

# **1. Introduction**

## **1.1. Document overview**

The World Health Organization (WHO) estimates that at least 95% immunity across all age groups, geographical regions and population subgroups should be achieved and maintained to sustain the interruption of measles transmission (1). Countries should attempt to identify immunity gaps, i.e. populations with below target immunity levels, and offer vaccination accordingly (2). For rubella, somewhat lower levels of immunity are adequate to eliminate infection due to the lower transmission rate of the virus, but it is important to ensure that women of reproductive age are protected from infection and the transmission of infection to a foetus.

There is no single perfect way to measure the proportion of a population that is immune and the proportion that remains susceptible to measles and rubella. However, three sources of data are useful in assessing the susceptibility profile (the proportion susceptible, stratified by characteristics such as age):

- measles case notifications (including outbreak investigations);
- vaccination coverage reports; and
- surveys that measure the proportion of a population that has antibodies to the relevant infection, known as seroprevalence surveys (1, 3 –6).

Each data source has advantages and disadvantages, but case notifications and coverage data are the most widely available (Box 1-1) and hence should be used to the greatest extent possible. The use of appropriate statistical analytic methods, data triangulation and mathematical modelling helps to compensate for some of the shortcomings in data quality (3, 4, 7).

The most direct and potentially least biased way to estimate the susceptibility profile of a population is via a suitably stratified, high-quality serological survey (henceforth called a *serosurvey*), where specimens obtained from selected populations are tested for antibodies to the respective viruses. Samples negative for viral antibody are interpreted as indicating susceptibility. Laboratory assays used to measure susceptibility must have adequate sensitivity and not falsely misclassify immune individuals as susceptible too often. This requirement is particularly important in low incidence/highly vaccinated populations, where there is no exposure to wild virus to boost immunity (8). Although high quality serosurveys have contributed to planning specific interventions (9, 10), confirming measles elimination (9) and monitoring the maintenance of elimination in a few highly developed settings such as the Republic of Korea, Australia, Japan and the United Kingdom (11, 12), there are many challenges that constrain implementation of serosurveys in low-resource settings.

High quality serosurveys require that serum samples be collected from individuals that accurately represent the target population, usually achieved by conducting a probability household survey; that appropriate, standardized laboratory methods with excellent quality assurance and control be used; and that data be appropriately analysed and interpreted. Deciding to perform a serosurvey that meets these criteria will therefore depend on available financial, logistical, laboratory and human resources. It is essential to consider whether a serosurvey can provide answers to programme questions in a desirable time frame, taking into account the typically long delay between data collection and availability of results. It is also important to note that serosurveys measure population immunity resulting from vaccination programmes (routine services and campaigns) or through natural infection (outbreaks).

**Box 1-1: Approaches to assessing population susceptibility profile: advantages and limitations of primary data sources**

Approach	Advantage	Disadvantage
<b>Immunization coverage data:</b> <b>Used to model population susceptibility</b>	<ul style="list-style-type: none"> <li>• Nationwide coverage collected routinely by Member States</li> <li>• Combined with effectiveness data to estimate proportion immune</li> </ul>	<ul style="list-style-type: none"> <li>• Requires reliable and accurate vaccination data, but in many Member States coverage data are not considered reliable</li> <li>• If field vaccine effectiveness is lower than assumed, may lead to overestimation of population immunity</li> <li>• May not identify highly susceptible sub-population groups</li> <li>• Population immunity may be overestimated using vaccine coverage if assume probability of second routine or SIA doses are independent of probability of past dose</li> <li>• Most useful in areas that have no measles circulation, but has lower accuracy for groups exposed to wild-type infections</li> </ul>
<b>Surveillance data:</b> <b>Analysing age-specific disease incidence rates over time</b>	<ul style="list-style-type: none"> <li>• Nationwide surveillance data reported to WHO for measles for all Member States and rubella for most Member States</li> <li>• Availability of qualitative data across subgroups relative to each other</li> </ul>	<ul style="list-style-type: none"> <li>• Need for advanced national surveillance systems with laboratory case confirmation</li> <li>• When incidence is low, gaps in immunity may remain undetected until virus is reintroduced</li> <li>• Does not detect subclinical cases (especially important for rubella)</li> <li>• Inability to provide quantitative data on susceptibility levels</li> <li>• Lack of reliable historic surveillance data for many Member States</li> </ul>

