



**World Health
Organization**

REGIONAL OFFICE FOR **Europe**

Guidance on conducting
serosurveys in support of
measles and rubella elimination
in the WHO European Region

ABSTRACT

The WHO Regional Office for Europe places a high priority on measles and rubella elimination. It calls on Member States to remain vigilant and take appropriate action to help achieve the regional goal to eliminate measles and rubella by 2015. Serosurveys can be an important tool for achieving and, in particular, for documenting the elimination of measles and rubella. The main purpose of conducting serosurveys in the context of measles and rubella elimination is to obtain additional information about the progress made towards elimination, identify areas in need of further efforts and help document and verify the elimination. To provide accurate and reliable results, serosurveys should be a collaborative effort between epidemiologists and laboratory scientists. Well-designed serosurveys can provide key information to help reduce susceptibility to measles and rubella and achieve the elimination goal.

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Abbreviations

EIAs	enzyme immunoassays
ESEN	European Sero-Epidemiology Network
HI	haemagglutination inhibition
IgG	immunoglobulin G
IgM	immunoglobulin M
LabNet	Measles and Rubella Laboratory Network
PRN	plaque reduction neutralization
SIAs	supplementary immunization activities
VE	vaccine effectiveness
VPD	vaccine-preventable disease

Introduction

The WHO European Region has a goal to eliminate measles and rubella by 2015 (1–3). Substantial progress has been made towards achieving this goal, but the transmission of these viruses persists and outbreaks of measles and rubella continue to occur in the Member States of the Region (4). In 2012, the WHO Regional Office for Europe established the Regional Verification Commission for Measles and Rubella Elimination and developed the framework for the verification process (5). Documentation of population immunity to measles and rubella is one of the principal lines of evidence in the framework. Serosurveys, or measurement of the presence of specific antibodies in a given population, provide information on population immunity and are being increasingly used in the Region to address the needs of the measles and rubella elimination programmes.

This document was developed for public health professionals and laboratory scientists in the Member States of the Region to support their decision-making related to the conducting of serosurveys in the context of the regional goal for measles and rubella elimination.

This report provides an overview of the potential utility and limitations of serosurveys and the epidemiologic and laboratory aspects of their design and interpretation. This report does not include a detailed description of specific epidemiologic and laboratory methods, which can be found elsewhere. The elements described below can serve as general guidance for using serosurveys and developing survey protocols that should be adapted to the specific population group or geographic area where a serosurvey is being considered.

1. Approaches to assessing population immunity

Immunization coverage and disease surveillance data are two primary sources of information for assessing population immunity (Box 1). Most Member States routinely collect these data as part of the measles and rubella elimination-related activities and preparation for the verification process. Despite this advantage, each of these data sources has certain limitations.

1.1 Using immunization coverage data

Coverage data can be used to model population immunity across age groups, taking into account expected vaccine effectiveness (VE), which is usually assumed to be 95% for 1 dose of a measles-containing vaccine given at >12 months of age, and 99% for 2 doses, with the first dose given at >12 months of age (6). VE for rubella-containing vaccines is assumed to be at least 95–97% for 1 dose and nearly 100% for 2 doses (7). But this approach has several limitations. Reliable and accurate immunization coverage data are essential. If coverage is overestimated, population immunity will also be overestimated. Quality of immunization programme delivery should be high so that optimal expected VE can be achieved. In settings with programmatic problems (e.g., vaccine quality and/or cold chain issues), actual VE may be lower than expected. In addition, reliance on nationwide coverage data creates the potential for missing high susceptibility in relatively small, low-performing geographic areas or population subgroups with suboptimal coverage.

Box 1. Approaches to assessing population immunity: advantages and limitations of primary data sources

Immunization coverage data

Immunization coverage data can be used to model population immunity across age groups, taking into account expected VE. The advantages are:

- nationwide coverage information routinely collected by Member States
- availability of expected VE estimates for measles and rubella vaccines.

The limitations are:

- need for reliable and accurate immunization coverage data;
- possibility of lower than expected VE in some settings, which could lead to overestimating population immunity;
- potential for missing high susceptibility in subnational units or population subgroups when relying on national coverage data;
- lower accuracy for older age groups exposed over time to wild virus circulation and outbreaks; and
- lack of reliable historic coverage data for many Member States.

Surveillance data

Analysing disease incidence data can help to identify gaps in population immunity. This has both

- advantages:
 - existence of nationwide surveillance for measles systems in all Member States and nationwide surveillance for rubella in most Member States;
 - ability to provide qualitative data on susceptibility across subgroups relative to each other; and
- limitations:
 - need for a highly sensitive surveillance system;
 - limited usefulness when the incidence is low or zero as immunity gaps might remain until the virus is introduced into the population;
 - inability to provide quantitative data on susceptibility levels; and
 - lack of reliable historic surveillance data for many Member States.

