African Union

AFRICA CDC
Centres for Disease Control and Prevention
Safeguarding Africa's Health

Africa Centres for Disease Control and Prevention

Generic protocol for a population-based, age- and gender- stratified sero-survey study for SARS-CoV-2

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Contents

Background

The novel coronavirus disease of 2019 (COVID-19) is a global pandemic, and the epidemic in Africa continues to rise at a rapid rate. Cases have been reported in 54 of 55 member states, most of which already experiencing community-spread hence are making it more difficult to contain the outbreak. With the expansion of testing, the cumulative number of cases reported in Africa has increased rapidly, however given the fact that majority of cases could be mild or asymptomatic, the true number of infections, their age and sex distribution, and the proportion that are severe or fatal is still uncertain using current surveillance methods.

The number of confirmed COVID-19 cases detected and reported in each country is influenced by many factors including limited access and/or utilization of healthcare and COVID-19 testing, limited surveillance, lack of knowledge amongst the population about when to seek testing, an asymptomatic presentation, and other unknown issues. This is true in all countries of the world, and not Africa specific, however there are factors unique to Africa which may also affect the way the virus behaves there. COVID-19 prevalence data are critical for planning effective mitigation strategies and understanding the true impact of the disease and relevant intervention measures in Africa, which might be quite different from regions with a different population age distribution or risk factor profile.

To address this knowledge gap and provide appropriate scientific guidance for the current and future epidemic prevention and control in Africa, Africa Centres for Disease Control (CDC) is putting in place this generic study protocol to provide guidance to the African Union Member States on standardized serological prevalence surveys of COVID-19. Sero-prevelance surveys will facilitate answering these questions and examining the differences between countries to understand what factors may have influenced recent transmission. This protocol is intended to be used as a template or guideline, adapted as needed for the local context and for the specific needs of individual countries.

Due to its novel origin, the baseline or pre-existing prevalence of antibodies to the virus in the population is assumed to be negligible. 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, even in the absence of pre-epidemic seroprevalence data.

Objectives

Primary objective

Define the age- and sex- specific cumulative incidence of COVID-19 in the general population up to the point at which the survey is carried out.

Secondary objectives

1. Identify the risk factors for severe disease.
2. Define the rates of symptomatic vs. asymptomatic infection in the population.
3. Provide a population-based denominator of infections to define the case-fatality rate of the disease.

Note that these secondary objectives will require some assumptions to be made and, in some cases, additional work to be carried out. For example:

- Identifying risk factors for severe disease will require both cross-tabulating results of the survey with PCR and other test results, and/or the assumption that most severe disease that has occurred in the recent past was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals who were positive. While this may seem like a large assumption, it is rather the relative rates of co-morbid conditions between people who are positive and negative, correlated with recent illness compatible with COVID-19 that will be analyzed. While not a precise measure, the results can be informative.
- Similarly, while it is not possible using this methodology to provide an accurate point estimate of the rate of symptomatic versus asymptomatic cases, the results of this survey can provide a lower boundary for asymptomatic infection rate using the proportion of seropositive individuals who have had no recent illness within the defined period of community transmission.
- The case-fatality rate will require additional work to estimate numbers of deaths in the population in which the survey is carried out. For most countries, this will involve an estimate of excess mortality calculated for the time of transmission in the community. There are several standardized methods[1] for doing this that have been used for influenza.

[1] Serfling RE. Methods for current statistical analysis of excess pneumonia-influenza deaths. Public Health Rep. 1963;78(6):494-506.
Reed C, et al. Estimating influenza disease burden from population-based surveillance data in the United States. PLoS One. 2015;10(3): e0118369.

Methods

Note: *The basic design outlined in this protocol represents a hypothetical situation in which a nationally representative sample is being collected to study seroprevalence by age and sex for the whole country. We have attempted to provide recommendations for countries where the setting, goals of the survey, or populations of interest might be different. These are highlighted in blue italicized text.*

Design

The study described in this protocol is a cross-sectional, population-based, age-stratified sero-survey. The study will provide a snap-shot view of cumulative incidence at the time of the survey. Participants will be asked questions regarding demographic information, signs and symptoms of any acute illness in the period since the outbreak started in the area or region under study, and existing co-morbidities. Blood will be collected to test for antibodies to SARS-CoV-2.

Study target population

The basic study design in this protocol is for a nation-wide cross-sectional survey designed to represent the entire population of the country.

