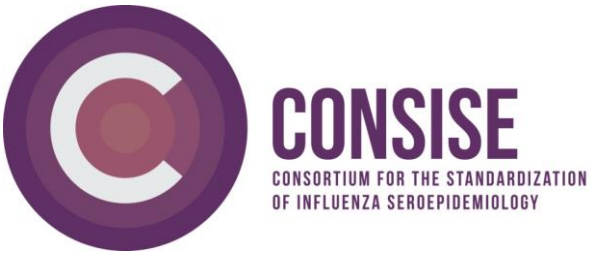
Cross-sectional seroprevalence study of novel Coronavirus (nCoV) infection prior and post epidemic periods

Working Document

Developed by

The Consortium for the Standardization of Influenza Seroepidemiology (CONSISE):

A Global Partnership to Develop Influenza Investigation Protocols and Standardize Seroepidemiology to Inform Public Health Policy



Date: working drat v1.1 8 May 2013

Contact: m.vankerkhove@imperial.ac.uk

LICENSE

PROTOCOL SUMMARY

Representative serologic studies are designed to collect information and the prevalence of antibodies to a new virus in a population. This information is critical to better estimate severity of the new virus. Outlined here is a cross-sectional serologic study which aims to collect serologic data from specific populations to estimate the prevalence of cross-reactive antibodies to the novel virus and to estimate cumulative incidence of the new virus using sera collected prior to the start of an epidemic and after an epidemic.

Assessments of close contacts and health care personnel of nCoV patients are treated in two separate protocols developed by CONSISE. This protocol outlines a cross-sectional serologic investigation in the same population in pre- and post-epidemic periods of nCoV.

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.

Details of who and how this protocol was developed are provided in section 5.0.

CONTENTS

1.0 SCIENTIFIC BACKGROUND AND RATIONNALE

As of 8 May2013, 31 laboratory-confirmed cases of human infection with novel coronavirus (nCoV) have been reported to WHO[1]: two from Jordan, two from Qatar, 23 cases from Saudi Arabia, two from the United Kingdom (UK), and two from the United Arab Emirates (one diagnosed in France). It is suspected that nCoV is a zoonotic virus, which may have arisen from animal exposures, and zoonotic transmission in the Arabian Peninsula, but information on exposures is limited and an animal reservoir is unknown. Human-to-human transmission is suspected and likely to have occurred in: one cluster of three familial cases in Saudi Arabia; one cluster among ICU health care workers in a hospital in Zarqa, Jordan involving 2 confirmed and 11 probable cases; a familial cluster in the UK, and the recent outbreak of 13 cases in eastern Saudi Arabia linked with one health care facility. Follow-up investigations by Ministry of Health officials have taken place for all cases from other cases and suggest that no further confirmed or probable cases occurred. At this stage, however, it is difficult to ascertain whether other primary zoonotic or secondary human-to-human transmission cases have been missed.

Coronaviruses are a large, diverse group of viruses that affect many animal species and infection in humans can cause a wide range of respiratory illnesses, typically with “common cold” symptoms. Genetic sequence data indicate that this nCoV is a beta-coronavirus similar genetically to bat coronaviruses, but antigenically and genetically distinct from any other coronavirus previously described in humans, including the coronavirus (SARS-CoV) that caused severe acute respiratory syndrome (SARS). Most cases have reported severe acute respiratory symptoms requiring hospitalization and intensive care. Eighteen of the 31 cases have been fatal.

The non-specificity of clinical case definitions for nCoV and unknown rates of symptomatic versus non-symptomatic infection means that infection attack rates cannot be estimated from case-based clinical surveillance. This information is critical to understanding the overall morbidity, mortality, and population-level severity of a novel influenza virus as it serves as the denominator for the estimation of severity indicators. Representative serological studies are designed to collect denominator data (i.e., number of infections) that can be used to estimate severity parameters such as the case fatality ratio (i.e., CFR, the total number of novel virus related deaths divided by the total number of infections) and hospitalization ratios (number of related hospitalizations divided by number of infections). Thus analysis of serological data can reduce the uncertainty around severity assessment and help inform the appropriate intensity and targeting of mitigation policies.

The gold standard for documenting infection rates is individually paired acute and convalescent sera collected with a longitudinal study design. However, these are rarely available during an outbreak situation particularly in the early stages of the event. An analogous method to estimate these infection rates on a population level is the pairing of sera from a representative cross-section of the population drawn before and after the event. This method uses pre-epidemic seroprevalence of

[1] http://www.who.int/csr/disease/coronavirus_infections/en/index.html

antibodies that react with the nCoV virus as a baseline value for comparison against the seroprevalence of antibodies in the same population after the event. A large number of countries used this type of cross-sectional study design to estimate cumulative incidence of the pandemic H1N1pdm virus (H1N1pdm09).[9] These methods can be applied to epidemics associated with other novel infectious agents with the availability of a validated serological assay.

As the sera collected before the event can be tested as soon as the appropriate laboratory methods are available, the seroprevalence of cross-reactive antibodies to the nCoV virus in different age groups and other at-risk populations in these sera can also be used to estimate the relative risk of infection between different groups of individuals, in an epidemic. While the presence of cross-reactive antibodies is not an exact correlate with either individual or population immunity, the difference in levels of pre-existing cross-reactive antibodies in different groups of a population can be helpful in predicting the relative susceptibility to infection between those groups. This might be of value in guiding vaccination or other intervention efforts. This will be very difficult for novel viruses, such as nCoV, where the association between seropositivity and immunity is not known.

1.1 STUDY OBJECTIVES

The data collected from this study can be used to refine/update recommendations for surveillance and case definitions, to characterize the key epidemiological transmission features of nCoV virus, help understand spread, severity, spectrum of disease, impact on the community and to inform operational models for implementation of countermeasures such as case isolation, contact tracing and quarantine.

PRIMARY OBJECTIVE

The primary objectives of this study are to determine the age-specific cumulative incidence of infection with nCoV in the population and to prevalence of cross-reactive antibodies to nCoV at baseline.