Population immunity estimates based on coverage data are more accurate in settings where immunity is largely derived from immunization rather than natural infection. This is particularly true where vaccination programmes have been in place for long periods and wild virus circulation has substantially declined. Also, assessment of population immunity based on historic coverage is less accurate for older age groups exposed over time to wild virus circulation and outbreaks (Box 1).

1.2 Using disease surveillance data

Analysing disease incidence data can help to identify gaps in population immunity. But this approach depends on the presence of a highly sensitive surveillance system, ideally case-based with laboratory confirmation and it is more useful when disease incidence is high. If the sensitivity of the surveillance system is low, underreporting of cases can lead to underestimating the extent of susceptibility; whereas preferential underreporting of cases, in particular geographic or demographic groups, can skew the susceptibility profile. In settings with low incidence or no ongoing transmission, even substantial immunity gaps might remain undetected for prolonged periods until the virus is introduced into the population. Disease incidence data can be useful for a qualitative assessment of susceptibility across subgroups relative to each other; however, surveillance data alone are not sufficient for a quantitative assessment of susceptibility (Box 1).

1.3 Using serosurveys

When historic coverage and surveillance data are unreliable or unavailable, or in settings with no ongoing circulation of measles or rubella viruses, serologic assessments can provide a direct measure of population immunity derived from both immunization and natural disease. In situations described above, information on measles or rubella immunity from a well-designed serosurvey can be more accurate than indirect estimates based on extrapolation of coverage and incidence data.

The 53 Member States of the Region have complex and diverse histories of measles and rubella immunization efforts. In different countries, measles- and rubella-containing vaccines have been introduced at different times with various schedules and target groups, and over time, numerous changes have been made to vaccination policies. The quality of immunization programmes and surveillance also has been variable. As a result, obtaining accurate historic surveillance and coverage data can be a challenge. There is therefore increasing interest throughout the Region to use seroprevalence data to estimate population immunity to measles and rubella. In many European countries, the interest is supported by a well-developed laboratory infrastructure, epidemiologic capacity and previous experience with seroepidemiology networks for assessing population immunity to vaccine-preventable diseases (VPDs), such as the European Sero-Epidemiology Network (ESEN) (8,9) and ESEN2 (10–12).

If designed and implemented appropriately, and with the understanding that they are resource intensive, serosurveys can provide key information to help reduce susceptibility to measles and rubella and help achieve the elimination goal. However, there has been great variation in both epidemiologic and laboratory methods used in previous surveys, which sometimes makes their interpretation and national and regional comparisons difficult (Box 1).

2 Utility and limitations of serosurveys

A serosurvey is the collection and testing of specimens from a defined population over a specified period of time to determine antibodies against a given etiologic agent as a direct measure of the population's immunity.

Serosurveys conducted routinely or periodically are often referred to as serosurveillance. However, they are not a substitute for epidemiologic or virologic surveillance for measles,

rubella and congenital rubella syndrome. Instead, they should be viewed as a supplementary source of information about the status of measles and rubella immunity (Box 2).

Box 2. Definitions

Serosurvey – the collection and testing of specimens from a defined population over a specified period of time to determine antibodies against a given etiologic agent as a direct measure of the population's immunity

Seropositivity – serologic evidence of the presence of an antibody of a specific type in the serum

Seroprevalence – the proportion of people in a population who test positive for serum antibodies against a specific disease or pathogen; it is often presented as a per cent of the total specimens tested

Serosurveillance – serosurveys conducted routinely or periodically

Although serosurveys can be extremely useful in certain situations, they have important limitations and should be undertaken after considering the added value from the information gained versus resources necessary for their implementation. If data sources allowing indirect assessment (e.g., coverage, surveillance and descriptive epidemiologic data on cases and outbreaks) provide clear, consistent and reliable evidence about population immunity, serosurveys can help to confirm these findings, but they are unlikely to yield much additional information.

Assessment of seroprevalence can be instrumental in situations where the above-mentioned data sources are insufficient or unreliable. Also, quantitative information on population susceptibility profiles is critical for decision-making about outbreak prevention or response strategies, or for verification of presumed population immunity levels. In some cases, conducting a small pilot study to assess the feasibility of a larger-scale serosurvey and to assess the feasibility of conducting a serosurvey in marginalized or hard-to-reach populations may be justified.

Seroprevalence data are useful to the elimination programme in a number of scenarios (Box 3) and can help in various ways.

