

## Annex 1: Protocol template

### Study protocol template

Primary investigator: Name, First Name, Institution

Co-investigator: Name, First Name, Institution

Laboratory Name: Contact person name

Organisation responsible for fieldwork: Contact person name

Version: date

### Background (introduction and justification)

The introduction must contain key background information that sets the stage for the survey question. It describes what is known about the situation relating to the control of disease in the country or region. Describe what information is unclear, not yet published, or otherwise unavailable. This background should lead to a justification for the survey and explains the research question. References to support the justification for the survey should be provided in this section.

*First paragraph of the introduction:* Review the background from a global perspective, including the global public health consequences in terms of death and disability and compare with the effectiveness and cost-effectiveness of interventions. Avoid generalized and unsupported statements. Provide quantified data when available. For example, rather than saying “Disease X is a major public health problem”, quantify the burden in terms of Disability Adjusted Life Years (DALYs).

*Second paragraph of the introduction:* Explain how the current situation may affect the nation and surrounding regions. Describe any issues particular to the population to be studied.

*Third paragraph of the introduction:* Describe how the situation presents itself in the national and local context. Provide information on completed, ongoing or planned prevention and control efforts to address this situation in the region or nation where the study will be conducted. Specify the data needed for effective prevention and control to address the situation – for example, vaccination coverage and disease surveillance trends over time, their variation by age group and region within the country, and why the currently available data is not sufficient. End the paragraph with the research question that the study will address.

### Proposed methods

#### Objectives

- Specify the primary objective, clearly stating the aim of the survey is to estimate seroprevalence and in how many strata, or to classify seroprevalence as above or below a certain threshold.
- Determine whether any comparisons of seroprevalence will be made (e.g. between different regions or provinces) and whether these are primary objectives for which sample size will be calculated.

- Specify any other objectives of the survey, if applicable.

## Survey population

Describe the population in which the survey will be conducted (country, state, district, population size) and specify inclusion and exclusion criteria.

## Study design

Describe the inferential goals of survey that will be conducted (estimating, classifying or comparing). Describe how specimens will be collected and whether new or existing specimens will be used. Note if participants will be recruited prospectively or retrospectively.

## Operational definitions

Define the criteria that will be used for key exposures and outcomes, how this will be measured, e.g. coverage by card or by card and recollection. Also need to define other critical operational aspects such as how seropositive, equivocal and seronegative results will be determined, i.e. which cut-off will be used. Cite references to any methodological guidelines used.

## Population sampling procedure

Describe the type of sample that will be used (simple random sample, systematic sample, cluster sample, stratified cluster sample). Describe the step-by-step procedure that will be used to select that sample.

## Sample size

Explain how the sample size was decided and clarify any assumptions used in the calculation and adjusted for non-response and design effect, if applicable. Make explicit reference to the software and/or the formulae used for the calculation.

## Data collection

### *Information collected*

Describe the information that will be collected through the questionnaire by providing an overall summary of the broad categories of items (demographic characteristics, socioeconomic status, vaccination history, travel history). There is no need to provide a detailed list of questions.

### *Data collection procedure*

Explain who will collect the data and the methods used (i.e. household survey, clinical records). Describe the instruments that will be used to collect information and provide details of these instruments in an annex.

### *Laboratory specimen collection, transport and analysis*

Describe the methods to be used for biological specimen collection, transport and analysis.

### *Other methods used to collect data*

Describe any other methods you plan to use to collect data and provide references as applicable.

## Data analysis

Describe the steps that will be followed for the data analysis, including:

- recoding of key exposure or outcome variables;
- indicators to be calculated for the descriptive epidemiology (seroprevalence);
- indicators to be calculated for the analytical epidemiology (hypothesis test to compare prevalence among different demographic or geographic groups);
- key main stratifications that are anticipated (e.g. stratifying by vaccination status and by age group);
- statistical software used;
- key shell tables and figures added to an appendix; and
- describe any modelling envisaged and collaborations established to do that modelling.

## Quality assurance

Describe the quality assurance procedures that will be used for:

- protocol development (e.g. peer review);
- field procedures (e.g. sampling methods);
- data collection methods (e.g. pilot testing, training of fieldworkers, translations, field supervision, cross checking);
- assays selection and laboratory methods (e.g. assay validation, SOP, training, EQAS, run control)
- data analysis;
- supervisory methods including numbers of supervisors per field team; numbers of external monitors; overall and laboratory coordination;
- use of GPS to log activities of field teams and supervisors; and
- automation of data transfer (e.g. using barcodes for specimen tubes).

## Bias and limitations

Enumerate the possible sources of bias and limitations of the proposed survey design and implementation. For each of these biases and limitations, describe:

- the nature of the bias and/or limitation
- possible consequences of the limitation on the data (e.g. over/underestimation of a parameter)
- steps taken to minimize the impact of the bias and/or limitation on the study.

## Ethical clearance

### *Vulnerable populations*

Note whether a vulnerable population will be studied. Such populations may include pregnant women, children or prisoners. Give adequate justification for including these populations.

### *Risks*

List the possible risks that participation in the survey may expose the participants. Do not downplay risks.

### *Benefits*

List the possible benefits that the participants or the community could receive through participation in the survey. Do not exaggerate benefits. Mention if a reasonable compensation will be given for participation (avoiding undue or inappropriate incentives), if results will be given to each individual participant and if vaccination will be offered to seronegative individuals.

### *Confidentiality*

Describe the practical steps taken to protect the confidentiality of survey subjects, such as use of de-identified codes or protection of identifying information.