This document was developed for public health professionals and laboratory scientists in Member States to support their decisions about conducting serosurveys in the context of achieving goals for measles and rubella elimination and control. It should be read with reference to the European guidelines for measles-rubella serosurveys (13) and the World Health Organization Vaccination Coverage Cluster Surveys: Reference Manual (14) for greater detail and examples. This document provides an overview of the potential utility and limitations of serosurveys and reviews the epidemiological and laboratory aspects of serosurvey design and interpretation. Detailed description of measles and rubella epidemiology can be found in the Global Measles and Rubella Strategic Plan: 2012–2020 (15) and laboratory methods in the Manual for the Laboratory Diagnosis of Measles and Rubella Virus Infection (16). These and other relevant documents as indicated throughout the text should be read in conjunction with this document, which aims to provide general guidance for implementation of serosurveys and the adaptation of survey protocols for specific population groups or geographic areas in which the serosurvey is being considered.

In this document, the areas covered are:

- Definition of serosurveys
- Considerations when deciding to conduct a serosurvey, including:
  - an overview of potential uses of serosurveys
  - advantages and disadvantages of serosurvey
  - cost of serosurveys.
- Methods for conducting serosurveys, including
  - ways to increase the efficiency of serosurveys
  - methods to clearly define the objectives and estimate the sample size required to meet survey objectives
  - references to guidelines on how to conduct high-quality, probability household surveys.
- Laboratory methods, including:
  - the selection of assays and specimens
  - specimen collection, transport and storage
  - documentation of processes and training of staff
  - guidance on standardizing laboratory assays and interpretation of test results.
- Data management, analysis and result reporting.
- Overview of how data from serosurveys can contribute to mathematical modelling of the epidemiology and control of measles and rubella infection.

A set of standard definitions used in this document is presented below (Box 1-2).

#### **Box 1-2: Standard definitions**

**Serosurvey:** Collection and testing of serum (or proxy such as oral fluid) specimens from a sample of a defined population over a specified period of time to estimate the prevalence of antibodies against a given specific infectious pathogen as an indicator of immunity.

**Seropositivity:** Detection in a specimen of an antibody level above a given protective threshold (which varies according to the sensitivity of the assay and the purpose of the analysis) for a specific infectious pathogen.

**Seroprevalence:** Proportion of people in a population who test seropositive for a specific infectious pathogen; often presented as a weighted percentage of the total number of specimens tested.

**Seroprotection:** Detection of antibody above a postulated immune-protective threshold.

**Serosurveillance:** Serosurveys conducted on a periodic basis or through ongoing collection and testing of specimens to assess changes in seroprevalence over time.

## 1.2. Potential uses of serosurvey results

A well designed serosurvey using sufficiently sensitive and specific assays can provide information on the proportion of population which has seroprotection and the proportion which is susceptible (non-immune). These immunity profiles may be more accurate than profiles inferred from imperfect measures of vaccination coverage and insensitive disease surveillance (7), where historical data on vaccination coverage and measles surveillance would need to be incorporated in mathematical models to estimate the effect of routine or targeted vaccination programmes on achieving seroprevalence. Before a vaccine programme is introduced, serosurvey data can be used in mathematical models to estimate the burden of disease, as is done particularly for rubella (17). In settings where infections are eliminated or near elimination and there are very few disease notifications, serosurveys can detect immunity gaps before outbreaks occur and therefore guide vaccination activities in high-risk population subgroups (13, 18). Serosurveys can also be done to provide cross-sectional evidence of the effectiveness of a supplementary immunization activity (SIA), a programme that targets at-risk populations for vaccination (10, 19, 20). In elimination settings, serosurveys can be used to monitor immunity over time and verify that elimination is sustained (8, 9, 21). The potential uses of serosurveys and the utilities, critical requirements and limitations of serosurveys are described below (Box 1-3) and (Box 1-4) respectively.

***Overall, serosurveys can be an important supplemental tool for achieving, documenting and sustaining the elimination of measles and rubella. However, serosurveys are associated with considerable costs and human resources and should be undertaken only in cases in which they provide clear added value and if appropriate design and laboratory procedures can be ensured. It is therefore important to carefully assess the need for a serosurvey and to determine whether other, less expensive and/or more rapidly obtained data can provide the information needed by the vaccination programme.***

## 1.3. Disadvantages of serosurveys

The main limitations to conducting serosurveys include the high financial cost, substantial staff and resource commitment, and logistical challenges. Choosing appropriate laboratories and assays can be challenging, as many laboratories use commercial assays that are developed for diagnostic purposes at the individual level rather than for population epidemiological studies (see **Laboratory Methods Section 4**). It can be difficult to obtain high community participation in serosurveys, and if many people refuse to provide a specimen, the survey results may not represent the population seroprevalence. Most importantly, serosurveys require substantial technical expertise in their design and implementation (especially probability household surveys) and in obtaining high-quality laboratory testing and statistical analyses required for the survey.