- Provide information about population immunity profiles. This can be an important line of evidence for documenting and maintaining elimination of measles and rubella; it is particularly valuable for older children and adults for which other methods of assessing population immunity may not be feasible or reliable.
- Assess the risk of outbreaks and identify high-risk population subgroups, which is particularly valuable in the absence of virus circulation.
- Guide immunization policies and strategies (e.g., decision-making regarding the need for supplemental immunization activities (SIAs) or changes to immunization schedules) and assess their effectiveness (e.g., impact of SIA implementation or effectiveness of an immunization programme, particularly in the case of continued outbreaks despite high reported coverage in the affected cohorts).
- Monitor population immunity over time, which is particularly useful in the absence of virus circulation.

Box 3. Utilities, critical requirements and limitations of serosurveys

The **utilities** of serosurveys are:

- provide information about population immunity profiles
- help assess the risk of outbreaks and identify high-risk population subgroups
- guide immunization policies and strategies
- help monitor population immunity over time.

The **critical requirements** are:

- collaboration between the epidemiologists and laboratory scientists;
- appropriate survey design and sufficient sample size;
- existence of adequate laboratory capacity;
- selection of appropriate laboratory methods; and
- appropriate standard operating procedures, training, quality control and oversight of the survey implementation.

The **limitations** are:

- high costs
- logistical challenges
- substantial time commitment
- limited utility for extrapolating immunization coverage levels.

Serosurveys may help validate and support epidemiologic findings and coverage estimates, but they are difficult to use for directly extrapolating vaccination coverage. With currently available laboratory testing methodologies, serosurveys are unable to distinguish between natural and vaccine-induced immunity. In addition, results of coverage assessments based on serologic testing can be influenced by waning immunity, ongoing wild virus circulation and any variations in VE. The overall direction of the effects of these factors on seroprevalence will vary depending on the situation, but the end result will likely be diminished correlation between immunity levels measured and immunization coverage being assessed. Also, coverage is calculated for birth cohorts eligible to have received a given vaccine dose in a given year, therefore the serosurvey must be designed to have sufficient statistical power for individual birth cohort analysis, rather than for wider age groups.¹

Some of the limitations of implementing seroprevalence surveys include high costs (e.g., fieldwork for specimen collection and transportation, laboratory reagents and supplies, and labour), logistical challenges and the substantial time commitment required. Cost considerations can sometimes be critical for decisions about the selection of the survey design. If previously collected specimens are available, or if prospective sampling can be conducted in conjunction with other studies, the serosurvey cost may be reduced. For example, if concurrent population-based studies such as demographic and health surveys, multiple indicator cluster surveys or other surveys are being undertaken in the country, adding measles-rubella serosurvey modules could

¹ For example, assessing by serosurvey the coverage for 2012 with the first dose of measles- and rubella-containing vaccine given at 12 months of age would require a sufficient sample size for children aged 12–23 months in 2012.

be considered. In such cases, it is important to ensure that the design of the primary survey is valid for the serosurvey purposes. At present, household surveys often include modules for collecting blood specimens to be tested for various biomarkers. Nevertheless, it is important to explore the possibility that the blood collection requirement may compromise acceptability of the original survey to potential participants before the decision to add additional modules is taken. If data on other VPDs are also needed, it is reasonable to include these additional components, to increase overall cost–effectiveness of serosurveys.

Logistical challenges potentially encountered during serosurveys include handling problems with availability of a reliable sampling frame; securing access to remote locations; managing difficulties of specimen storage and transportation in field conditions; training field workers; and ensuring the smooth operation of laboratories when large numbers of specimens are received over a short time.

Planning and implementing serosurveys are time consuming. Testing large numbers of specimens, particularly if cell-culture-based methods are used, is also time consuming. Therefore, it is necessary to ensure a balance between programmatic needs for rapidly collecting and utilizing the serosurvey data and resource constraints, feasibility and eventual utility of the survey.

3. Epidemiologic aspects of serosurvey design

A serosurvey should be a collaborative effort between epidemiologists and laboratory scientists. Both are needed to obtain accurate and reliable results. To ensure a proper survey design and consider potential biases, epidemiologists should be involved early on at the planning stage. Otherwise, it may be difficult to interpret the results, even with the most sophisticated laboratory testing. Before beginning serosurvey implementation, a written protocol should be developed and shared with all partners.

3.1 Defining the survey population

The specific objectives of the serosurvey will determine populations that need to be included (e.g., general population, age groups targeted in recent SIAs, birth cohorts with low coverage, special groups for whom new or updated immunization policies may be needed) and the geographic area of the serosurvey. Study groups should be defined in a way that would allow meaningful analysis and interpretation of results related to key variables. Birth cohorts eligible for the same immunization schedule should be grouped together. For example, if in a country where the first dose of measles and rubella vaccine is given at 12 months of age and the second at 3 years of age, a serosurvey that includes children 0–5 years of age would mix 3 different categories (unvaccinated children aged < 1 year, 1–2-year-olds eligible for 1 vaccine dose, and 3–5-year-olds eligible for 2 doses) together and complicate interpretation of the results and identification of underlying programmatic issues (e.g., problems with delivery of the first dose versus problems with providing the second dose).