#### *Biological specimen*

List the biological specimens that may be collected and how they will be used. Specify the duration of storage and how remaining specimens will be managed and/or disposed. Ensure that these proposals match the ethics approval.

#### *Informed consent*

Describe the procedures used to obtain consent from survey subjects and the key elements that will ensure that the consent will be fully informed. If informed consent is not needed for this survey, explain why.

#### *Ethical committee clearance*

Determine whether the protocol requires full ethical committee review, expedited review or no review because the protocol is exempt (e.g. programme evaluation). If ethical committee review is needed, specify the committee from which approval will be sought.

The protocol needs to specify what will be done with the dataset and with laboratory samples after completion. Who will be responsible for storing and access these? Define the public sharing of the dataset, e.g. by sharing with WHO.

### **Practical considerations**

#### *Fieldwork*

Describe practical arrangement for the fieldwork (e.g. logistics).

#### *Timeline*

Provide a timeline with the key milestone, best presented as a Gantt chart.

### **Communication of Results**

The protocol should describe what steps will be taken to communicate results to the different stakeholders including communities. Describe the different kind of report – executive report briefly summarizing key outcomes; technical reports for funders, implementers and survey partners; governmental reports for ministries of health, lay reports for peripheral health workers and communities.

### **Budget**

Detail the summary budget outlining proposed expenditure by presenting key activity expenditure items such as labour costs, capital equipment, consumable costs, laboratory testing, logistics, legal and specialist fees, overheads etc. The proposed budget should incorporate all expected expenditure and contain contingencies for unforeseen occurrences. Any assumptions associated with the budget should be documented for future reference.

### **Annexes**

A protocol is considered complete and can be submitted to an ethical committee only if it includes annexes that contain shell tables, instruments, consent forms and other information necessary to understand how the survey and analysis are to be conducted.

## **Data collection instruments**

All data collection instruments and consent forms associated with study subjects must be available in local language with an English back-translation.

### *Other data collection instruments*

Attach non-questionnaire data collection instruments that will be used (e.g. tools used for supervision and quality control chart abstraction forms or observation records as appropriate).

### *Identifier collection sheet*

Attach the identifier collection sheet that will be used to connect identifying information to the codes on each questionnaire and specimen label.

## **Informed consent form**

Attach a consent form based on a standard template, checking that it contains all items on the WHO checklist. Ensure that the consent form is in plain language and does not contain any jargon. Avoid unfamiliar or technical words. If such words are unavoidable, define them in a glossary.

## **Others**

Attach any other forms or documents required for the completion of the study.

## **References**

Include a list of references to support key points made in the introduction and provide additional information or documentation about specific methods adopted.

## **Annex 2: Primary roles of the survey coordinator, field supervisor and laboratory supervisor**

### **Survey Coordinator**

The survey coordinator has authority over all the people involved in the survey and has direct access to the survey commissioning authorities. The coordinator is responsible for:

- overseeing the implementation of the survey
- ensuring the cooperation of relevant government agencies
- providing the sampling frame and facilitating the selection of enumeration areas and households
- making budget estimates prior to identification of sources of funds for the survey
- ensuring that pre-survey assessments of the field and laboratory capabilities are conducted
- obtaining ethics committee approval
- ensuring procurement and customs clearance of all supplies
- selecting field supervisors,
- select the laboratories and approve the assay(s) and logistics
- periodically review fieldwork and laboratory work
- reporting survey results.

### **Field Supervisor**

Under direction of the survey coordinator, the field supervisor is responsible for:

- explain the purpose of the survey to local health and administrative facilities
- ensuring that field staff are fully familiar with their task
- providing the team member with the necessary materials for their daily activities
- ensuring that collection and handling of specimens are collected according to the protocol
- ensuring all potentially infectious materials (sharps, syringes, used wipes) are disposed of correctly
- ensuring that forms are fully completed and data checked before leaving the survey area
- giving the completed data collection forms to those responsible for data processing
- confirming specimens are labelled, packaged and transported to the laboratory according to the protocol
- ensuring the welfare and security of the team members.

### **Laboratory coordinator**

Under direction of the survey coordinator, the laboratory coordinator is responsible for:

- conducting pre-survey assessments of laboratory capabilities
- establishing and communicating roles and relationships if more than one laboratory is involved
- selecting, validating and approving assays and sample types as required
- ensuring appropriate quality assurance to monitor laboratory performance
- ensuring the competency of laboratory staff
- reviewing equipment validation
- procure materials and reagents to undertake planned activities

- review and approve protocol for the processing of specimens, including rejection criteria
- ensuring that laboratory testing and reporting is carried out according to the protocol
- reviewing results for completeness and checking for errors before analysis
- referring specimens for further testing according to instructions or storage for later use; and
- ensuring the safety and security of laboratory staff.

## Annex 3: Collection, storage and shipment of specimens for measles and rubella serosurveys

### List of blood collection materials (per person)

- 2 x alcohol swabs
- 2 x sterile gauze
- 1 pair of gloves
- Tourniquet
- Steel needle or butterfly:
  - For adults: 18–20 gauge
  - For children: 21–24 gauge
- 1 x syringe
- Vacutainers: 5-10 ml for older children and adults and 2.5 - 5 ml for younger children
  - Red capped vacutainers: serum needs to be pipetted into cryovials after centrifugation
  - Gel separator vacutainers: serum is separated in the vacutainer after centrifugation and can be transported without pipetting
- Pre-printed adhesive labels with unique identifying code for tubes (one series of 10 barcodes per individual)
- Needle disposal container, waste bags
- Cold box and ice packs

### Specimen types for serosurveillance

The most common specimen collected for serosurveys is **whole blood**, collected by venepuncture. Depending on the specific conditions of the country, alternative sampling methods, such as **dried capillary bloodspots** on filter paper, or **oral fluid** or could be used. Below are guidelines for the collection and handling as well as the storage and shipment of these three specimen types.