Adaptation: Depending on resource availability, the need to test and improve the research tools, as well as generating a proof of concept, countries may decide to start with a specific population group such as residents of suspected high disease burden areas, urban dwellers, refugee camps, health care workers, teachers etc. For geographically defined sub-populations, such as urban dwellers or camps, the basic study design will be similar to the one described in this protocol. For special populations such as teachers or healthcare workers, significant modifications will need to be made to the approach for selection of participants to limit sample bias and ensure representativeness.

The target population for the nation-wide study is household members aged 5 years or above, regardless of their previous or current infection with COVID-19, who resided in the country[1] during the period of transmission of SARS-CoV-2.

Sample size

The following is an example of a sample size calculation for a nationally representative study, stratified by age and sex, in country with a diverse population living in both urban and rural areas. For the purpose of sample size estimation, seroprevalence is estimated at 5%.

[1]Or target area if the survey is not national.

Considering 95% confidence interval, design effect of 3[2] and ~10% non-response rate, the minimum sample size is calculated to be 1200 households with one member of each household selected. This is designed to give a total of 240 individuals for each of the age strata 5-9, 10-19, 20-39, 40-59 and ≥60 years old.

Adaptation: This sample size may be large enough to compare seroprevalence in urban versus rural areas, if all ages and genders are combined. However, it should be remembered that generalizing the comparisons of two different proportions from a study to the overall population requires a larger sample size than a single point estimate for a study population. If this comparison is a primary goal of the study, sample size and sampling procedures should be modified to ensure sufficient power to demonstrate a difference, if one exists.

Alternatively, if a smaller geographic area is chosen for study, or several areas that are very similar in their likelihood of level of transmission, the sample size may be much smaller than this. For example, if only urban areas are studied, it is likely that the differences in seroprevalence between clusters will be smaller than that used for the above calculation, thereby reducing the design effect and resulting in a smaller sample size.

For any notable deviations from the example provided, sample size projections should be rechecked. A free online sample size calculator is available here: http://www.openepi.com/Menu/OE_Menu.htm

Table: Estimated sample sizes at different levels of sero-prevalence and design effect, a precision of ± 5%, and 5 age strata (note: does not account for non-participation or dropout).

Prevalence of SARS-CoV-2 antibodies (%)	Design effect		
	2 Sample size	3 Sample size	4 Sample size
2	305	455	565
5	730	1095	1460
10	1385	2075	2770
15	1960	2940	3920
20	2460	3690	4920

[2]We have chosen a design effect of 3 based on the observation that rates of infection appear quite different in different areas of a country, especially when comparing rural and urban areas. This variability will increase the design effect, and subsequently, the sample size. However, if the area under study is thought to be much more homogenous in terms of seroprevalence of SARS-CoV-2 antibodies, a smaller sample size could be used. However, ideally, increasing the sample size would involve collecting data from a higher number of *clusters* rather than increasing the number of individuals per cluster as this would improve the statistical reliability of the results.

Sampling

The following is an example of how a sample might be selected in an average-sized country with multiple states or sub-national administrative units (regions, provinces, counties, etc.): The sampling frame will be the most recent national census[1] projection data. Sampling will be a randomized multi-stage cluster sampling, with the first stage of sampling being proportionate to the most recent population size estimate. The first stage will be the sampling of 12 sub-national administrative units which will be selected from the total with likelihood of selection proportionate to the estimated population of the unit. Within each selected sub-national unit, 10 communities will be randomly selected using the simple random sampling. Within each selected community, 10 households will be randomly selected from a list of all households. One member of the household over the age of five years will be randomly selected from each selected household for participation. The likelihood of selection will be calculated for each stage for later analysis.

Households are defined as a group of people (two or more) living in the same residence[2]. Sampling will exclude residential institutions, such as boarding schools, dormitories, hostels or prisons.

Selection of individuals will be constrained to give the appropriate number of individuals for each age stratum.

Adaptation: The principle that should be adhered to is to keep the number of individuals in a single cluster relatively small – ideally ≤10 – in order to decrease the association between individuals included in the overall sample. If a country has a small number of states or first-level administrative units, use the next level down as the first sample stage. For example, Rwanda has only 5 states, divided into 30 districts. If the survey were to be done in Rwanda, the first stage of selection would be to choose 12 districts from amongst the 30 districts using the method of probability proportionate to population size. A different number of districts or states could also be used – the goal is to provide a representative sample and keep the individual clusters small.

For the selection of households at the final stage of the sample, the ideal is to randomly select from a list of all households in the community. However, if this is not available, other methods can be used. For example, selection could be done using GPS coordinates or using the Enumeration Areas (EA) from the last census.