The geographic area included in a serosurvey will also be determined by the survey's objectives. If a serosurvey targets the entire population of a particular age group, the sampling sites should be selected from locations throughout the country. By contrast, targeting only one or a few

geopolitical unit/s for sampling would be sufficient if high-quality surveillance determines that disease is only occurring in that particular area.

3.2 Sampling

Sampling is the process of selecting a portion of the population to determine characteristics (e.g., rubella susceptibility) of the overall population. It is important to select the appropriate sampling method for serosurveys and consider the limitations, particularly human resources, financing and time constraints.

Sampling can either be probability or nonprobability based. Commonly used sampling methods are summarized in Boxes 4–5. The gold standard is probability sampling (Box 4) because the results are most likely to be representative of the population surveyed. In probability sampling, each person's probability of being selected from the survey population is known and is greater than zero, and sampling involves random selection at some point. The estimate of seroprevalence for an entire population is produced by weighing results for sampled units according to their probability of selection.

Box 4. Probability sampling methods

Simple random sampling

Each unit from the sampling frame has an equal probability of being selected and selection is entirely by chance.

This type of sampling can be a step in more complex sampling methods.

Systematic sampling

Each unit in the sampling frame has an equal probability of being selected but selection is based on a predefined rule.

The starting point in the sampling frame is chosen randomly and from there people are selected at certain regular intervals (e.g., every tenth person).

Stratified sampling

The survey sample is divided into mutually-exclusive subgroups based on their common characteristics with the stratification variable, which is likely to correlate with the outcome.

Sampling is performed separately for each stratum using simple or systematic random sampling (e.g., stratifying samples into males and females, children and adults).

Sampling with probability proportional to size

The probability of being selected for each unit is proportional to the value (size) of another variable believed to be correlated with the outcome.

This method concentrates the sample on large elements, which have the greatest impact on population estimates.

This is advantageous when there is a wide range of values in the size variable (e.g., when the sample is to be selected from schools of various numbers of students, or from settlements with various numbers of residents).

Box 4. Probability sampling methods contd.

Sampling is performed systematically from a randomly selected starting point in intervals obtained by dividing the total number of elements in the sampling frame by the total number of units to be selected. As a result, units from larger-size elements are given higher probability of being selected than those from smaller elements.

Cluster or multistage sampling

A sample is selected in groups (clusters) rather than in individual units. Generally, sampling is clustered by geography.

Cluster sampling generally requires a greater sample size than simple random sampling to achieve the same level of precision. The degree of difference (design effect) depends on the degree of differences between clusters and within clusters.

This method is often implemented in multiple steps (complex multistage sampling) with selection of clusters at each stage. First, a random sample of units at the first level is performed, and then the second-level units are randomly selected from the units randomly selected at the first stage, etc. The final units to be surveyed (e.g., serosurvey participants) are randomly selected at the last step.

There are substantial advantages to using cluster, particularly multistage, sampling, such as reduced travel and administrative costs and elimination of the need of a full sampling frame of the entire population under survey. Instead, the sampling frame for the elements selected at the last stage is sufficient.

Box 5. Nonprobability sampling methods

Convenience sampling

The sample is selected from part of the population, which is readily available and convenient. In this case, the probability of selection is unknown and generalizations about the total population are difficult because the sample may not be representative.

Although not preferred, convenience sampling is sometimes the only available option for a serosurvey.

Quota sampling

The eligible survey population is divided into mutually exclusive subgroups, as in stratified sampling, and a predetermined number of units are selected from each subgroup (e.g., 200 females and 100 males).

Selection is based not on random sampling, but on the judgment of a person selecting the sample.

Probability samples (random samples) can be selected using various methods ranging from simple random sampling to complex multistage cluster sampling. For population-based sampling to be feasible, a sampling frame must be available. A sampling frame is a list that includes all people eligible for participation and from which selection will be made. More information on sampling frames is in Box 6.

Box 6. Sampling frames

The sampling frame should include, depending on the survey design, the entire population of a given geographic or administrative unit (country, region, district or locality) of target age groups, or specific population groups (e.g., health care workers, students, military, pregnant women)

Various sampling frames can be used: health facility registries, housing registration lists, voter lists, school rosters, etc.

Sampling frames may not be completely accurate. Commonly encountered sampling frame deficiencies include omitted or incorrectly included people, duplicate records or factual errors.

Choosing the optimal sampling frame and getting access to it can be challenging and sometimes very time and resource consuming. Multistage cluster surveys are less demanding in this regard, because actual sampling frames are only needed for selected clusters, thus sparing the arduous effort of collecting the detailed information for the entire population under survey.