#### Whole blood

##### *Collection, handling, transport of blood samples and separation of serum:*

Blood should be collected by venepuncture in a sterile tube (at least 5 ml of whole blood for older children and adults and at least 2.5 ml for infants and younger children) and labelled with in the manner described in the protocol, but including the collection date. Tubes must be placed in upright position. A laboratory request form should be completed at the time of specimen collection and must accompany all specimens sent to the laboratory.

Whole blood can be stored at for up to 48 hours before the serum is separated, but it must not be frozen as red blood cells will lyse, causing haemolysis.

Whole blood should be allowed to clot and then centrifuged at 1000 × g for 10 minutes to separate the serum. If there is no centrifuge, the blood should be kept in a refrigerator (2–8°C) until there is complete retraction of the clot from the serum (no longer than 24 hours).



The serum should be carefully removed with a fine-bore pipette to avoid extracting red cells and transferred aseptically to a sterile labelled vial with the patient's name or identifier, date of collection and specimen type. Use 1 single-use pipette for each person's blood.

#### *Storage and shipment of sera:*

Separated serum should be stored at 2–8°C for a maximum of 7 days or until shipment takes place. Serum specimens stored for longer periods must be frozen at –20°C or lower and transported to the testing laboratory on dry ice. Repeated freezing and thawing should be avoided as this may have detrimental effects on the stability of IgG antibodies.

As a general rule, serum specimens should be shipped to the laboratory as soon as possible. Do not unnecessarily delay shipment in order to wait for the collection of additional specimens. Uniquely labelled serum specimens should be placed in sealable plastic bags or pouches containing absorbent materials such as cotton wool to soak up any leakage that may occur. Styrofoam boxes or an insulating (vacuum) flask should be used to contain the sealed bags or pouches. The specimen form and investigation form for each specimen should be placed in a separate plastic bag and taped securely to the inner surface of the top of the Styrofoam box or on the outside of the vacuum flask.

If using ice packs, make sure they are frozen. Place them at the bottom and along the sides of the Styrofoam box. The samples should then be placed in the centre and more ice packs placed on top.

A shipping date should be arranged between the sample collectors, logistics personnel and the laboratory. When arrangements have been finalized, the receiver should be informed of the time and manner of transportation.

More details on the packaging and transportation of samples refer to the Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital Rubella Syndrome (1).

### **Dried blood spot**

#### *Collection and handling:*

Clean each subject's finger (or heel in the case of very young children) with alcohol swab and prick with a sterile, disposable microlancet. Collect up to four drops of whole blood on standardized filter paper (such as Whatman Chromatography paper no 3, Schleicher and Schuell #903, or other high-quality paper).

The filter paper should be marked, either by hand or laser printer, in a standard format that includes 10–15 mm circles within which blood drops are placed. Spaces to write the unique identifier code of the subject, with a space provided to write the laboratory or specimen number should be marked on the paper.

Subject unique identifier: .....
Subject information: .....
<div style="border: 1px solid black; padding: 5px; min-height: 20px;">Laboratory Number:</div>
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="border: 1px solid black; border-radius: 50%; width: 40px; height: 40px; margin: 5px;"></div> <div style="border: 1px solid black; border-radius: 50%; width: 40px; height: 40px; margin: 5px;"></div> <div style="border: 1px solid black; border-radius: 50%; width: 40px; height: 40px; margin: 5px;"></div> <div style="border: 1px solid black; border-radius: 50%; width: 40px; height: 40px; margin: 5px;"></div> </div>
Date of collection: ...../...../.....

The filter paper should be allowed to thoroughly dry for at least 60 minutes at room temperature. Filter papers may be placed in a slide holder or similar receptacle during the drying process.

#### *Storage and shipment:*

Each dried filter paper should be wrapped individually in paper, foil or plastic to prevent possible cross contamination. Filter papers should be stored out of sunlight, inside a plastic bag to protect from dust and moisture. Dried blood spot samples are not considered biohazardous and can be shipped from the site of collection to the laboratory without special requirements or special documentation. Although dried blood spot samples do not need to be kept refrigerated or frozen during transport, it is advisable to store in a cool place and transport to the laboratory as soon as possible.

### **Oral fluid**

#### *Collection and handling:*

Crevicular fluid exuded from the interface between the gums and teeth contains fluid that has low levels of immunoglobulin. A number of swab collection devices (such as the Orocol) have been developed specifically to collect these fluids from the mouth. Follow the specific instructions provided by the device manufacturer. The swabs are designed to be used like a toothbrush and should be rubbed along the gum until the swab is wet. This usually takes about one minute. The wet swab should be placed inside the clear plastic transport tube that has a label to write the identifying code for the subject and the collection date.

#### *Storage and shipping:*

Once a sample has been collected, seal the device according to manufacturer's instructions. If the daily ambient temperature is below 22°C, samples should be shipped to the laboratory within 24 hours. At higher temperatures, samples should be kept in a refrigerator (2-8°C) until they are shipped to the laboratory chilled. The samples are usually not considered biohazardous and can be shipped from the site of collection to the laboratory without special requirements or special documentation.