SECONDARY OBJECTIVES

Comprehensive study investigations, such as the one described below, can provide rich data to assess numerous secondary outcomes including:

- To identify specific groups (e.g. age groups, minority groups, or other at-risk populations) with highest levels of susceptibility for infection based on pre-epidemic titers of antibody.
- To identify specific at-risk groups with higher incidence of infection.
- To describe the changes of population antibody levels over time [additional, optional objective if surveys are repeated over a prolonged period of time]

COMMENT: For novel viruses other than influenza, achieving these objectives depends on the availability of a validated serological test for which the sensitivity and specificity is approximately known. It should be noted that even for influenza, the relationship between seropositivity and immunity is not well correlated and susceptibility patterns based on seroprevalence can only be understood in a relative sense.

2.0 STUDY PROCEDURES

2.1 STUDY DESIGN AND TIMING

Two cross sectional samples of sera will be used to determine the cumulative incidence of nCoV. The first sample will be pre-epidemic sera, collected before widespread community transmission of the nCoV occurs (Figure 1, time indicated by A or B) and the second sample, or post-epidemic sera, will be collected after the epidemic wave or waves are over, ideally during the period starting at least 2 weeks after the end of the wave (Figure 1, time indicated by C or D). The cumulative incidence of infection will be determined by taking the difference between the two seroprevalence values. Sample size will be sufficient to determine incidence for each age stratum (see section 3.1 below).

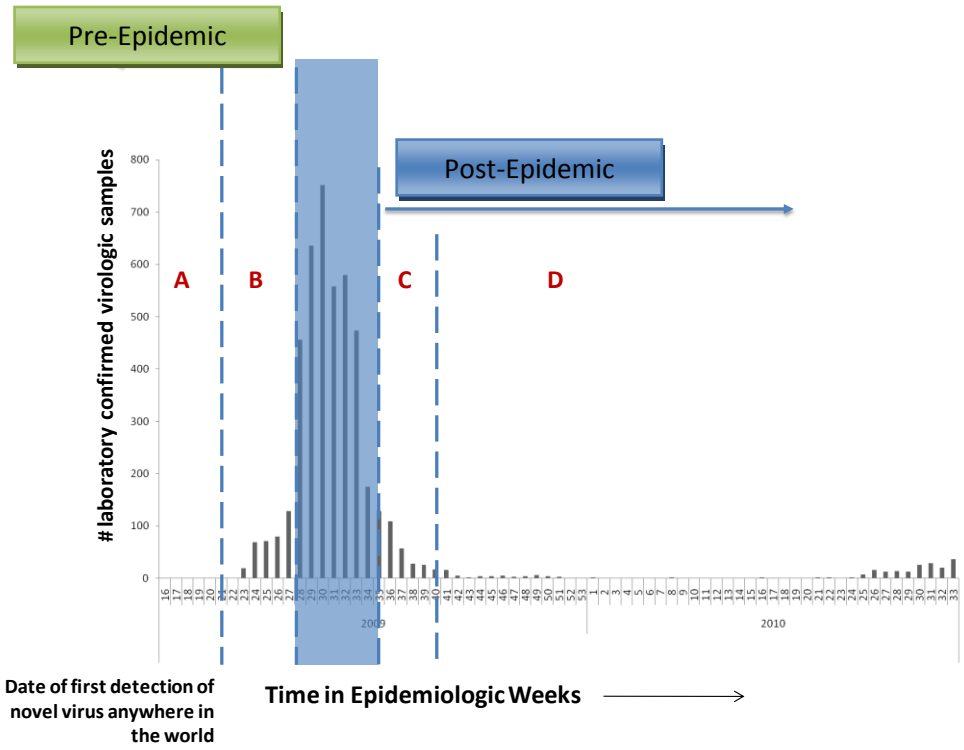


Figure 1 Characterization of sera collection timing in relation to national epidemic curve

Legend: The legend shows a hypothetical curve of disease activity in a country. The arrows indicate when sera could be collected for this protocol. “A” and “B” indicate timings when pre-epidemic sera could be collected, the shaded area indicates example of peak activity and “D” represents the time when post-epidemic sera should ideally be collected. “C” indicates the time period in which an early sample of sera could be collected for a preliminary, early estimate although it

should be noted that collecting sera in this time period will underestimate the infection rate. The time period "C" might be used if, for example, a vaccination campaign is being planned which could complicate later interpretation of results.

Three scenarios for data collection are provided depending on the source of **baseline sera** (see Table 1):

[Scenario 1] Use of residual sera for both the pre- and post-epidemic seroprevalence. This study design will make use of anonymized, stored sera drawn for other purposes but from the same sources (or populations) both before and after the epidemic (Figure 1, time period A). A sufficient number of samples from each age stratum will be taken to produce age-specific incidence rates for each (see sample size calculations in section 3.1 below). Potential sources of sera could be blood banks, diagnostic laboratories (e.g. microbiological laboratories), or clinical chemistry labs. This study design will have the inherent limitation of being able to only use available data which will not likely include important information on co-morbid conditions and vaccination status, for example. In addition, each source of sera will have different potential biases because of the population from which they are drawn. One variation on this scenario that could produce additional data is to use residual stored sera for pre-epidemic seroprevalence but for the post-epidemic sera prospectively and randomly select individuals who present for blood collection at locations where pre-epidemic sera were collected and administer a structured questionnaire to all subjects. This variation has the advantage of allowing a comparison of the proportion of sero-positive and sero-negative individuals with potential risk conditions in the post epidemic group.

[Scenario 2] Use of residual sera for the pre-epidemic baseline seroprevalence with a serosurvey after the epidemic to measure post-epidemic seroprevalence. The baseline values will be determined in the same way as scenario 1 above and so will be subject to the same limitations and biases. The post-epidemic survey can be designed in several ways (see section 2.3) but should be drawn from the population of the same geographical area and age groups as the pre-epidemic sera. This method has the advantage of allowing the collection of collecting additional data (e.g., data on co-morbid conditions, vaccination history, symptoms) but may introduce unforeseen spurious results due to the use of sera from a population with different characteristics than the baseline population. Exclusion criteria for the post-epidemic survey can be designed to help decrease these differences.