There are also many challenges with interpreting survey results. First, defining appropriate cut-off values for test results to distinguish between susceptible and immune individuals can be difficult (22), although statistical mixture models can be used to address individual variability in cut-offs (23). Serosurvey results are cross-sectional and cannot distinguish the source of immunity as the results are estimates of population immunity at a particular point in time, reflecting an aggregate of both historical vaccination coverage and disease incidence. When interpreting serosurvey results for driving programmatic action, vaccination coverage and case-based surveillance data should also be considered. Analysing these data together is critical for accurate survey interpretation and outbreak risk estimates, and for effective planning of SIAs or other interventions that focus on immunity gaps identified, such as susceptible age groups or individuals in geographical areas (7, 24, 25).

### Box 1-3: Potential uses of serosurvey data on measles and rubella

*adapted from Cutts and Hanson (11)*

Potential Uses	Comments
Pre-vaccine introduction  Estimate burden of disease and theoretical herd immunity thresholds	Age-profiles of seroprevalence are used to estimate age-specific rates of infection.  Estimate the burden of congenital rubella syndrome, in part because acquired rubella surveillance is insensitive due to the high proportion of subclinical cases.
Post-vaccine introduction  Identify which age groups to include in supplementary immunization activity	Account for waning antibody levels after vaccination, especially in the absence of natural boosting. Depending on the assay used there may be many false negative results.
Before measles and rubella elimination  Monitor progress towards elimination and identify population gaps in immunity	Understand clinical and epidemiological relevance of waning antibody levels after vaccination, otherwise population immunity may be under-estimated by seroprevalence data.  Monitor progress towards targets for population prevalence of immunity by age group.
Evaluate impact of SIAs	Comparison of seroprevalence before and after a SIA is the most direct but least feasible method.  A single post-SIA survey can be used to determine if target immunity prevalence has been reached.  Incorporate historical data on vaccination coverage and measles incidence in mathematical models to estimate the effect of the SIA.
Estimate vaccine coverage	Difficult to use serosurveys to estimate vaccine coverage for measles and rubella because:  it is difficult to exclude natural infection in many Member States;  presence of antibody does not indicate the number of doses received nor whether received via routine programme or SIAs;  absence of antibody does not mean non-vaccination;  poor vaccination practices can reduce effectiveness; and

antibody levels wane over time.

## 1.4. Ways to increase the efficiency of serosurveys

Post measles and rubella elimination – Serosurveys can complement data on vaccination coverage to confirm that elimination is sustained. Cost considerations can be critical when designing the serosurvey. Ways to increase the cost-effectiveness of serosurveys include:

- Detect immunity gaps and allow preventive action before measles is introduced and outbreaks could occur.
- Using previously collected specimens.
- Conducting measles and rubella serosurveys in conjunction with other surveys/serosurveys

- Using multiplex assays to test simultaneously for immunity to a number of diseases
- Employing non-probability sampling for certain objectives and in certain settings.

#### 1.4.1. Using previously collected specimens or existing data

Most commonly, the objectives and methods of a serosurvey are designed prior to specimen collection and data generation. However, sometimes archived specimens can be used. If using archived specimens, it is critical to ensure that the original protocol and consent forms allow for the storage of samples and additional future testing in the manner defined by the new serosurvey protocol. The use of existing specimens or data is explored in more detail in **Section 2: Design the survey and develop the protocol**.

#### 1.4.2. Conducting measles and rubella serosurveys in conjunction with other surveys

Combining measles and/or rubella serosurvey with surveys being conducted for other purposes can greatly reduce the costs of fieldwork. At present, multipurpose household surveys such as the Demographic and Health Survey (DHS) often include modules for collecting blood specimens from a subsample of participants to be tested for various biomarkers. Testing these specimens for measles and/or rubella serology can be considered if specimens are collected from the appropriate target population. Other periodic surveys such as Multi Indicator Cluster Survey (MICS), AIDS Cluster Survey or Malaria Cluster Survey could be linked to vaccine-preventable disease (VPD) serosurveys. Sometimes, serosurveys are conducted for other VPDs such as tetanus (18) or hepatitis B (26) and it may be possible to extend the survey to include the relevant age groups and sample sizes to meet the measles and/or rubella objectives.