**Note:** Use of dry blood spot, oral fluid or any specimen type not specified by the assay manufacturer, must be validated prior to use in the serosurvey. Generally, these specimen types obtain lower levels of serum and therefore change the sensitivity and specificity of the assay. This may cause the seroprevalence to be underestimated.

## Annex 4: Calculation and use of survey weights

To make appropriate population level estimates of seroprevalence and to estimate meaningful prevalence confidence intervals, it is necessary to use estimation methods that incorporate survey weights. Survey weights are based on the probability of selecting each unit in all stages. This annex introduces the principle of survey weights and the information required to calculate them but will not give adequate instructions to guide the calculations. Consult with a statistician to calculate survey weights.

A survey respondent's survey weight quantifies the number of eligible respondents in the population who are represented in the study. The weight is calculated using the probability that the respondent was selected to participate in the survey. If the sample design is stratified, the dataset will include a variable to identify from which stratum each sample was collected. If cluster sampling was used, the dataset will include a variable to identify from which cluster each sample was collected.

Survey weight calculation is usually a multi-stage calculation:

- What was the probability the cluster was selected?
- Given that the cluster was selected, what was the probability that the respondent's household was selected?
- Given that the household was selected, what was the probability that the respondent was selected?

Once the full multi-stage probability has been calculated, the weight is the inverse of the probability.

***Example: Calculate the sampling weight for an individual with these three probabilities:***

- Probability that the cluster was selected =  $1/1000$
- Given that the cluster is selected, probability that this HH was selected =  $1/22$
- Given that this HH is selected, probability that this respondent was selected =  $1/7$

Probability (P) that this respondent was selected into the sample for this stratum:

$$P = 1/1000 \times 1/22 \times 1/7$$

$$P = 1/154,000$$

***Sampling weight for this individual =  $1/P = 154,000$ .***

The weights are sometimes adjusted to account for nonresponse of some selected respondents. The members of the population who those respondents would have represented are re-assigned to respondents who did respond. In other situations, the nonresponse is handled with other missing data methods. Consult with a statistician when drafting an analysis plan to select an appropriate method to document and adjust for nonresponse in the survey.

The weights are sometimes further adjusted to adjust to the known stratum-level sum or national-level sum of the number of eligible respondents. This is called post-stratification. For example:

- If the sum of eligible respondents in the country is 25,890,000 (e.g. from census information), then the sampling weights can be scaled to that figure.

- If the ratio of males and females are known, the weights can be adjusted to the appropriate ratio.
- If the populations of eligible respondents in regional strata are known, then the weights can be adjusted so the regions are represented proportionally in the calculations. This is helpful if, for some reason, they are not represented proportionately in the survey dataset.

**Consult with the sampling statistician to decide whether these adjustments are possible and appropriate for your study.**

## Annex 5: Proposed report outline

Generally, the report will follow the same format as the protocol. Where there have been deviations from the protocol, a description of the deviation and the reasons why the deviation was necessary is required.

### 1. High level executive summary

### 2. Historical background section

- The EPI, including vaccination schedule(s) that cover all birth cohorts targeted by the survey and health sector in the country
- Any recent changes in the national immunization programme, such as the introduction of new vaccines or changes in delivery strategy
- Any recent changes in the health sector, such as the introduction of universal health insurance
- Summary of recent administrative coverage data or disease outbreak description
- Summary of SIAs conducted in the country (year, age group, vaccines included, coverage achieved) including the dates of the most recent SIA
- Summary of results for previous vaccination coverage surveys or serosurveys
- Justification for this serosurvey

### 3. Serosurvey objectives

- Primary and secondary

### 4. Serosurvey methods

- Sampling
  - target population and exclusions
  - sampling frames
  - sample size calculations
  - selection methods at each stage.
- Profile of implementing personnel
- Training and piloting
- Fieldwork (data collection tools)
- Blood collection, labelling, storage and transport
- Laboratory assays and quality assurance measures
- Ethical considerations
- Data management (data collection, checking, storage, security)
- Weighting
  - Overall base weight calculation
  - Weight adjustments (for nonresponse or other reason).
- Summary of survey participation numbers (unweighted or weighted depending on the tables)
  - Final number of clusters by stratum
  - Final number of households (initial, replaced, non-respondent) by stratum
  - Final number of children (initial, replaced, non-respondent) by stratum.
- Analyses done

## 5. Results section

- Summary of available information on those not included in the analysis (refusals, partial completes)
- Description of the sample, including a summary of respondent background characteristics
- Main results (tables, graphs, maps)
  - Estimated seroprevalence by:
    - i. place: national, provinces, districts
    - ii. person: age groups, ethnicity, religion, or any other demographic category, vaccination status and history of illness
  - Vaccination coverage, if vaccination status (routine or SIA) is assessed, including reasons for no vaccination and factors associated with no/incomplete vaccination.

## 6. Discussion section

- Strengths and limitations and implications of limitations
- Limitation in the design stage (sample size, sampling methods)
- Limitation in the implementation stage (excluded or inaccessible data, high nonresponse or refusal rate).

## 7. Implications and recommendations

- Main recommendations based on the results
  - Areas or groups of people with an “alarmingly low immunity”
  - Significant lower immunity in some districts compared in the rest of the country.

## 8. Annexes

Include all survey materials including:

- questionnaires
- SOPs developed
- terms of reference for field teams
- training agendas and tools
- informed consent form
- tools for quality control of fieldwork
- communication materials
- final ethical review approval
- correspondence.