Although probability sampling is the gold standard, it is not always feasible, and nonprobability samples are sometimes sought instead (Box 5). In nonprobability 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 predetermined 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. Although nonprobability sampling is considerably less expensive, it can be difficult, or sometimes inappropriate, to extrapolate the findings from nonprobability samples to an overall population. In these cases, other lines of evidence may be needed to supplement the results of the serosurvey so that the conclusions about the larger population can be validated.

Efforts should be undertaken to reduce the limitations of nonprobability sampling as much as possible. Known sources of potential biases should be considered and taken into account in the analysis. Collecting detailed information on selected participants, particularly information related to potential sources of bias will help to better describe the sample and help assess how closely it approximates the survey population. Another potential approach is to diversify sources of samples. If the results from different sources are consistent with each other, there can be a higher degree of reassurance that these samples, although not based on random selection, reasonably reflect seroprevalence in the population of interest. These approaches will not totally eliminate bias, but they can help to get a better sense of the representativeness of the sample and the generalizability of results.

3.3 Sample size

The sample size must be large enough to produce an estimate with an acceptable degree of precision (margin of error). Increasing the sample size generally results in increased precision, but at some point the added costs associated with collecting a larger sample size do not justify additional expenses. Factors to consider for determining the required sample size in the study protocol include the size of the overall population eligible to be included in the serosurvey, the acceptable level of precision, the degree of certainty that the acceptable precision is achieved, the

expected seroprevalence and the design effect. These parameters and their impact on a sample size required to achieve the serosurvey objectives are described in Box 7.

Box 7. Parameters to consider when estimating the required sample size

Population size

This is the size of the population eligible for inclusion in the survey.

With other parameters constant, required sample size increases when the population increases. However, the sample size changes very little after population exceeds 10 000 and reaches a plateau for populations greater than 1 million.

Acceptable precision/margin of error

The precision of +5% to +7% is usually considered the minimum acceptable level in measles-rubella serosurveys.

Greater margins of error correspond to lower precision and vice versa. The lower the acceptable margin of error, the larger the sample size needed to achieve it.

Confidence level

This reflects the probability of achieving the desired precision of the survey.

The acceptable level of uncertainty is usually the 95% confidence level (90% or 99% may also be used in some cases).

The need for a higher confidence level leads to an increase in the required sample size.

Expected seroprevalence

This is the expected frequency (percentage) of seropositives (or seronegatives, depending on the study question) in a study population.

The expected distribution of 50% requires the largest required sample size. Therefore, if expected seroprevalence is unknown, the 50% value should be used for sample size calculations. This will ensure sufficient sample size for any seroprevalence levels found in the survey.

Design effect

The effect of the cluster sampling on the required sample size reflects potential differences between the clusters and the overall population.

Values vary with cluster sampling, depending on the survey design and populations studied (equals 1.0 in non-cluster sampling). For sample size estimation purposes, values between 1.5 and 2.0 are often used.

To account for the design effect, the sample size is inflated accordingly (multiplied by the value of design effect); therefore, a higher design effect will require a larger sample size.

Sample size calculations can be performed by using statistical formulas or, more conveniently, by using tools available online or in standard statistical packages. Table 1 provides examples of variations of required sample size depending on acceptable precision and expected seroprevalence.

Table 1. Examples of minimum required sample sizes by acceptable precision and expected seroprevalence^a

Acceptable precision (%)	Expected seroprevalence (%)					
	50	70	80	90	95	99
± 3	1056	889	679	383	203	43
± 5	383	322	246	139	73	16
± 7	196	165	126	71	38	8

^a assumes population size 100 000; confidence level 95%; design effect 1.0.

Source: Estimated using the sample size calculator for proportion from OpenEpi: Open Source Epidemiologic Statistics for Public Health (13).

The number resulting from these calculations is a minimum required sample size for a given unit of analysis (e.g., age stratum). It is important to consider that it may not necessarily allow sufficient statistical power for subgroup analysis as well, particularly if seroprevalence is close to 50%, when the required sample size is the largest. Therefore, if subgroup analysis is planned, sampling should be done for individual subgroups and the overall sample size should be a total of samples for all subgroups. For example, if a serosurvey to determine measles seroprevalence among 1–19-year-olds also needs to estimate seroprevalence for individual age groups (e.g., for 1–4, 5–9, 10–14 and 15–19-year-olds), sample size should be calculated for each age stratum separately. This may result in a substantial increase in the total sample size and has to be balanced with feasibility and available resources.

4. Laboratory aspects

To define an adequate laboratory testing strategy for any proposed serosurvey, engaging the laboratory during the planning stages is essential. Testing strategy should include a description of how the samples will be processed, labelled and stored, how testing will be performed and how data will be analysed.

The plan should provide the validation criteria for the assay used and include a protocol for re-testing samples from invalid assays. While some laboratories have experience testing large numbers of samples and have electronic data collection and analysis tools in place, some of the laboratories may require additional resources to scale up to high volume testing.