Eligibility criteria

Inclusion criteria: All individuals over the age of 5 years[3] identified for recruitment into the investigation, irrespective of known acute or prior COVID-19 infection who resided in the area under study during the period of SARS-CoV-2 transmission and who are able to give consent, or in the case of minor children, whose legal guardian is able to give consent for them.

Exclusion criteria: All individuals who refuse or are unable to give informed consent, or present contraindication to venipuncture. Prisons, dormitories, camps for refugee or internally displaced peoples will also be excluded from study. Each of these special situations requires specialized approaches for study.

[1]Alternatively, recent Demographic and Health Surveys (DHS), Multiple Indicator Cluster Surveys (MICS), or population-based HIV impact assessment (PHIA) can be used if they are more recent and reliable.
[2]In practice, the exact definition of "household" may vary due to social, political and cultural practices. However, the principle is that households however defined will be selected in an unbiased way.
[3]If it is desirable to add children under the age of 5 years, the additional age stratum will require an increase in sample size of approximately 240 individuals in that age group. Each additional stratum added will be approximately the same, unless large differences are expected in the likely proportion that are sero-positive in the stratum.

Informed consent

Each survey participant will be requested to sign an informed consent before being enrolled. The survey worker shall explain to participants the specific purpose, content, method, benefits and risks of the survey. After the participants fully understand the content and significance of this survey, they will be asked to sign the informed consent form before the interview begins. If the selected participant is illiterate, a minor child, or otherwise unable to sign, they can give verbal consent with a proxy designated to sign on their behalf. Children over the age of 9 years[4] will be asked for their assent to participate, in addition to written consent being given by a legal guardian. (See annex for information statements and consent and assent forms).

Sample collection

Approximately 6 milliliters of blood will be collected by venipuncture from the selected member of each household upon recruitment into the investigation and consent being given. Blood will be drawn by a trained phlebotomist who will be responsible for labeling and packing the specimen for transport.

Adaptation: Rapid antibody test can also be employed for the sero-survey in which case finger prick specimen can be collected and testing done on site. This would greatly decrease logistical demands on specimen transport and allow immediate reporting back to the participant. However, the antibody test should have high performance characteristics (more than 98% sensitivity and specificity). There are tests currently in development that may meet these criteria; survey planners should consult current Emergency Use Listing of WHO[5] or the U.S. Food and Drug Administration's Emergency Use Authorization list[6] for details. As with any test, it is important to know the performance characteristics of the test used and whether it detects IgG only, IgM only, or both in order to be able to interpret the resulting data.

Specimen transport, reception and storage

The blood specimens shall be labelled with the participants unique identification number, packaged and transported in a sealed bag with the specimen transport form (see annex for the attached form). Blood samples will be stored on ice in a portable cooler at the time of collection until they arrive at the storage facility where they will be refrigerated (4℃ or frozen to -40℃) the same day and transported to a local designated laboratory to separate the serum from the whole blood. The serum will be divided into two tubes, A (for testing) and B (for storage).

After the blood samples are separated, they should be transported to the designated laboratory with testing capability in cold storage as soon as possible. The receiver shall store the B tube (storage tube) at -40℃ and send the A tube (test tube) to the laboratory for storage at 4℃ and complete the test within 2 days. After testing, the serum specimens of tube A will be stored at -40℃.

[4]Or the age considered appropriate for the area where the study is being done.
[5]WHO EUL list available at: https://www.who.int/diagnostics_laboratory/EUL/en/
[6]FDA EUA list available at: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance

Repeated freezing-thawing of specimens should be avoided. Samples should be divided equally before freezing to minimize freezing-thawing cycle.

Adaptation: As discussed above under in the sample collection section, specimen collection and transportation to the central level may not be necessary if rapid antibody tests are used for the sero-survey.

Serological testing

Serum samples will be screened for the presence of both IgM and IgG SARS-CoV-2 virus specific antibodies using a validated enzyme-linked immunosorbent assay or similar methodology[7].

All tests will be performed according to manufacturer's instructions and approved survey standard operating procedures (SOPs) including the use and interpretation of appropriate quality control (QC) samples. Test results will be interpreted according to manufacturer's instructions, unless otherwise stated. SOPs will be adhered to during the entire survey period, including, but not limited to, procedures for specimen collection, testing, sample storage and transportation, biosafety, and waste disposal. Reference panels from NiBSc or equivalent panel will be incorporated as part of initial test verification.

Test results will be notified by mail or text message to all participants (or their legal guardian in the case of a minor child).

[7]Multiple tests are available for use. The specific test selected should have a WHO Emergency Use Listing (EUL) and have known performance indicators including sensitivity and specificity.