[Scenario 3] The third scenario occurs when baseline residual sera are not available or the investigators desire to generate data without the inherent biases of residual sera. It is the most cost and labor intensive of the three as it requires two separate cross-sectional serosurveys, one before and one after the epidemic. In this

scenario, a pre-epidemic cross-sectional serosurvey is performed in the community to collect baseline sera (see below for sampling methods) prior to widespread community transmission in the population to be surveyed (Figure 1, time period B). Post-epidemic sera are collected with a cross-sectional survey in the same population 2 weeks or more after the epidemic has finished. Populations could include:

- SARI subjects from representative geographic locations throughout a country
- A random selection of individuals from one (or many) geographic areas for the first sampling of pre-epidemic sera, and a second random selection of individuals from the *same* geographic area(s) for the second post-epidemic sera collection

  A number of locations will be selected at random using a standard geographical information system (GIS) such as Google Maps (www.maps.google.com)[10]. A visual census will be conducted at those points and the addresses (or other unique descriptors) of the closest [insert number] households recorded. Individuals from households that agree to participate will be enrolled as described in the section below on study procedures.

- A random selection of individuals from specific populations, such as animal market workers, veterinarians, cullers, etc.

COMMENT: The third scenario may be most appropriate for nCoV since serologic tests are still being developed, and because the route of transmission is unclear. Note, that if you choose the third option, the sample size must be sufficiently large to have the power to detect differences in exposures.

For all 3 scenarios, study participants should be matched by age and sex to the residual sera, at least within the age groupings described in section 4.2.

2.2 ETHICAL CONSIDERATIONS

Ethical approval must be sought in accordance with local, regional and national authorities.

COMMENT: It is strongly recommended that ethical approval is obtained in advance from relevant ethical or institutional review boards (e.g., national Ministries of Health, Agriculture, etc) using a generic protocol such as this one prior to an outbreak. Once an outbreak occurs, the study design, questionnaires, sampling and consent forms can be modified rapidly to the actual situation. This may still have to be resubmitted for ethical approval, but as the generic protocol including this final step has already been approved, this could be a very rapid process, without substantial delay to the start of the investigations.

2.3 STUDY POPULATION

Study participants will be selected from the same geographic area as the population from which the baseline sera are taken.

SELECTION OF STUDY PARTICIPANTS

Three options for selection of study participants are described below. These apply to both pre and post epidemic baseline sera:

Option 1 *Convenience sample using residual sera:*

If residual sera are used for the baseline seroprevalence, a second set of residual sera collected after the epidemic from the same source can be randomly selected to determine post-epidemic seroprevalence. Post-epidemic sera should be selected to match the pre-epidemic sera in terms of age and sex distribution and should ideally come from the same sources. Potential sources of sera are diagnostic laboratories, screening laboratories, microbiological laboratories, and blood banks. There are also a few periodic nationally representative general health surveys that may include blood collection and could be used as baseline, in which case the post-epidemic sera would have to be collected through a survey as described in the next two sections.

One variation of this approach is to randomly recruit participants who present for blood collection at the same sites that provided the pre-epidemic sera. Study participants will be well individuals referred by their physicians for routine screening blood draws and should be age and sex matched with the original pre-epidemic sera.

COMMENT: The methods described above are the same as presented in the CONSISE Protocol: Seroepidemiology of human influenza infection using residual sera/convenience samples for establishing baselines and/or monitoring trends over time.

Option 2 *Random digit dialing*:

Following a media campaign to advertise the study, households will be randomly called and invited to participate. Only one individual per household should be selected and included in the study.

Option 3 *Geographical sample:* The same geographic areas chosen for the pre-epidemic sera collection should be used again for the post-epidemic sera collection.

Table 1 Advantages and disadvantages of participant selection methods

Section method	Advantages	Disadvantages
Convenience sample of residual sera	Easy, least labor intensive Similar profile of study participants as in pre-epidemic group Easier to recruit children as the venopuncture is for another purpose. Much easier to do repeatedly over time to measure the decline in population levels of antibody	Potential for bias in case selection because of the profile of individuals from whom the sera were collected.
Random digit dialing	Random selection, less potential for bias	Land-lines are less used than cell phones; cell phones difficult to localize geographically and lists are harder to acquire. Different group from pre-epidemic sera, if residual sera are used for baseline. More challenging to recruit children
Geographical selection	Random, least potential for bias	Most labor intensive Requires more technical capacity, mapping skills More challenging to recruit children because of venopuncture.

ELIGIBILITY CRITERIA

Inclusion Criteria: any individual who gives informed consent or has the consent of a parent will be eligible for inclusion. Children aged 10-18 will be asked to assent.

COMMENT: The age of consent and assent may vary by country. Check with local IRB requirements.

Exclusion criteria:

- Any contraindication to venopuncture (doesn't apply if using residual sera).
- Age less than 6 months (doesn't apply if using residual sera)
- Vaccinated with vaccine known to be cross-reactive with the currently circulating novel virus. (note, these could either be excluded from the study or just excluded from the calculations but in the latter case the sample size would have to be adjusted accordingly.)
- For residual sera studies, exclude those with acute febrile illness.

2.4 SAMPLE COLLECTION TIMINGS: PRE- AND POST-EPIDEMIC SAMPLING

PRE-EPIDEMIC SERA

Ideally, the pre-epidemic sera should be collected before circulation of nCoV is widespread in the population under study (Figure 1). Individuals will not likely develop antibodies to an infective organism within the first 10 days after infection.

POST-EPIDEMIC SERA

Post-epidemic sera collection will be done between 2 and 12 weeks of when circulation of nCoV returns to low levels. As described in Figure 1, there may be situations in which sera collection would be drawn somewhat earlier but this will decrease the estimate of infection rate depending on the collection time in relation to local viral activity.

2.5 SUBJECT RECUIRTMENT AND DATA COLLECTION

For each sampling scheme:

- For each of the three scenarios described in section 2.3, sufficient numbers of samples will be taken to enable age-specific estimates of sero-prevalence for each age stratum: 0 to <2 years; 2 to 5 years; 5 to <15; 15 to <25 years; 25 to <35 years; 35 to <50; 50 to <65 years; and ≥ 65 years. More age strata can be added, increasing the total sample side accordingly, but maintaining these major break points will aid in making comparisons to other studies.
- Samples should be age matched within a ± 6 month window for children under age 5 years, ± 1 year for children 5 – 18 years, and ± 5 years for adults over 18 years, if possible. At a minimum, the number of specimens in each of the age strata above should be approximately equal and analysis carried out on each stratum independently.
- For all three of the selection methods described, the number of people that are requested to participate and the number that refuse will be recorded.

COMMENT: The age strata described are based on the assumption that individuals in each of the strata will generally have similar types and degree of exposures, and similar rates of infection within the strata. As such, they provide convenient strata for matching of controls, however, the local situation may suggest that other strata make more sense epidemiologically or that closer matching is desired. For reporting purposes, these standard age strata are recommended in order to improve the comparability of data with other studies.