## Annex 6: Use of serological data for modelling

Mathematical modelling allows for cross-sectional data such as serological surveys and with basic principles of the underlying biology, project backward to understand the past and forward to predict the future. Below, typical age profiles of serology are illustrated and the difficulty of interpreting these data without the use of mathematical models is demonstrated. Some of the key opportunities presented by modelling data from serological surveys are introduced; first addressing issues related to interpreting the present by understanding the past and second to projecting the future.

### Illustrative age profiles of serology and the problem of interpretation

The age profile of seroprevalence for measles and rubella generally reflects a high probability of a positive titre in infants, resulting from transplacental transmission of maternal antibodies to the baby and a subsequent exponential decline as the infant ages. After these first months of life, the proportion of children with positive titres generally rises, at a rate determined by the rate at which children either acquire the infection or are vaccinated. Figure A6-1 illustrates a range of possible patterns of seropositivity over age over a time-course of increasing control.

Since natural and vaccine-associated immunity cannot be distinguished, the profile of seroprevalence by age obtained via a post-vaccination serological survey is inevitably a combination of vaccination and infection history (Figure A6-1, Figure A6-2). Furthermore, these two determinants of seropositivity will interact: if vaccination levels are high, the rate of acquisition of measles in unvaccinated children is likely to be lower. The age specificity of SIA campaigns can also result in very distinct profiles across age, with multiple cohorts having very high titres (Figure A6-1, B through D).

There are some possible seropositivity changes not addressed in Figure A6-1. Sometimes seropositivity in infants deviates from the usual pattern, suggesting both low infection incidence and low vaccine coverage (such that mothers and their newborns are typically not immune). Babies of vaccinated mothers have lower antibody levels than those with mothers whose antibodies were induced by natural infection. This implies that in newborns of vaccinated mothers, antibodies may decline to undetectable levels sooner. Additionally, seropositivity at late ages may be low if vaccine-induced levels of antibody titres wane (2).

### Evaluating campaign vaccination coverage

Because it is currently not possible to distinguish between immunization resulting from natural infection or from vaccination (Figure A6-2), it is ideal to obtain both a pre- and post-SIA serological survey when estimating coverage achieved by a vaccination SIA. Without the baseline provided by the pre-SIA survey, it is impossible to directly distinguish seroprevalence following a large outbreak (e.g. from a successful vaccination SIA). However, if a pre-SIA survey is not feasible, it may be possible to develop inferential tools to provide coverage estimates, as long as there are reliable data on the history of vaccination and age-specific disease incidence.

For example, the probability that an individual of age  $a$  is seronegative in a survey taking place in year  $y$  can be framed as the probability that the individual:

- was not vaccinated via routine vaccination in year  $y-a$  (reflecting the first year of life);
- was not vaccinated in any of the SIAs for which the individual might have been eligible in years between  $y-a$  and  $y$ ; and

- was not infected between  $y-a$  and  $y$ .

This logic can be used to construct a likelihood framework for every individual and thereby infer vaccination coverage in a particular SIA if sufficient information is available on routine vaccination, coverage of previous SIA, or age-specific disease incidence and if appropriate assumptions can be made.

## Estimating the force of infection over age

An age-serology profile allows us to estimate the force of infection (FOI); the rate at which susceptible individuals acquire infection. This allows the probability of infection for individuals at every age to be calculated, defined by the probability of remaining susceptible up to that age and then becoming infected. It can also be used to identify at-risk age groups. For rubella, this approach provides the means for inferring the risk of infection for women of childbearing age (3). Because the burden of congenital rubella syndrome (CRS) is extremely difficult to measure directly due to the complexity of diagnosis, this approach has been the only way to obtain an estimate of the CRS burden (3).

In the absence of vaccination and assuming that the FOI is constant over age, a catalytic model indicates that the proportion of seropositive individuals at age  $a$  is defined by

$$P(a) = 1 - \exp[-\text{FOI} \times a]$$

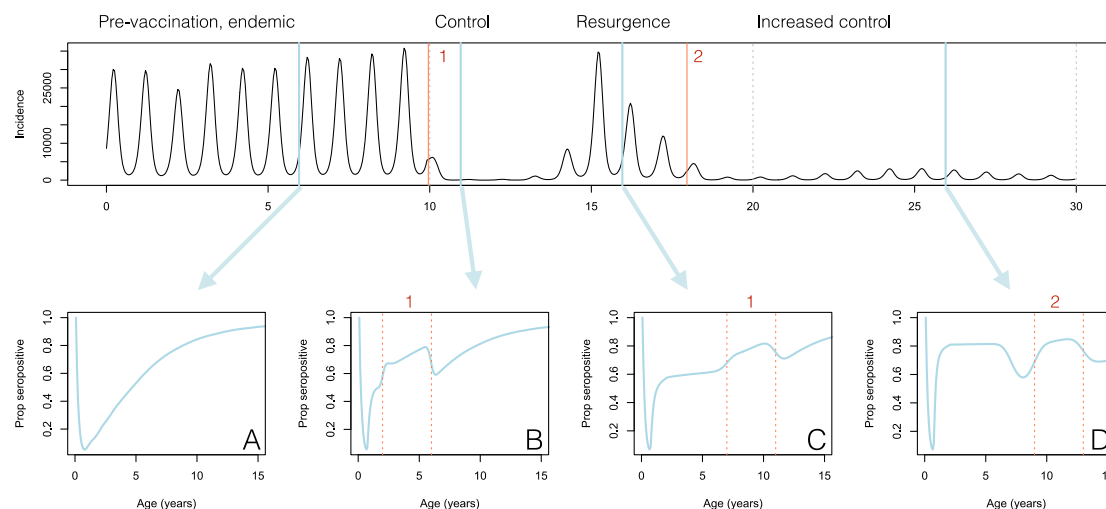
Intuitively, at every age, the FOI is the risk that each individual has of getting infected. It is relatively straightforward to modify this model to account for changing transmission over age (4) (see Figures A6-3 and A6-4), or to incorporate vaccination. This approach has also been used to infer patterns of contact over age (5) and extract changes in age-dependent mixing patterns, based on changes in the age-specific trends of the FOI (6).