4.1 Biological samples

Serosurveys conducted for measles and rubella typically use the quantitative detection of antibodies in a single serum sample. The standard specimen for measles and rubella population immunity studies is serum or plasma. Use of alternative samples obtained by less invasive means (e.g., dried blood spots and oral fluid samples) could simplify specimen collection, handling and storage requirements, and reduce costs, particularly for large-scale surveys. The use of alternative samples has been well validated for diagnostic testing based on the detection of immunoglobulin M (IgM). However, their use in serosurveys based on detection of immunoglobulin G (IgG) or neutralizing antibodies requires further validation. As always,

appropriate biosafety precautions and procedures for biohazard management should be in place at sampling sites and laboratories where the testing is performed, even though the risks from specimens obtained from apparently healthy people are considered low.

4.2 Assays

Both antibody-mediated (humoral) and cellular responses contribute to immunity to measles and rubella. However, measurement of antibodies against measles and rubella is the preferred approach to assessing immunity status because of demonstrated good correlation with protection and the availability of standardized assays. In contrast, cellular responses to these viruses and their respective vaccines are less well understood, and there are no standardized assays for their measurement, which would be suitable for testing many hundreds or thousands of specimens.

A number of immunological assays are available to measure antibodies to measles and rubella viruses (Box 8). For measles, measurement of antibodies by the plaque reduction neutralization (PRN) assay provides the best correlate of protection from infection (6,14). PRN assay provides a quantitative measurement of the level of neutralizing antibodies and is considered the gold standard for measuring protective antibody levels. However, the assay is expensive, time consuming, labour intensive and unsuitable for testing very large numbers of samples. Therefore, for serosurveys, enzyme immunoassays (EIAs) to measure IgG antibodies, which persist for long periods after exposure to virus or vaccine, have become a commonly used alternative to PRN assays. Neutralizing antibodies are the best correlate for protection against rubella too, but many previous rubella studies have used the more convenient and highly accurate haemagglutination inhibition (HI) assay. At present, similar to measles, EIAs are more widely used assays for rubella antibody detection (7,15).

Box 8. Immunological assays to measure antibodies against measles and rubella viruses

PRN assay

The advantages are:

- allows quantitative measurement of the level of neutralizing antibodies
- provides the best correlate of protection and is considered the gold standard for measles.

The limitations are:

- expensive, time consuming, and labour intensive
- unsuitable for testing very large numbers of samples.

EIAs to measure IgG antibodies

The advantages are:

- good correlation with results of PRN and HI assays
- high sensitivity and specificity
- commercial availability
- relatively low cost
- requires small amount of specimen

Box 8. Immunological assays to measure antibodies against measles and rubella viruses contd

- faster and less labour-intensive than PRN assays
- suitable for testing large numbers of specimens.

The limitations are:

- less sensitive than PRN and HI assays at low levels of antibodies; and
- difficult to include a standard calibration sample with a known IgG concentration for direct comparison of results obtained with different EIA kits.

Other assays (HI, complement fixation, immunoprecipitation, etc.)

HI assays have certain characteristics.

- They are used in most early studies as the standard assay for rubella.
- The advantages are high sensitivity and good correlation with neutralization and EIA results.
- The limitations are high cost and labour intensity.

HI assays were more widely used in the past but have largely been replaced by EIAs because of the higher sensitivity and specificity, as well as wide availability and convenience of use.

Currently available EIAs are highly sensitive and specific, commercially available, relatively inexpensive, require a very small amount of specimen, and are substantially faster and less labour intensive than PRN assays. Generally, IgG levels measured by EIAs correlate well with antibody levels in PRN assays, but EIAs may be less sensitive at low levels of antibodies (6). EIAs are particularly suitable for large-scale studies when the testing of large numbers of specimens over a short period is required.

In the past, other assays to detect antibodies have also been used (e.g., complement fixation, immunoprecipitation). However, because of the higher sensitivity and specificity, and wide availability of EIAs, they are rarely used at present.

A number of commercial and in-house EIAs to detect measles and rubella IgG antibodies are available. Their sensitivity and specificity vary, but some of the commercial assays have been validated relative to the gold standard (neutralization assays).

4.3 Quality and comparability

High-quality laboratory testing is as important for the validity of serosurvey results as is appropriate selection of the survey sample. To facilitate high-quality laboratory investigations for measles and rubella in the Region, the Regional Office coordinates the Measles and Rubella Laboratory Network (LabNet), established in 2002 (16). As of 2013, LabNet comprises 71 laboratories, including national measles and rubella reference laboratories in 49 of 53 Member States (16). Since EIA is routinely used by LabNet to detect measles- or rubella-specific IgM to provide laboratory confirmation of measles or rubella infection, LabNet laboratories are well suited to perform the EIA for IgG in support of serosurveys. Another advantage is that to

maintain a very high standard for overall laboratory performance, the Regional Office conducts annual accreditation of LabNet laboratories and provides advice about standardized testing procedures and reagents.