CONVENIENCE SAMPLE

Local laboratories that are used by community physicians for routine screening blood tests will be recruited to participate in the study and phlebotomists at each will be trained in administering the questionnaire [alternatively, study personnel could be positioned at each site]. Materials for collection of study data and specimens will be provided to the participating laboratories with appropriate labels for both questionnaires and sera collection tubes.

[alt] An alternative method is to take residual sera after it is drawn for other purposes, however, this would severely limit the information collected with the sample.

RANDOM DIGIT DIALING

Phone numbers in the same geographic area as that from which the pre-epidemic residual sera are taken will be randomly selected for calling.

If the phone is not answered on the first attempt, two subsequent attempts will be made for each randomly selected number at different times of the day. Phone numbers of businesses will be excluded. All attempted calls will be recorded.

When the phone is answered, the person answering will be asked if he/she is over the age of [local age of consent]. If not, the person will be asked to bring and adult to the phone. Only one participant per volunteer household will be selected for participation until the desired sample size is achieved for each age group. Volunteers will be invited to come to the local collection point for the blood draw (alternatively, study personnel could be sent to the home of the participant) and appropriately reimbursed for their travel.

GEOGRAPHIC SAMPLING

Households will be identified as described above from a random sample of GIS coordinates within the area from which the pre-epidemic residual sera are drawn. The households will then be approached in a random order.

When somebody answers the door, they will first be asked whether the respondent is over the age of [local age of consent]. If they are not, they will be asked how many household members who are over the age of consent are present and to name them. A random name will be chosen from those present and interviewers will ask to speak to the selected subject. The study will be briefly described to the responding subject or guardian and a time arranged to return and formally recruit them to the study.

If no one is at home at the first visit, each randomly selected household will be visited 2 additional times at different times of the day and each visit recorded.

INFORMED CONSENT

During the first interview with the volunteer study subject, the purpose of the study will be explained and consent obtained by a trained member of the investigation team. Consent for children less than 18 years or younger will be obtained from their parents. Assent will also be obtained for children 10 to 18 years old.

COMMENT: The age of consent and assent may vary by country. Check with local IRB requirements.

MINIMUM DATASET

After enrollment and informed consent is obtained, we recommend that a standardized minimum data set be collected with any specimens for nCoV testing (and date of specimen collection) including: age, gender, location, exposure (date of first and last known exposures, duration, proximity) to confirmed case-patient, occupation, signs and symptoms, underlying conditions, risk factors (i.e., exposures) for infection and severe disease, a history of the use of respiratory protection, any treatments or other potentially relevant medication.

A template of the study questionnaire for the use of all cases and contacts can be found in Appendix B.

COMPENSATION AND INCENTIVES TO PARTICIPATE

COMMENT: Participants can be offered reimbursement for reasonable out of pocket expenses; however, the level of compensation should not be such that participants are unduly influenced into consenting to participate.

PREVENTION OF NCOV INFECTION IN FRONT-LINE STAFF

Front-line staff including all study personnel will be trained in infection control procedures including proper hand hygiene and the correct use of surgical face masks, if necessary, not only to minimize their own risk of infection when in close contact with patients during home visits and elsewhere, but also to minimize the risk of the personnel acting as a vector of infection between household members or between households.

2.7 SPECIMEN COLLECTION AND LABORATORY EVALUATIONS

The precise test protocols need validation but it is likely that the Haemagglutination-Inhibition (HI) assay and a virus neutralization protocol will be used in seroepidemiological studies of influenza. Serological assays for other novel agents could potentially include additional methods such as Enzyme Linked Immunosorbent Assay (ELISA) or Immunofluorescent antibody assays.

2.7.1 SPECIMEN COLLECTION, TRANSPORTATION

Cut and paste guidance from WHO lab guidance, here:
http://www.who.int/csr/disease/coronavirus_infections/LaboratoryTestingNovelCoronavirus_21Dec12.pdf

Additional records should be kept for each biological sample, including the time of collection, the conditions for transportation and the time of arrival at the study laboratory.

2.7.2 LABORATORY PROCEDURES

VIROLOGIC TESTING

To consider a case as laboratory-confirmed, one of the following conditions must be met:

- positive RT-PCR or other validated molecular assays for at least two different specific targets on the nCoV genome

OR

- one positive RT-PCR assay for a specific target on the nCoV genome and an additional different PCR product sequenced, confirming identity to known sequences of the new virus.

A positive PCR assay for a single specific target without further testing is considered presumptive evidence of nCoV infection. Final classification of cases will depend on clinical and epidemiological information combined with laboratory data. Case definitions can be found at:

Ehttp://www.who.int/csr/disease/coronavirus_infections/case_definition/en/index.html

Member States are requested to immediately notify WHO.

See full details for virologic laboratory testing of nCoV can be found here: http://www.who.int/csr/disease/coronavirus_infections/LaboratoryTestingNovelCoronavirus_21Dec12.pdf.

SEROLOGIC METHODS

COMMENT: The development and validation of serologic assays for nCoV are currently limited but are being pursued by a small number of laboratories across the globe. Here we provide details of the only published serologic testing available for nCoV[1,2], but we are aware of pending publications from Public Health England (formerly the UK Health Protection Agency).

COMMENT: Only a limited number of laboratories have the facilities for nCoV serologic testing and therefore collaboration between countries without current capacity and designated reference laboratories is possible. Collaboration is up to the discretion of member states carrying out the research, but WHO/EMRO strongly support such collaboration and would willingly facilitate collaboration and possible shipment elsewhere for testing.

The following laboratory assay results are currently available for defining a case as nCoV antibody positive and full details can be found in[1,2].

- Screening for antibodies reactive to nCoV by indirect immunofluoresence assay (IFA) described by [1,2]

- It is strongly recommended that confirmatory serologic testing should be done using microneutralization or ELISA-based assays using appropriately timed sera (ideally paired acute and convalescent sera)[1,2]

COMMENT: If appropriately timed and collected paired acute and convalescent sera are collected, a four- fold rise in titer is indicative of seroconversion.

