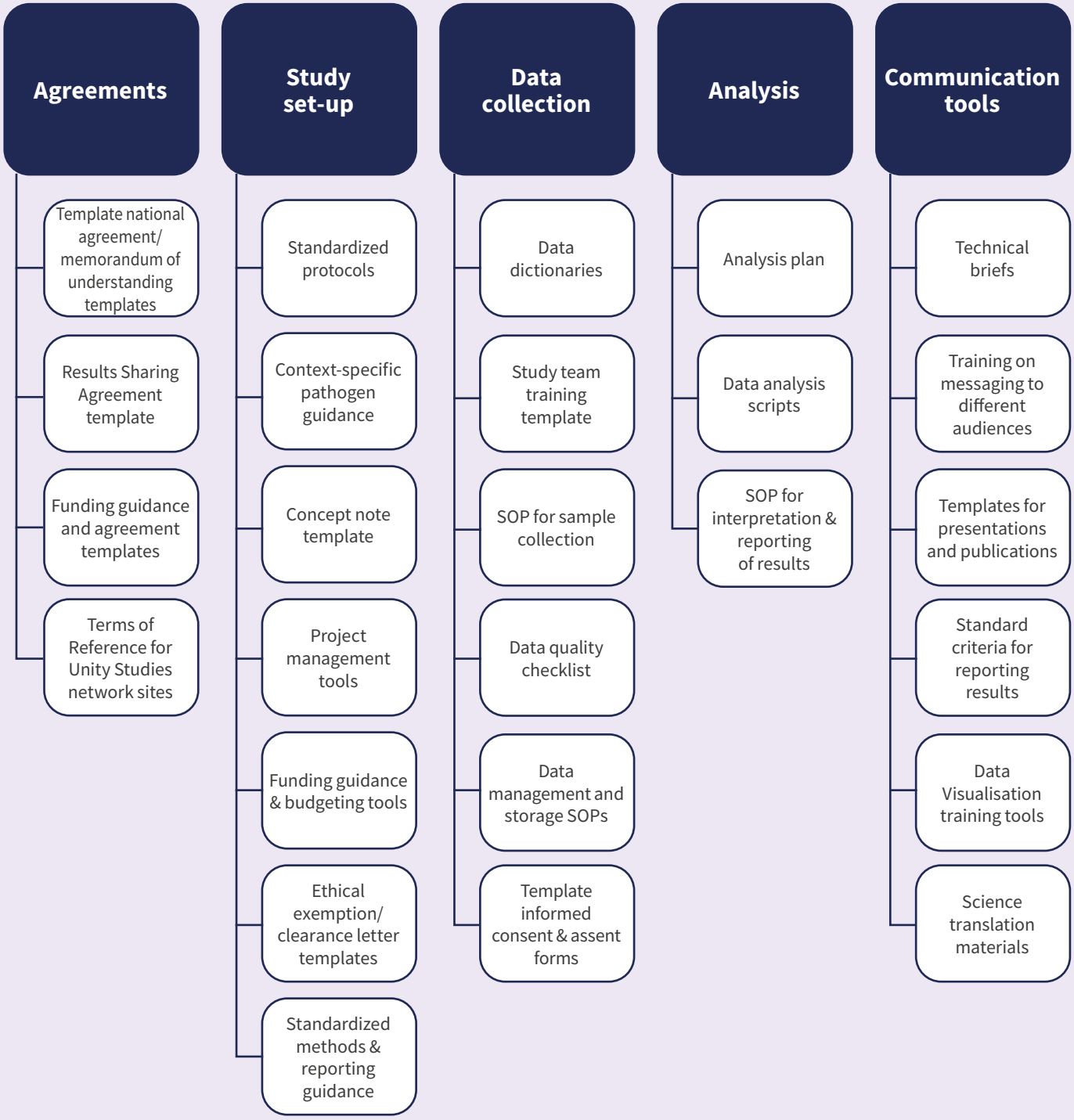
Toolkit items will be available on the WHO website. CLICK TO VIEW

5. Protocol Toolkit

5.1 Protocol Toolkit

The timely and quality implementation of this protocol should be supported by the use of toolkits developed by WHO and implementing partners. These will include components such as pre-planned agreements, study set-up resources, data collection, analysis and communication tools as shown in **Figure 2** and highlighted throughout the protocol.

Figure 2: Proposed toolkit components to support quality implementation of Respiratory Investigations and Studies protocols



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- University of Hong Kong: Ben Cowling
- EpiConcept: Tony Nardone

This document has been adapted from previous protocols developed and used during the COVID-19 pandemic through the COVID-19 WHO Sero-epidemiological Investigations initiatives (Unity Studies), which built on the global Consortium for the Standardization of Influenza Seroepidemiology (CONSISE), and the WHO's Pandemic Influenza Special Investigations and Studies initiative. Thus the protocols benefitted from extensive input from a range of colleagues and partners listed below (in alphabetical order): Anne von Gottberg (National Institute for Communicable Diseases, South Africa), Rebecca Grant (WHO HQ), Maria Van Kerkhove (WHO HQ), Jodie McVernon (The Peter Doherty Institute for Infection and Immunity, Australia), Anthony Nardone (Epiconcept), Michael Parker (University of Oxford), Malik Pereis (Hong Kong University), Max Smith (University of Oxford), Marta Valenciano (Epiconcept), John Watson (US CDC) and Joseph Wu (Hong Kong University).