A number of other factors affect the overall quality of laboratory testing, including adequate training of staff, standard operating and external quality assurance procedures being in place, and adequate oversight of laboratories. It is important, particularly for large-scale serosurveys, that the laboratories have the logistical capacity to handle large numbers of specimens over short periods. Because of variations in reagents used, direct comparison of results obtained with different EIA kits is difficult. Therefore, all specimens in the given serosurvey should ideally be tested with test kits from the same source, preferably from the same lot, and include in testing a standard calibration sample with a known concentration of anti-measles or rubella IgG (14). The serum standard for measles is available from the National Institute for Biological Standards and Control, Hertfordshire, United Kingdom.

Comparability of laboratory results and consistent testing procedures are crucial for the ability of serological surveys to serve as helpful tools in measles and rubella elimination. Therefore, collaborative networks have been established to harmonize testing practices and increase comparability of results. In the Region, ESEN and ESEN2 collaborations were established to coordinate and harmonize the serological surveillance of immunity to a variety of VPDs across participating laboratories (8–12). The methodologies for testing were successfully harmonized for five different antigens, including measles and rubella, in seven European countries in ESEN and 21 European countries and Australia in ESEN2. Standardization was achieved by testing a panel of reference serum samples by all of the participating laboratories with EIAs used in a given country and by regressing each country's results against the reference laboratory's results. These equations were then used to standardize the national results.

Details of WHO recommendations for serological testing for measles and rubella, including specimen collection, transportation, storage and laboratory assays and their interpretation, can be found in the *Manual for the laboratory diagnosis of measles and rubella virus infection* (17). Information on biohazard management can be found in the *WHO Laboratory biosafety manual* (18).

4.4 Interpretation of laboratory results

The levels of measles- or rubella-specific antibodies correlate with immunity. The level of antibodies determined in neutralization assays provide the best correlate of protection, as only functional antibodies involved in virus neutralization are measured. While EIAs detect antibodies, including those not responsible for neutralization, their results serve as a good practical surrogate to levels of neutralizing antibodies, especially when large numbers of samples are being tested.

Antibody levels, expressed in international units per millilitre (IU/mL), above 120 IU/mL for measles (6,14) and above 10 IU/mL for rubella (7,15) are considered protective for the majority of people. However, people with antibody levels below these cut-off levels can sometimes still be protected. This is likely due to cellular mechanisms involved in protection and the long-term presence of memory B cells mediating immunological memory established after primary infection or vaccination, even after the decline of circulating antibodies (waning antibody titres). Very low levels of serum antibodies, e.g., <40 IU/mL for measles (6) and <10 IU/mL for rubella

(7) in most cases reflect susceptibility, and the lack of detectable antibodies is very well correlated with susceptibility. Positive results in very young infants are generally due to the presence of maternal antibodies, which wane in a few months and therefore do not indicate long-term protection.

In serosurveys, results are usually expressed as the proportion of seropositive or seronegative people among the survey population. Qualitative determination of antibody presence dividing specimens into positive, negative or indeterminate categories is often sufficient. These categories are defined according to the recommendations of assays and test kits used. To simplify analysis, the indeterminate group is often combined with seronegatives to avoid overestimating population immunity. However, in some cases, indeterminate levels in EIA correspond to low levels of antibodies that might still provide protection. Combining them with seronegatives can lead to underestimating population immunity. Therefore, careful judgment should be exercised and quantitative values of antibodies corresponding to each category should be taken into account when categorizing and interpreting results.

In addition to qualitative results, serologic assays can deliver quantitative results expressed as dilution titres or international units per millilitre (IU/mL). Quantitative determination is particularly useful amidst concern about potential waning immunity in vaccinated populations or about lower levels of vaccine-induced antibodies. The method of determining quantitative values, particularly for EIA methods, must be carefully considered. Since EIA is generally less sensitive than PRN, samples giving negative or equivocal results in the EIA are sometimes retested by PRN.

5 Human subject issues

Human subject regulations vary from country to country. Appropriate procedures for adequate protection of human subjects in accordance with all relevant regulations should be in place for any serosurvey that collects personally identifiable data. Privacy of the participants should be assured and individuals asked to participate in the serosurvey should be given clear, easily understandable and comprehensive information about objectives, procedures and implications of the survey, including risks and benefits of participation, so that they can make an informed decision on whether or not to participate. In many cases, obtaining formal informed consent (written or verbal) will be required. The only situation in which these steps will not be required is when a serosurvey uses samples that have been delinked from personal information and individual participants can therefore not be identified.