3.0 SAMPLE SIZE, STUDY ENDPOINTS AND STATISTICAL ANALYSES

3.1 SAMPLE SIZE CONSIDERATIONS

The effect of sample size on statistical precision is illustrated in the tables below. With this sample size the 95% CI for the estimation of prevalence within each age group and overall is shown in the table below for various observed prevalences.

COMMENT: If investigators desire to demonstrate either susceptibility or incidence of infection in specific at-risk groups, sampling will need to be done in such a way as to ensure sufficient sample size in the group of interest.

Table 2 95 % confidence intervals as a function of sample size for various observed prevalences

Prevalence	N=200	N=1000
0%	0.0-1.8	0.0-0.4
5%	2.4-9.0	3.7-6.5
10%	6.2-15.0	8.2-12.0
15%	10.4-20.7	12.8-17.4
20%	14.7-26.2	17.6-22.6
25%	19.2-31.6	22.3-27.8
30% 35%	23.7-36.9 28.4-42.0	27.2-32.9 32.0-38.0
40%	33.2-47.1	36.9-43.1
45%	38.0-52.2	41.9-48.1
50%	42.9-57.1	46.9-53.1

To estimate incidence from prevalence, the difference between the prevalence at two time points can be calculated. When combining all age groups this would give reasonable precision for estimating incidence from prevalence as shown in the table 3 below.

Table 3 Precision (95% CI) of change in overall prevalence

Prevalence at time point 1 (P1)	Prevalence at time point 2 (P2)	Difference in P2-P1	95% CI
5	10	5	2.7-7.3
10	15	5	2.1-7.9
15	20	5	1.7-8.3
20	25	5	1.3-8.7
5	15	10	7.4-12.6
10	20	10	6.9-13.1
15	25	10	6.5-13.5

Within each age group the precision would be much lower if incidence were calculated this way. However if incidence is modelled as a function of time and age then this sample size will still give good precision within age groups.

3.2 STUDY OUTCOME MEASURES

The following section discusses the endpoints – that it, what can be measured and calculated using the data that is collected in this study – for the primary objectives, including statistical advice.

3.2.1 PRIMARY OUTCOMES

The following will be assessed as study endpoints corresponding to the study's primary objectives:

- Prevalence of cross-reactive antibodies to nCoV at baseline
- Determine the age-specific cumulative incidence of infection with nCoV in the population.

3.3 STATISTICAL ANALYSES

3.3.1 FOR PRIMARY OBJECTIVES

PREVALENCE OF CROSS-REACTIVE ANTIBODIES TO NCOV AT BASELINE

For both pre-epidemic and post-epidemic samples, participants who are antibody positive as described in section 2.7.2 will be considered seropositive. The prevalence of cross-reactive antibodies (P) to the novel virus at baseline will be determined for each age group and overall using pre-epidemic serologic results, as follows:

P = (number of seropositive cases among pre-epidemic sera / sample size of pre-epidemic sera) X 100%.

CUMULATIVE INCIDENCE OF NCOV

The cumulative incidence of nCoV infection for each age group will be:

CI = number of prevalent non-vaccinated cases in post-epidemic sample – number of prevalent cases in pre-epidemic sample.

ASYMPTOMATIC INFECTION RATE

Individuals in the post-epidemic survey that do not recall having influenza-like illness (ILI) symptoms during the period of the pandemic will be counted as probable asymptomatic infections. The lowest limit of asymptomatic infections is:

Asymptomatic infection rate = number of seropositive cases with no history of symptoms ÷ total number of seroposirive cases

Bivariate associations between risk factors and seroconversion will be determined by chi-square statistics or 2-sided Fisher's exact test and expressed as odds ratios with 95% confidence intervals. Multivariate logistic regression will be used to further analyze the associations.

4.0 REPORTING OF FINDINGS

4.1 REPORTING OF FINDINGS

Any deviations of the study methodologies should also be reported to aid in the interpretation of findings.

5.0 BACKGROUND OF CONSISE

This protocol *Cross-sectional seroprevalence study of novel Coronavirus (nCoV) infection prior and post epidemic periods* was developed by CONSISE, the Consortium for the Standardization of Influenza Seroepidemiology,[4,5] a global partnership aiming to develop influenza investigation protocols and standardize seroepidemiology to inform public health policy for pandemic, zoonotic and seasonal influenza. This international partnership was created out of a need, identified during the 2009 H1N1 pandemic, for better (standardized, validated) seroepidemiological data to estimate infection attack rates and severity of the pandemic virus and to inform policy decisions[4-6].

Recognizing this gap, several institutions including the World Health Organization (WHO), the Public Health Agency Canada (PHAC), European Centres for Disease Control (ECDC), US Centers for Disease Control and Prevention (USCDC), Imperial College London (ICL), UK Health Protection Agency (UKHPA), University of Hong Kong, the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne, Australia, and many other research institutions formed a partnership to develop best practices and standardize influenza seroepidemiological methods. Members of the steering committee are listed in Appendix I. Two global meetings have been held to date, the first in Canada hosted by PHAC in early 2011 and the second in Stockholm Sweden in December 2011 hosted by ECDC, with a third meeting planned for January 2013.

During the December 2011 meeting, it was decided that seven generic detailed protocols should be developed that can be used in pandemic outbreak settings and for routine serologic collection during non-pandemic seasons (Table 4). In doing so, our aim is to adopt a common framework for serological studies, standardize methodology and reporting. With the emergence of novel Coronavirus (nCoV) in the Middle East in 2012[7,8], CONSISE modified their zoonotic and household protocols to create a protocol specific to investigate the epidemiology, serologic and virologic of close contacts of confirmed and probable nCoV patients.

This study protocol was developed by CONSISE as a tool to be modified and adapted to local needs during the event of a human outbreak with a novel respiratory virus, notably nCoV. It was created in consultation with and reviewed by an ad hoc group of technical experts and has undergone preliminary review. Individuals who have reviewed this protocol are listed in Appendix A. We suggest that seroepidemiologic studies which are part of a comprehensive outbreak investigation of contacts, as proposed in this protocol, will be most productive.