8. Appendix

Appendix A: Questionnaires

Population-based age-stratified seroprevalence investigation protocol for respiratory pathogens with pandemic potential

Form 1: Reporting form for each participant

Unique Participant ID

1. Data Collector Information

Name of data collector	
Data collector Institution	
Data collector telephone number	
Data collector email	
Form completion date (dd/mm/yyyy)	____/____/____
Date of interview with informant (dd/mm/yyyy)	____/____/____

2. Participant identifier information

First name*	
Family name*	
Sex	☐ Male ☐ Female ☐ Non-binary ☐ Not known
Date of birth (dd/mm/yyyy)*	____/____/____ ☐ Unknown
Age (years, months)	____ years ____ months ☐ Unknown
Telephone (mobile) number	
Email	
Address*	
National social number/identifier (if applicable)*	
Country of residence	
Nationality	
Ethnicity (optional)	☐ Arab ☐ Black ☐ East Asian ☐ South Asian ☐ West Asian ☐ Latin American ☐ White ☐ Aboriginal/First Nations ☐ Other: _____ ☐ Unknown
Occupation COMMENT: Should be a categorical variable with options suitable for Country Y context.	
Have you had contact with anyone with suspected or confirmed pathogen X infection in the past 10 days?	☐ Yes ☐ No ☐ Unknown If Yes, dates of last contact (dd/mm/yyyy): ____/____/____

* These identifiers are commonly accepted as personally identifiable information and must be kept confidential, however these may vary by country and should be updated by Country Y according to national guidelines.

Form 1: Reporting form for each participant (continued)

3. Symptom history

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

Comment: (X) period to cover time since emergence or re-emergence of pathogen X 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 pathogen X infections as well as other infections that might have been circulating during the relevant period.

Comment: This portion of the form may require revision according to the clinical presentation and case definition of pathogen X.

Comment: If age and date of birth are considered sensitive information, it might be possible to ask individuals which age group they fall within, and specify a list of age groups.

Fever (≥38 °C) or history of fever	☐ Yes ☐ No ☐ Unknown
Sore throat	☐ Yes ☐ No ☐ Unknown
Runny nose (rhinorrhea)	☐ Yes ☐ No ☐ Unknown
Cough	☐ Yes ☐ No ☐ Unknown
Shortness of breath (dyspnea)	☐ Yes ☐ No ☐ Unknown
Chills	☐ Yes ☐ No ☐ Unknown
Loss of smell (anosmia)	☐ Yes ☐ No ☐ Unknown
Loss of taste (ageusia)	☐ Yes ☐ No ☐ Unknown
Vomiting	☐ Yes ☐ No ☐ Unknown
Nausea	☐ Yes ☐ No ☐ Unknown
Diarrhoea	☐ Yes ☐ No ☐ Unknown
Headache	☐ Yes ☐ No ☐ Unknown
Rash	☐ Yes ☐ No ☐ Unknown
Conjunctivitis	☐ Yes ☐ No ☐ Unknown
Muscle aches	☐ Yes ☐ No ☐ Unknown
Joint ache (myalgia)	☐ Yes ☐ No ☐ Unknown
Loss of appetite	☐ Yes ☐ No ☐ Unknown
Nose bleed	☐ Yes ☐ No ☐ Unknown
Fatigue	☐ Yes ☐ No ☐ Unknown
Chest pain	☐ Yes ☐ No ☐ Unknown
Seizures	☐ Yes ☐ No ☐ Unknown
Altered level of consciousness	☐ Yes ☐ No ☐ Unknown

Other neurological signs	☐ Yes ☐ No ☐ Unknown If Yes, specify:
Other symptoms	☐ Yes ☐ No ☐ Unknown If Yes, specify:

4. Patient symptoms: complications

Did any of these symptoms require you to seek medical attention?	☐ Yes ☐ No ☐ Unknown
Did any of these symptoms require you to miss work or school?	☐ Yes ☐ No ☐ Unknown
Have you needed to use supplemental oxygen?	☐ Yes ☐ No ☐ Unknown
Emergency department: Did any of these symptoms require you to go to an emergency department?	☐ Yes ☐ No ☐ Unknown
Hospitalization: Did any of these symptoms require you to be hospitalized?	☐ Yes ☐ No ☐ Unknown
Intensive care unit: Did any of these symptoms require you to be admitted to an intensive (critical) care unit?	☐ Yes ☐ No ☐ Unknown

5. Patient vaccination

Have you previously received a vaccination for pathogen X? COMMENT: this field will need to be adapted to include a vaccine for pathogen X (if one is available), or to include others that may be associated with pathogen X	☐ Yes ☐ No ☐ Unknown
If yes:	
Type of report	☐ Verbal ☐ Vaccination card or other documentation ☐ Other ☐ Unknown
What is the date of administration and product name of vaccine dose 1?	____/____/____ dd/mm/yy Product name _____
What is the date of administration and product name of vaccine dose 2?	____/____/____ dd/mm/yy Product name _____ ☐ Not received vaccine dose 2

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.

Serology testing methods and results:

Complete a new line for each specimen collected and each type of test done:

Laboratory identification number	Date sample collected (dd/mm/yyyy)	Date sample received (dd/mm/yyyy)	Type of sample	Type of test	Result (according to assay thresholds/ cut-offs - please report these)	Result date (dd/mm/yyyy)	Specimens shipped to other laboratory for confirmation
	____/____/____	____/____/____	☐ Serum ☐ Whole blood ☐ Dried blood spot ☐ Other, specify:	Specify type (ELISA/IFA/ RDT, IgM/IgG/IGA/total Ab, microneutralization, PRNT), etc.):	☐ Positive ☐ Negative ☐ Indeterminate/ probable Titre (irrespective of result):	____/____/____	☐ Yes If Yes, specify date ____/____/____ If Yes, name of the laboratory: ____ ☐ No