## From the force of infection over age to risk of infection as a function of age

The FOI over age further allows the evaluation of the risk of infection as a function of age. For example, the probability of being infected between ages 5 and 10, or during childbearing years, is the probability of not being infected up to the start of the chosen age class (since individuals can only be infected once) and then being infected during that age class. This calculation can help identify targets for SIAs (Figure A6-4). However, these simple analyses ignore stochastic dynamics, local extinction and heterogeneity in coverage, which may affect patterns of risk over age.



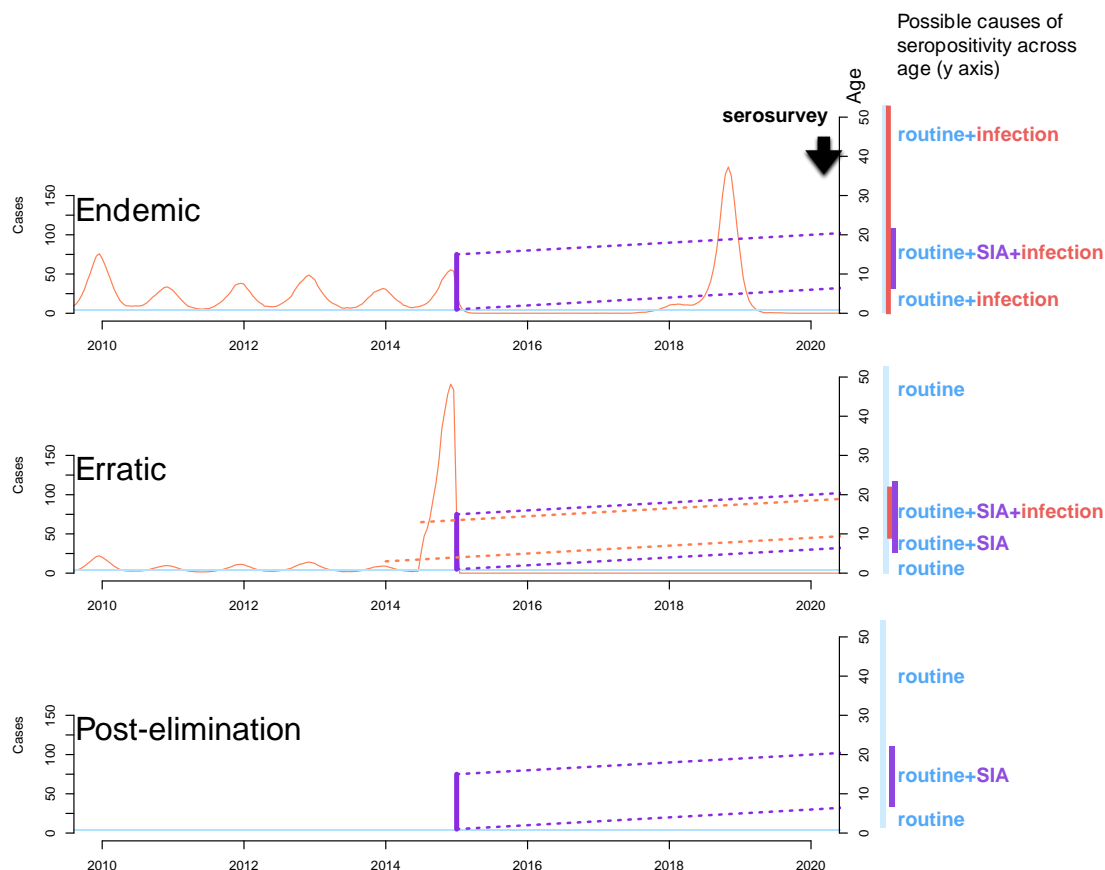
**Figure A6-1: Simulated time-series of incidence (y axis) of an immunizing infection through a changing context of control (top panel) and corresponding proportion seropositive (y axis) as a function of age (x axis) at various points across this time-course (lower panels A through D).** Routine coverage (affecting children around 12 months) shifts from no vaccination to coverage of 50% (after 10 years) and then to coverage of 80% (after 20 years, both shifts marked by dashed vertical line). SIAs targeting children 1 to 5 years of age with coverage levels of 60% occur after 10 years (SIA 1) and 18 years (SIA 2, both marked by red vertical red lines). Serological surveys occur after 6, 11, 16 and 26 years (blue vertical lines). Corresponding age profiles of seropositivity are shown below, with red vertical lines indicating the age ranges affected by the SIAs in preceding years – indexes show which SIA is relevant. A) Proportion seropositive in the absence of vaccination shows a gradual increase over age after the decay of maternal immunity. B) Vaccination slows the rate of acquisition of seropositivity via natural infection, but vaccine-acquired seropositivity increases at rates reflecting routine vaccination delivery and SIAs may result in further age-specific increases at particular ages (affecting individuals ages 2 to 6 years here). C) During periods of control, seropositivity in relevant age classes will reflect routine vaccination coverage (ages 1 to 6, corresponding to very low incidence between year 11 and year 14 on the time series above, but also the youngest age classes would have experienced the resurgence; above age 6, individuals would have experienced the first SIA). D) This pattern is also in evidence under increased routine coverage (seropositivity in ages 1 to 6 closely reflect the 80% vaccination coverage). The dip in seven-year-olds reflects that these individuals were old enough that the second SIA reduced incidence and therefore they had a low risk of acquiring seropositivity via natural infection, but they were too young to experience the increase in routine coverage that occurred in the 20<sup>th</sup> year.