Specifically, this protocol *Cross-sectional seroprevalence study of novel Coronavirus (nCoV) infection prior and post epidemic periods* was drafted by CONSISE (Appendix A) members with input from many partners and influenced by the following protocols, shared with CONSISE for the purposes of developing this protocol:

- *Numerous Cross-sectional influenza seroepidemiology protocols from several countries shared with WHO for the following publication:* Estimating Age-Specific Infection Rates for the 2009 Influenza Pandemic: a Meta-Analysis of A(H1N1)pdm09 Serological Studies from 19 countries[8]
- *A seroprevalence study of novel swine influenza A H1N1 among Ontarians.* Crowcroft N, et al. Canada (Public Health Ontario)
- *Retrospective Study of the Sero-prevalence of Human Influenza A (H5N1) among Anonymized Stored Specimens in China. Shared by Yu Hongjie,* Yuelong Shu, (National Influenza Center, China), Sager P [U.S. National Institutes of Health] PIs
- *Protocol for the assessment of baseline age-specific antibody prevalence and incidence of infection to novel influenza A (H1N1)v.* Miller E, Pebody R, Andrews N, Hoschler K, Stanford E (Public Health Protection Agency of England).

Table 4 – CONSISE Protocols Under Development

	Protocol	Primary Objectives
Epidemic/Pandemic	1. Prospective longitudinal cohort study of influenza virus infection during epidemic periods	Determine age specific cumulative incidence of infection during an influenza epidemic
	2. Cross sectional seroprevalence study of a novel influenza virus infection prior and post epidemic periods	Determine age specific cumulative incidence of infection with a novel influenza virus in the population Measure prevalence of cross-reactive antibodies to the novel virus
	3. Household transmission studies for pandemic influenza	Estimate household secondary infection risk, and factors associated with variation in the secondary infection risk Characterize secondary cases including clinical presentation and asymptomatic fraction Investigate serological response following confirmed influenza infection
	4. Closed setting outbreak investigation protocol for pandemic influenza	Describe the clinical spectrum of infection including the asymptomatic fraction Estimate overall clinical attack rates (by subgroup and clinical risk group) Describe correlation between infection, disease and serology
	5. Assessment of Health Care Personnel	Detect the presence of human-to-human transmission of a novel virus within a health care setting
Seasonal Influenzas	6. Seroepidemiology of human influenza virus infection using residual sera/convenience samples for establishing baselines and/or monitoring trends over time	Estimate population immune status/susceptibility to relevant influenza viruses Estimate incidence in previous-seasons for the different relevant influenza viruses
Zoonotic Influenzas	7. Investigation of Zoonotic Influenza Infection in Humans	Measure age-specific infection in relation to zoonotic exposure Identify (modifiable) risk factors for human infection

Source: [4]

Questions about the generic protocol should be directed to m.vankerkhove@imperial.ac.uk, while questions related to the country-specific protocols for which this protocol was based on should be directed to the contact points mentioned for those protocols.

We hope you find this protocol helpful.

CONSISE
CONSORTIUM FOR THE STANDARDIZATION OF INFLUENZA SEROEPIDEMIOLOGY

www.CONSISE.tghn.org

REFERENCES

1. Buchholz U, Müller MA, Nitsche A, Sanewski A, Wevering N, Bauer-Balci T, Bonin F, Drosten C, Schweiger B, Wolff T, Muth D, Meyer B, Buda S, Krause G, Schaade L, Haas W. Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October-November 2012. Euro Surveill. 2013;18(8):pii=20406. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20406 2013.

2. Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, Kreher P, Lattwein E, Eschbach-Bludau M, Nitsche A, Bleicker T, Landt O, Schweiger B, Drexler JF, Osterhaus AD, Haagmans BL, Dittmer U, Bonin F, Wolff T, Drosten C. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill. 2012;17(49):pii=20334. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20334> 2012.

3. Laurie KL, Huston P, Riley S, Katz JM, Willison DJ, Tam JS, Mounts AW, Hoschler K, Miller E, Vandemaele K, Broberg E, Van Kerkhove MD, Nicoll A. Influenza serological studies to inform public health action: best practices to optimise timing, quality and reporting. Influenza and Other Respiratory Viruses 2012;doi: 10.1111/j.1750-2659.2012.0370a.x Online 30 April 2012.

4. Van Kerkhove MD, Broberg E, Engelhardt OG, Wood J, Nicoll A, on behalf of the CONSISE steering committee. The Consortium for the Standardization of Influenza Seroepidemiology (CONSISE): A Global Partnership to Standardize Influenza Seroepidemiology and Develop Influenza Investigation Protocols to Inform Public Health Policy. Influenza and Other Respiratory Viruses 2012;Published online: 2012 Dec 26. doi: 10.1111/irv.12068. [Epub ahead of print].

5. WHO. Seroepidemiological studies of pandemic influenza A (H1N1) 2009 virus. Weekly Epidemiologic Record 2010;24:229-236.

6. WHO. Novel coronavirus infection in the United Kingdom, 23 September 2012; Available at: http://www.who.int/csr/don/2012_09_23/en/index.html. 2012.

7. Albarrak AM, Stephens GM, Hewson R, Memish ZA. Recovery from severe novel coronavirus infection. Saudi Med J 2012;33(12):1265-9.

8. Van Kerkhove MD, Hirve S, Koukounari A, Mounts AW, for the H1N1pdm Serology Working Group. Estimating Age-Specific Infection Rates for the 2009 Influenza Pandemic: a Meta-Analysis of A(H1N1)pdm09 Serological Studies from 19 countries. Influenza and Other Respiratory Viruses *in press*.

APPENDIX A PROTOCOL AUTHORS AND REVIEWERS

Dr. Anthony W Mounts
World Health Organization
Geneva, Switzerland

Dr. Maria Van Kerkhove
MRC Centre for Outbreak Analysis and Modelling
Department of Infectious Disease Epidemiology
Imperial College London
London, United Kingdom

Dr. John Wood (retired)
Division of Virology
National Institute for Biological Standards and Control,
United Kingdom

Dr. Othmar Engelhardt
Division of Virology
National Institute for Biological Standards and Control,
United Kingdom

Dr. Angus Nicoll
Influenza and Other Respiratory Viruses Program
European Centres for Disease Prevention and Control

CONSISE STEERING COMMITTEE

CONSISE's steering committee is composed of individuals (Table A1) from several organizations including the World Health Organization, the US Centres for Disease Control and Prevention, the European Centres for Disease Prevention and Control (ECDC), Public Health England (Formerly the UK Health Protection Agency), Imperial College London, the WHO Collaborating Centre for Reference and Research on Influenza (Melbourne, Australia), University of Hong Kong, Oxford University Clinical Research Unit in Hanoi, and Public Health Agency of Canada.