6. Staffing and training

Measles and rubella serosurveys are usually led by epidemiology and laboratory scientists responsible for survey design and planning, including development of protocol and standard operating procedures and staff training, as well as survey implementation and data analysis. In addition, required personnel include staff responsible for enrolment and specimen collection, laboratory technicians, supervisory staff, data managers, survey coordinators and others as needed. For the most effective participation at all levels, serosurvey protocols should clearly define the roles and responsibilities of each staff member. To ensure compliance with defined

survey procedures, trainings outlining relevant aspects of the survey should be conducted for all levels of staff involved.

7. Data collection and analysis

Depending on the rationale for conducting the serosurvey, data collection elements may vary in scope. For measles and rubella serosurveys, the minimum required set of variables would include the participant's sex, age, residence (urban/rural or more specifically, region/district/locality) and vaccination history, if available and reliable. In addition, information on factors potentially associated with the risk of transmission or with the likelihood of being immunized should be collected, if possible. Detailed descriptive information on the setting should also be obtained, particularly if the serosurvey deals with specific population groups. Data collected need to be entered into a database that has appropriate procedures for quality assurance and data cleaning in place.

In data analysis, the main outcome measure is seroprevalence—the percentage of survey participants with at least a given level of antibodies (e.g., >10 IU/mL). If the survey design allows it, 95% confidence intervals around these estimates should be calculated. Basic descriptive analysis should include immunity levels overall and by sex, age, region or any other category the survey was intended to assess. The complexity of the analysis will vary depending on the survey design and the sampling methods used. If the study subgroups have been weighted in the process of the survey design, the results need to be adjusted in the analysis to account for weights so that the final result reflects the true seroprevalence in a study population. Risk factor analysis using appropriate statistical methods can be conducted if information on factors potentially associated with immunity has been collected.

8. Interpretation of serosurvey findings

The principal reason for conducting serosurveys in the Region for the purpose of measles and rubella elimination is to obtain additional information about the progress made towards elimination, identify areas in need of further efforts and help document the elimination. Therefore, interpretation of serosurvey findings should be done with the elimination goals in mind.

The primary question to be answered is whether the level of susceptibility found in the serosurvey is consistent with achieving and maintaining interruption of measles and rubella virus circulation (i.e., if, in the event of virus introduction, the immunity would result in values of $R_0 < 1$).² Because transmission rates, which vary by age group and by exposure setting, are an important contributor to overall risk of disease, along with population immunity, there are no universally adopted cut-off levels for seroprevalence consistent with interruption of circulation. The seroprevalence threshold required for herd immunity has been estimated to be very high (92–95%) for measles (6) and somewhat lower (85–87%) for rubella (7). Based on statistical modelling, in 1999, the first Strategic framework for the elimination of measles in the European Region proposed that to achieve elimination, population susceptibility levels by age group in the

² R_0 , or basic reproduction number, is the number of cases one case generates on average over the course of its infectious period. If $R_0 > 1$, sustained spread of the infection will be possible; if $R_0 < 1$, the infection will die out in the long run.

Region should not exceed the following targets: 1–4 years: 15%; 5–9 years: 10%; 10–14 years: 5%; >15 years: 5% (19). Of note, these levels have been used as targets when interpreting the results of several serosurveys in the Region, including those conducted through ESEN2 (11). The Americas Region’s strategy for measles elimination set 25% among 1–4 year olds as the maximum acceptable susceptibility level without specifying levels for older groups assumed to be immune following the catch up and follow-up immunization campaigns (19,20).

Documentation of serosurvey results should present a very clear description of sampling methods, including their type (probability or nonprobability) and steps followed, as well as of laboratory assays and the criteria for immunity used (e.g., cut-off values for seropositivity). Limitations of the survey design and laboratory methods should be described and taken into account when interpreting serosurvey findings. Possible biases should be noted and addressed as much as possible. The generalizability of results or how applicable they are to other populations should also be discussed.

Serosurvey findings should be interpreted in the light of current and historic data on the disease incidence and the policies and performance of immunization programmes (schedules, coverage), including any past SIAs. This approach will help with validating survey findings (particularly important for surveys based on convenience samples) and might pinpoint areas for improvement. For example, higher seroprevalence in birth cohorts targeted by SIAs than in birth cohorts relying only on routine immunization can be an indicator of the weakness of the routine programme. An analysis by age group aligned with the immunization schedule allows for an overall assessment of the effectiveness of delivery of vaccine doses applicable to the given age group (e.g., 1 dose of measles and rubella vaccine in 1–4 year age group). Also, if other VPDs have been included in the survey, comparison of the results across different antigens can help determine if the identified problems are related to a given vaccine or are of systemic nature related to the immunization programme in general.

9. Conclusion

Overall, serosurveys can be an important supplemental tool for achieving, documenting and maintaining elimination of measles and rubella. However, they 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.

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