Survey data collection

A questionnaire will be administered through a face-to-face interview with each participant to collect information about demographics, recent illnesses with signs and symptoms, recent admissions to hospital, and co-morbid conditions. (see annex for questionnaire).[8]

The questionnaire will be piloted before finalized on a small number of volunteers in both rural and urban areas. The pilot will be used to test the clarity of the questions, the appropriateness of the consent form, and the ease of use by the field investigators.

Data management

Data will be entered and stored on a secure, password protected computer or cloud-based database. Each participant will be given a unique identification number that will be used for linking data to laboratory results.

Data analysis

Descriptive statistical analysis will be conducted. Proportions will be calculated for categorical variables; means and standard deviation will be analyzed for continuous data. Chi-Square will be applied to evaluate the association between categorical variables; all the estimates will be weighted according to the sampling probabilities.

[8]Note: if specific tools are being used such as digital tablets or other devices, provide some information about training, how they will be used and how the data will be transferred to the central database.

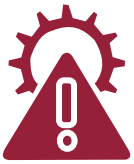
Prevention of COVID-19 infection among investigation personnel and household members

All personnel involved in the investigation will be trained in infection prevention and control procedures (standard contact and droplet precautions, as determined by national guidelines). These procedures will include proper hand hygiene, physical distancing and the correct use of surgical/cloth face 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.

Confidentiality

Participant confidentiality will be maintained throughout the investigation. All study participants 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 will not be disclosed elsewhere.

If the data are shared by the implementing organization with any other agency or institution providing support for data analysis, data shared will include only the study identification numbers and not any personably identifiable information.



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 COVID-19 infection and may prevent further transmission of the virus. Participants will also be informed of their test results, but the interpretation of those in terms of benefit to the participant is unknown at this time.

Annexes

1. Consent form

Hello. My name is _____. I am working with the Ministry of Public Health. I am inviting you to participate in a research study about the COVID-19 illness that so many people have had recently in our country. This study will tell us how many people have been infected with the virus that causes COVID-19 in our country. It will help us to understand what causes some people to get very sick with it, while others are only mildly affected. In addition, it will help us to understand the differences in how it affects children, adults and elderly adults. The information we collect will help ministry of health officials to better plan future measures for COVID-19 control and prevention.

Your household has been randomly selected – by chance - for the survey to represent other households in the community. If you agree to participate I would like to ask you some questions about your health and any recent illnesses you may have had, and to draw a small amount of blood from your arm - about 2 teaspoons full - to test to see if you may have already had COVID-19. There may be some slight pain with the blood drawing, but it will be very brief. The amount of blood taken will not be enough to affect your health although there may be a small amount of bruising at the site afterwards. The entire process will take about 30 minutes. We will send the results of your test back to you by (mail/SMS/or other to be determined). This information may not have direct benefit for you at this time but will help us to better plan how to control the virus.

All of the information you give, and your test results will be kept secret other than what we share with you and others who are directly involved in analyzing the information we are collecting. The results of this study may be used in reports, presentations, or publications but your name will never be used. The information will be stored on a safe computer to which only the researchers involved in the study will have access.

Your participation is completely voluntary and there's no penalty if you say no. In fact, even if you say yes, you can still change your mind at any time during the process. If there are any questions you don't want to answer, just let me know. You can also stop the interview at any time.

Do you have any questions?

Please sign below to indicate that you understand, have had the opportunity to ask the interviewer about this study and agree to participate.

Name of the Participant (Write your name in the line): _____

Signature of the Participant (Put your signature in the line):_____

Name of Interviewer: _____

Signature of interviewer _____

Date: _____

May I begin the interview now?

a. Yes

b. No

2. Assent form (for minor children)

Assent Form

My name is [provide your full name]. I work for the [provide]. I am inviting you to participate in a research study about the COVID-19 illness that so many people have had recently in our country.

Your parent knows about this study and gave permission for you to be involved. If you agree, I will ask you to let me to draw a small amount of blood from your arm to test to see if you may have already had COVID-19. You may feel a little bit of pain when blood is taken, but this won't last long. I will also ask your parents some questions about your health.

You do not have to be in this study. No one will be mad at you if you decide not to do this study. Even if you start the study, you can stop later if you want. You may ask questions about the study at any time.

If you decide to be in the study, I will not tell anyone else about the results of your test, other than your parents.

Signing here means that you have read this form or have had it read to you and that you are willing to be in this study.