Table A1 CONSISE Steering Committee Members

Name	Institution
Angus Nicoll Eeva Broberg	European Centres for Disease Control
John Wood Othmar Engelhardt	NIBSC, Medicines and Healthcare Products Regulatory Agency, UK
Maria Van Kerkhove Steven Riley	MRC Centre for Outbreak Analysis and Modelling, Imperial College London, UK
Anthony Mounts Wenqing Zhang	World Health Organization
Karen Laurie	WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia
Jackie Katz Tim Uyeki	US Centres for Disease Control and Prevention, Atlanta, United States
Malik Peiris Benjamin Cowling	The University of Hong Kong, School of Public Health , Department of Community Medicine , Hong Kong
Katja Hoeschler Richard Pebody	Public Health England, London, UK
Peter Horby	Oxford University Clinical Research Unit in Hanoi, Vietnam
Monique St-Laurent	Public Health Agency Canada
Marianne van der Sande	National Institute for Public Health and the Environment (RIVM), the Netherlands
Olav Hungnes	Norwegian Institute of Public Health, Norway

APPENDIX B DATA COLLECTION FORM

The following questionnaire should be used for all subjects included in the above listed investigation. This questionnaire is separated into sections: General questions, Exposure questions, Symptom and Course of Disease questions and Background Demographic questions and represent the minimum questions that should be asked to all subjects. We encourage the user to keep these sections in the recommended order and to add additional exposure questions that are relevant to your cultures, situation, contexts, and understanding of the situation in your country.

Each subject should be allocated a unique identification number.

COMMENT: Once questionnaire is finalized, full instructions and skip patterns should be added. Comments throughout the questionnaire are highlighted with purple text.

COMMENT: Note that adding multiple choice answers will allow for easier data analysis.

SECTION 1 GENERAL QUESTIONS

General questions

1.1. What is your full name: _____

COMMENT: Many countries may have numeric identifiers and if possible and permission is obtained, please collect.

1.2. What is your relationship to the patient/case?

COMMENT: You could list the possible choices including different types of relationships, servants such as cleaners, cooks, drivers, etc. This should be a multiple choice question.

1.3. Place of primary residence: _____

1.3.1. Do you have homes elsewhere? Y/N

1.3.2. If yes, please specify where:

1.4. Did you spend any time with the confirmed nCoV case-patient in the 10 days before symptom onset (or specify time period here)? Y/N

COMMENT: If possible, identify the confirmed nCoV patient by name or other identifier.

SECTION 2 EXPOSURE QUESTIONS

COMMENT: Exposure history should be focused on a specified time period before the symptom onset of the nCoV case-patient. If the subject is a case, then exposure should be focused 10 days prior to symptom onset or a time period should be specified.

RECENT TRAVEL HISTORY AND ANIMALS ENCOUNTERED

The following questions relate recent travel history and the animals you encountered during these travels.

Recent Travel History

1.1 List the areas within the country you travelled to between (specify time period here).

_____

_____

_____

List all countries you travelled to between (specify time period here).)

1.2. Country Name:

1.2.1. Dates Travelled: _____ to _____

1.3. Country Name:

1.3.1. Dates Travelled: _____ to _____

1.4. Country Name:

1.4.1. Dates Travelled: _____ to _____

1.5. Country Name:

1.5.1. Dates Travelled: _____ to _____

1.6. Country Name:

1.6.1. Dates Travelled: _____ to _____

Animal exposures during these travels

1.7. Did you have contact (touching) with domestic or wild animals during any of these travels?

[Be more specific questions based on the activities the family participated in there]

1.8. Did you physically touch an animal of any kind during these trips?

If yes, what kind of animal(s) _____ and in which country: _____

If yes, what kind of animal(s) _____ and in which country: _____

If yes, what kind of animal(s) _____ and in which country: _____

If yes, what kind of animal(s) _____ and in which country: _____

If yes, what kind of animal(s) _____ and in which country: _____

1.9. Did you visit a market selling live animals? Y/N

1.10. Did you physically touch an animal of any kind at these markets? Y/N

If yes, what kind of animal(s) _____

1.11. Did you visit any other venue at which live animals were present? Y/N

(COMMENT: specify examples depending on the country: e.g., farm, camel race or falconry events)

1.11.1.1. If yes, please specify venues_____

1.11.1.2. Please specify any animals you touched.

1.11.2. Did you eat anything while at these events?

1.11.2.1. Event 1:_____ Food consumed: _____

1.11.2.2. Event 2:_____ Food consumed: _____

1.11.2.3. Event 3:_____ Food consumed: _____

1.11.2.4. Event 4:_____ Food consumed: _____

1.11.3. Did you have direct contact with animals (either alive or dead?) while there? If yes, what type of animals?

1.11.3.1. Did you touch any items such as fences, textiles, or other physical objects that may have had contact with animals while there? If yes, please specify.

1.11.3.2. Did you have contact with any body fluids, secretions, or excrement of animals o while there? If yes, please specify.

FOOD EXPOSURES

The following series of questions are focused on food exposures in the 10 days prior to the case-patient's symptom onset (or specify time period).

1.12. Did you eat any of the following food items raw, that is uncooked? (Answer Yes/no)

1.12.1. fresh fruits, if yes, specify type

1.12.2. dried fruits, if yes, specify type

1.12.3. vegetable , if yes, specify type

1.12.4. salads, if yes, specify type

1.12.5. other? Please specify _____

1.13. Did you drink fresh (i.e. not canned or processed) fruit juices? If yes, please specify.

1.14. Did you eat any uncooked meat?

1.14.1. If yes, specify species consumed

1.14.2. If yes, specify body part consumed (e.g., flesh, blood, etc)

1.15. Did you drink any unpasteurized milk? Y/N

1.15.1. If yes, specify from which species

ANIMAL EXPOSURES IN AND AROUND THE HOME

The following questions address animal exposures during the period of a few days before the patient's illness until a few days after he went into hospital.

COMMENT: specify time period

Animal husbandry

2.1. Were any pets, including work animals or hunting animals, kept in or around your home during this period?

2.1.1. If yes, what kind and how many?

2.1.2. Were you aware of any other animals present in or outside around your house during this time (e.g. bats, rodents)?