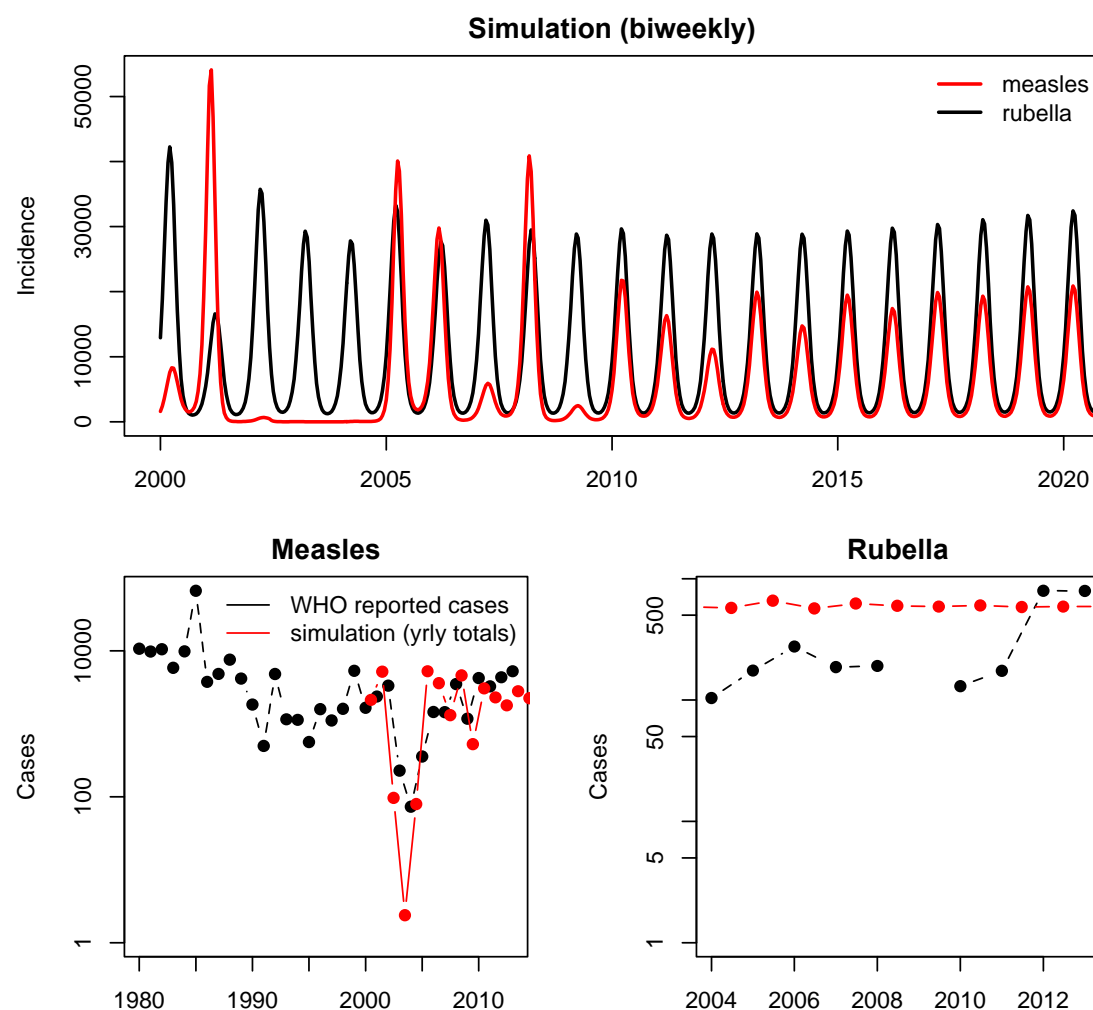
**Figure A6-2: Illustration of the complexities in inferring campaign coverage from serological surveys.** The history of incidence (y axis, left) through time (x axis) and over age (y axis, right) will combine with vaccination to affect the age profile of seropositivity. An individual may be seropositive due to one of these:

- routine vaccination, assumed here to occur around 9 months and to have constant coverage over time (shown by the light blue horizontal line);
- natural infection, assumed to affect all individuals from around 6 months in an endemic situation (top panel) and individuals between age 3 and 11 in an outbreak situation in the year of the outbreak only (middle panel); the range protected in subsequent years is indicated by red dashed lines); or
- the SIA targeted individuals from ages 1 to 15 and occurred in 2015 (purple vertical line, with range protected in subsequent years shown by purple dashed lines).