The advantage of reducing fieldwork costs by combining surveys must be weighed carefully against the potential disadvantages:

- The existing survey may not have the same objectives or target population, or it may be too small, or use a sampling plan that is not sufficiently representative to meet the new study's inferential goals.
- The logistics may be too complicated. Potentially, the combination of several biomarkers will complicate the survey implementation because it may require more logistics, several testing kits and different laboratory procedures. Adequate coordination among different departments and experts will be required.
- The specimen collection requirement may compromise acceptability of the original survey to potential participants, e.g. addition of blood collection may not be acceptable to participants of a survey that was predominantly data collection by interview.

If there is excellent overlap in demographics of eligible respondents, the sample size is adequate and the logistics amenable to collection, storage and processing of appropriate specimens, collaboration between surveys might be very fruitful. It is important to begin planning and coordination a long time in advance. For example, key decisions about what will be included in DHS surveys are usually taken 18–24 months ahead of fieldwork and the Ministry of Health sometimes has limited input into survey design. It is therefore important to establish early collaboration with the Census Office or whichever authority is supporting the DHS planning. Work with a sampling statistician early in the process to understand the power and precision that the other survey will yield for your seroprevalence inferential goals. In some cases, the other survey may be much larger than is needed to meet your serosurvey goals and it might be possible to save resources by sub-sampling survey respondents, using a disciplined random selection process, to take advantage of the DHS or other serosurvey.

#### **Box 1-4: Utilities, critical requirements and limitations of serosurveys**

##### **Utilities of serosurveys:**

- Provide information about population immunity profiles
- Help assess the risk of outbreaks
- Identify high-risk population subgroups
- Guide immunization policy and strategies
- Monitor population immunity over time

##### **Critical requirements of serosurveys:**

- Collaboration between epidemiologists and laboratory scientists
- Appropriate survey design and sufficient sample size
- Excellent logistics and laboratory capacity
- Selection of appropriate laboratory testing methods
- Standard operating procedures, training, quality control and oversight
- Community participation and acceptance of specimen collection

##### **Limitations of serosurveys:**

- High cost
- Difficult logistics
- Substantial time commitment
- Limited utility for extrapolating immunization coverage levels because of the impact of natural infection

See the World Health Organization Vaccination Coverage Cluster Surveys: Reference Manual (14) and **Section 2.6** below for details about sampling design.

#### **1.4.3. Using multiplex assays to test simultaneously for a number of infections**

Another potential opportunity for cost savings is through multiplex testing (5, 27, 28). Using multiplex assays can make the laboratory component more cost-effective as they test and report results on several different analytes simultaneously on the same sample. Some multiplex assays use small volumes of specimens (e.g. 1 µl) and may also be validated for use with a dried blood sample. These technologies can reduce the time of testing and streamline processing and associated labour costs. In effect, more data can be obtained from a single survey, resulting in greater efficiency (5). However, there are no commercial multiplex assays, and therefore any assay needs to be developed and validated prior to use.

#### **1.4.4. Employing non-probability sampling**

Although probability sampling is the gold standard approach to serosurveys, it is not always feasible. Non-probability sampling is sometimes used instead. In convenience samples, the sample is selected from the population which is readily available and convenient (e.g. collection of sera from women

attending antenatal clinic check-ups for rubella serology). Although not ideal, convenience sampling or quota sampling is sometimes the only feasible option for a serosurvey (13).

In non-probability sampling, the probability of selection of a given unit from the entire survey population cannot be accurately determined, or it might be zero. Because sampling is done based on certain pre-determined criteria rather than randomly, there is a possibility of selection bias that makes it unclear how representative the selected sample is of the survey population. It is therefore inappropriate to extrapolate findings from non-probability samples to populations that had no chance of being part of the sample. Data from women attending antenatal clinic, for example, cannot be assumed to represent women who never attend antenatal clinic. In countries where almost all women attend antenatal clinic at least once during pregnancy, the potential bias on overall estimates may be small, but in many developing countries the potential for bias would be important as they may have many socio-economic and cultural differences from clinic attendees. Nonetheless, sentinel surveillance based at clinics is one way to monitor changes over time, always remembering that the data do not provide evidence about those who never attend the clinics involved in the surveillance.

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