2.1.3. Did you notice any animal feces or urine in or outside around your home during this time? Y/N

2.2. Did you have contact (touch) with domestic or wild animals? Y/N

2.3. Did you physically touch an animal of any kind?

2.3.1. If yes, what kind of animal(s) _____

2.4. Did you visit a market selling live animals?

2.5. Did you visit any other venue at which live animals were present (e.g. farm, camel race or falconry events?

2.6. Did you visit the case-patient's farm?

If yes,

2.6.1. Did you get out of the car while there?

2.6.2. Did you eat anything while there? If yes, please specify

2.6.3. Did you have direct contact with animals while there?

2.6.4. Did you touch any items such as fences, textiles, or other physical objects that may have had contact with animals on the farm?

2.6.5. Did you have contact with any body fluids, secretions, urine or excrement of animals on the farm while there?

Questions for people who live on a farm (Skip if subject does not live on a farm)

2.7. What is your job on the farm? _____

2.8. How many years have you worked on the farm?

2.9. Do you sleep at the farm?

2.10. Do you have physical contact with animals on the farm?

2.10.1. If yes, how often? Many times/day, daily by not many times, less than daily

2.11. Do you wear any protective equipment while working on the farm? Y/N

2.11.1. If yes, what do you wear?

2.11.2. How often do you wear protective equipment? Always/sometimes/Never

2.12. Do you have contact with body fluids, secretions, or excrement of animals on the farm?

2.13. Have you personally been involved with the slaughter or sacrifice of an animal on the farm during the period [some period just before onset of illness of the patient]

2.14. Do you consume any products from the farm (e.g. milk, meat)?

2.14.1. If yes, please specify____ were these cooked/uncooked? _____

2.14.2. If yes, please specify____ were these cooked/uncooked? _____

2.14.3. If yes, please specify____ were these cooked/uncooked? _____

2.15. Have you ever seen bats at the farm? If yes, please state when and where

Other Animal Exposures

2.16. Do you own any animals as pets?

2.16.1. If so, what species and how many? _____

2.16.2. Do you care for these pets? _____

2.16.2.1. If so, how do you care for these pets? _____

2.17. Do you participate in any animal sports racing or watching?

2.17.1. If so, what animals (E.g., camel racing)? _____

SECTION 3 SIGNS, SYMPTOMS AND COURSE OF DISEASE QUESTIONS

The following questions address the symptoms you experienced during (specify time period of interest). If the subject had no symptoms, skip to section 4.

3. Did you experience any signs or symptoms during (specify time period of interest)? If the subject had no symptoms, skip to section 4.

3.1. Did you have any of the following signs/symptoms in the last six months?

3.1.1.	Dry Cough	0 Yes	0 No	0 Don't know	
3.1.2.	Productive Cough	0 Yes	0 No	0 Don't know	
3.1.3.	Phlegm	0 Yes	0 No	0 Don't know	
3.1.4.	Runny nose	0 Yes	0 No	0 Don't know	
3.1.5.	Sore throat	0 Yes	0 No	0 Don't know	
3.1.6.	Fever ____°C	0 Yes	0 No	0 Don't know	if yes, max. temp:
3.1.7.	Shortness of breath	0 Yes	0 No	0 Don't know	
3.1.8.	Muscle pain	0 Yes	0 No	0 Don't know	

3.1.9.	Diarrhea	0 Yes	0 No	0 Don't know
3.1.10.	Chest Pain	0 Yes	0 No	0 Don't know
3.1.11.	Vomiting	0 Yes	0 No	0 Don't know
3.1.12.	Rashes	0 Yes	0 No	0 Don't know

3.2. Did you seek medical care? Y/N

3.2.1. If yes, what kind of medical care did you seek?

COMMENT: Add multiple choice for options – outpatient care, GP visit, etc

3.3. Where you hospitalized during the course of your illness?

3.3.1. If yes, when were you hospitalized (DD/MM/YYYY): ____/___/_____

3.3.2. If yes, which hospital did you receive treatment(s)? _____

SECTION 4 BACKGROUND

The following questions are addressing your background medical history and other background questions.

Health

4.1. Is there any hereditary disease running in your family? Y/N please specify

4.2. Do you have any underlying chronic diseases (eg diabetes): _____/

4.2.1. If yes, how long/what age where you diagnosed?

4.3. Do you have any known allergies: _____

4.4. What medications do you regularly take: _____

4.5. What medications do you sporadically take: _____

4.6. If female, are you pregnant? Y/N

4.6.1. How many weeks?

4.7. If female, are you post-partum? Y/N

4.7.1. How many weeks post-partum?

Personal living situation

4.8. What is your current family status? (single, married, living with a partner, other…)

4.9. Do you live alone?

4.9.1. If no, do you live together with someone else?

4.9.2. If yes, whom do you live with? (wife/wives, children, other relatives, …)

4.10. Do you have servants?

If yes,

4.10.1. how many?

4.10.2. for what kind of service do they provide? Please specify

4.10.3. do they live in your house?

4.10.4. what nationality are they?

4.11. What type of dwelling do you live in? Apartment, detached house, other, please specify

4.11.1. Do you have air-conditioning in your house?

Relationship to other nCoV patients

4.12. Do you know any of the other novel Coronavirus case-patients?

4.12.1. If yes, whom?

4.12.2. What was your relationship to them?

4.12.3. Is there anything you can think of that you have in common with the other novel Coronavirus patients?

4.13. What is your profession: _____

4.13.1. How long have you been in this profession? ____ years

Other Questions

4.14. Do you practice any sports?

4.15. Do you own a helicopter?

4.16. Do you own a car?

4.17. Do you currently smoke: _____

4.17.1. If yes, for how many years?

4.17.2. If yes, what do you smoke? _____

4.17.3. If yes, how many cigarettes (or other) per day do you smoke

4.18. Did you previously smoke?

4.18.1. If yes, for how many years?

4.18.2. If yes, what did you smoke? _____

4.18.3. If yes, how many cigarettes (or other) per day did you used to smoke?

4.19. Do you consume alcoholic beverages?

4.19.1. if yes: how much and what beverage?: Daily/weekly/monthly?

4.20. Are you using any illicit drugs?

4.20.1. if yes: which drugs: how much?

4.21. How old are you:

4.21.1. What is your date of birth (DD/MM/YYYY): ____/___/_____

4.22. What is the highest education level you finished? (add mult choice)

4.23. What is your household income level (provide ranges and circle best fit)?