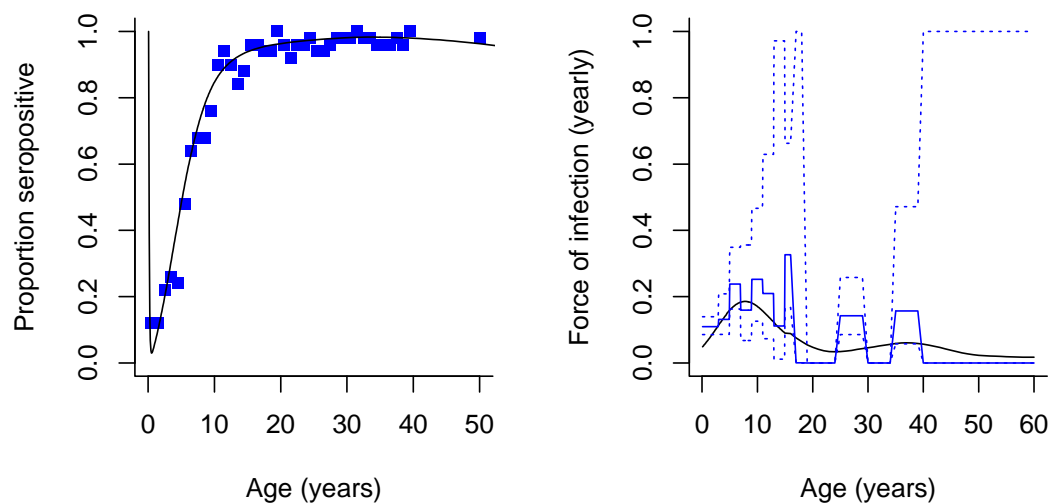
Based on this, the far right column indicates the possible causes underlying seropositivity in 2020 for individuals at different ages in the three scenarios. Note that we are ignoring waning of titres acquired by vaccination, which may further complicate interpretation of results (2).



**Figure A6-3: Simulations of measles and rubella dynamics in Ethiopia (top panel) and comparison with yearly totals from WHO reports (bottom panel).** The model (described in (7)) uses known aspects of the demography and history of vaccination (e.g. measles SIAs occurring during 2002–2003), as well as basic descriptions of the biology of measles and rubella. Assumptions regarding the pattern of contact over age are based on diary studies (8).  $R_0$  for measles was set to 20 and for rubella was set to 5. The populations were initiated in the year 2000 after a few years to remove transients. Since disease surveillance is not 100% sensitive, especially for rubella, reported cases will reflect a fraction of simulated cases. Simulations are accordingly adjusted by a reporting rate equal to 0.02 for measles and 0.002 for rubella. Note that since many different sets of parameters might yield approximately the same result, we cannot be sure that we are accurately capturing reality. Serological surveys could serve to validate the model.



**Figure A6-4: Estimating the force of infection over age** A simulated serological survey (blue squares) from the data from 2012 from the time series of rubella shown in Figure A6-3 (true value based on the simulation shown in black); and the corresponding estimated pattern of force of infection over age (blue line) with confidence bounds (dashed lines) as well as the 'true' value based on the simulation (black line). The estimated probability of infection based on the estimated FOI for individuals aged greater than 5 is 0.47, with confidence intervals (CI) from 0.38 to 0.53; the true value is 0.48. The estimated probability of infection between 5 and 10 years of age is 0.27 (CI from 0.18 to 0.36); the true value is 0.35. The estimated probability of infection of those aged 5 to 15 years is 0.42 (CI from 0.34 to 0.49); the true value is 0.44. The estimated probability of infection in women of childbearing ages is 0.04 (CI from 0.01 to 0.06); the true value is 0.03. The estimated value of  $R_0$  obtained, combining the FOI over age with demographic patterns, is 5.98 (CI from 2.2 to 12.9). The simulated age bins used for the serological survey here are very fine and the sample size within each bin is 100. With coarser bins and smaller sample sizes, the precision of inference will decline. Note also that finer age classes and larger sample sizes will improve inference.



## References for Annexes

1. World Health Organization. 2019. Manual for the laboratory-based surveillance of measles, rubella, and congenital rubella syndrome on World Health Organization. [https://www.who.int/immunization/monitoring\\_surveillance/burden/laboratory/manual/en/](https://www.who.int/immunization/monitoring_surveillance/burden/laboratory/manual/en/). Accessed 22 February 2019.
2. De Melker H, Pebody R, Edmunds W, Lévy-Bruhl D, Valle M, Rota M, Salmaso S, Van Den Hof S, Berbers G, Saliou P. 2001. The seroepidemiology of measles in Western Europe. *Epidemiology and Infection* 126:249-259.
3. Cutts FT, Vynnycky E. 1999. Modelling the incidence of congenital rubella syndrome in developing countries. *International Journal of Epidemiology* 28:1176-1184.
4. Farrington CP. 1990. Modelling forces of infection for measles, mumps and rubella. *Statistics in Medicine* 9:953 - 967.
5. Edmunds WJ, Gay NJ, Kretzschmar M, Wachmann H. 2000. The pre-vaccination epidemiology of measles, mumps and rubella in Europe: implications for modelling studies. *Epidemiology and Infection* 125:635–650.
6. Anderson RM, May RM. 1985. Age related changes in the rate of disease transmission: implications for the design of vaccination programmes. *Journal of Hygiene of Cambridge* 94:365-436.
7. Metcalf CJE, Lessler J, Klepac P, Cutts FT, Grenfell BT. 2012. Minimum levels of coverage needed for rubella vaccination: impact of local demography, seasonality and population heterogeneity. *Epidemiology and Infection* 16:1-12.
8. Mossong J, Hens N, Jit M, Beutels P, Aranen K, Mikolajczyk R, Massari M, Salmaso S, Scalia Tomba G, Wallinga J, Heijne J, Sadkowska-Todys M, Rosinska M, Edmunds WJ. 2008. Social Contacts and Mixing Patterns Relevant to the Spread of Infectious Diseases. *PloS Medicine* 5:e74.