Name of the Participant (Write your name in the line): _____

Signature of the Participant (Put your signature in the line): _____

Name of Interviewer: _____

Signature of interviewer: _____

Date: _____

3. *Parental information and consent form* (to be used when participant is a minor child)

Dear Parent [or caregiver, legal guardian],

My name is [provide your full name] and I am working with the Ministry of Public Health. We are conducting a research study to better understand the current epidemic of COVID-19 in our country. The results of this study will help the Ministry of Health make better decisions about how to control the spread of the virus. The purpose of this form is to inform you and provide information about the study.

What is this research study for?

This study will tell us how many people have been infected with the virus that causes COVID-19 in our country. It will help us to understand what causes some people to get very sick with it, while others are only mildly affected. In addition, it will help us to understand the differences in how it affects children, adults and elderly adults.

What does the study involve for my child?

If you agree to allow your child to participate in the study, we will draw a small sample of blood from his/her arm – about 2 teaspoons full – and ask you some questions about his/her health and any recent illness. There may be some slight pain with the blood drawing, but it will be very brief. The amount of blood taken will not be enough to affect his/her health although there may be a small amount of bruising at the site afterwards.

Why is my child being invited to take part in a research study?

Your child has been chosen at random – by chance – to represent other children in the country. By learning how many children have already been infected with this virus and comparing this to the numbers in adults, we will better understand how this virus is spreading.

What happens if I say yes, but I change my mind later?

Your child's participation in this study is voluntary. Your child may decline participation at any time. You may also withdraw your child from the study at any time; there will be no penalty. Likewise, if your child chooses not to participate or to withdraw from the study at any time, there will be no penalty.

What are the benefits for my child to be in this study?

We will send you the results of the test once it is completed. This will only tell you whether your child was infected in the past but will not tell you if he/she is now immune. This information will help us to better plan how to control the virus but will not have direct benefits for your child.

What happens to the information collected for the study?

Your child's information and the results of the test will be kept secret, other than what we share with you and others who are directly involved in analyzing the information we are collecting. The results of this study may be used in reports, presentations, or publications but your child's name will never be used. The information will be stored on a safe computer to which only the researchers involved in the study will have access.

Is there any way being in this study could be bad for my child?

There are minimal risks and inconveniences to participating in this study. These include:

- Having blood drawn
- Spending time answering questions about health and illness

By signing below, you are agreeing to your child's participation in this study:

Your child's name: _____

Parent's name: _____

Parent's Signature: _____

Date: _____

4. Data collection forms

Demographic and identifying information					
Date of Interview:	Study ID No.:				
Interviewer Name:					
Participant first/given name					
Participant surname					
Name of parent (if participant is a minor child):					
Sex	Male				
	Female				
Date of Birth	Month				
	Month				
	Year				
Telephone (mobile) number:					
Location:					
District/State:					
Community:					
Address/house number:					
Occupation:					
Number of people living in the household					

Symptom history	
Since March 2020, have you had any of the following sign and symptoms:	
Fever ≥38°C	□ Yes □ No
Chills	□ Yes □ No
Fatigue	□ Yes □ No
Muscle ache (myalgia)	□ Yes □ No
Sore throat	□ Yes □ No
Cough	□ Yes □ No
Runny nose	□ Yes □ No
Shortness of breath	□ Yes □ No
Wheezing	□ Yes □ No
Chest pain	□ Yes □ No
Other respiratory symptoms	□ Yes □ No
If yes, please specify	
Headache	□ Yes □ No
Loss of smell	□ Yes □ No
Loss of taste	□ Yes □ No
Diarrhea	□ Yes □ No
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
Did any of these symptoms require you to be hospitalized?	□ Yes □ No □ Unknown
If yes, what was the date of your hospitalization	Month Month Year

Pre-existing medical conditions	
Have you ever been diagnosed with any of the following?	
Diabetes	□ Yes □ No
Chronic lung disease	□ Yes □ No
High blood pressure	□ Yes □ No
Serious heart condition such as heart failure, previous heart attack, or similar problem.	□ Yes □ No
Neurological condition such as seizures, paralysis, or delayed development	□ Yes □ No
Chronic kidney disease	□ Yes □ No
HIV infection or AIDS	□ Yes □ No
If yes, on antiretroviral therapy?	□ Yes □ No
TB	□ Yes □ No
Sickle Cell anemia	□ Yes □ No
Cancer	□ Yes □ No
Other:	
Are you pregnant? (for women between the ages of 14 – 55 years)	□ Yes □ No
Do you smoke tobacco products?	□ Yes □ No

5. Laboratory transport Form

Date of collection:	**Study ID No:**
Phlebotomist Name:	
Participant first/given name:	
Participant surname:	
Name of parent (if participant is a minor child):	
Date of Birth	Day Month Year
Telephone (mobile) number:	
Location:	
District/State:	
Community:	
Address/house number:	

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