Toolkit for Integrated Serosurveillance of Communicable Diseases in the Americas

PAHO Pan American Health Organization
World Health Organization REGIONAL OFFICE FOR THE Americas

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Abbreviations and acronyms

CAPI	computer-assisted personal interviews
CDC	United States Centers for Disease Control and Prevention
DBS	dried blood spot
DEFF	design effect
DPT	diphtheria, pertussis, and tetanus vaccine
ELISA	enzyme-linked immunosorbent assay
EQAS	external quality assurance system
GVAP	Global Vaccine Action Plan
Ig	immunoglobulin
MBA	multiplex bead assay
MDA	mass drug administration
MFI	median fluorescence intensity
MMR	measles, mumps, and rubella vaccine
NID	neglected infectious diseases
NTD	neglected tropical diseases
PAHO	Pan American Health Organization
PAPI	paper and pencil interviewing
PCR	polymerase chain reaction
PE	phycoerythrin
QC	quality control
ROC	receiver operating curves
VPD	vaccine-preventable diseases
WASH	water, sanitation, and hygiene
WHO	World Health Organization

Introduction

Background

The Region of the Americas has a long history of elimination of diseases, including the eradication of smallpox, the elimination of polio and neonatal tetanus, and the elimination of endemic transmission of measles, rubella, and congenital rubella syndrome (*1, 2*). Until the first half of 2020, countries in the Region had approached the elimination of malaria. Seven countries in the Region were certified from 1962 to 1973 and Argentina, El Salvador, and Paraguay achieved malaria-free status in recent years. Several neglected infectious diseases were targeted to be eliminated at the regional level, including leprosy, trachoma, lymphatic filariasis, onchocerciasis, and dog-mediated human rabies.

Ongoing elimination efforts in the Region have also achieved a substantial reduction in the impact of Chagas disease, soil-transmitted helminthiasis, schistosomiasis, and fascioliasis in children and other populations at risk. Likewise, the elimination of mother-to-child transmission of hepatitis B, HIV, syphilis, and Chagas disease is also within reach, backed up by an integrated conceptual framework for quadruple elimination (*3*).

Despite the progress, national indicators mask inequalities within the countries, and health systems face a multitude of social, demographic, and epidemiological challenges that threaten the sustainability of achievements and progress toward reaching the Sustainable Development Goals (SDGs) by 2030. Member States of the Pan American Health Organization (PAHO) endorsed a Disease Elimination Initiative in 2019 to eliminate more than 30 communicable diseases and related conditions by 2030 (Resolution CD57.R7) (*4*) by using innovative tools and approaches (*3, 5*). This initiative articulates four lines of action, including to strengthen strategic health surveillance and information systems, and calls for a multi-disease approach for mapping, control, elimination, prevention, and post-elimination monitoring at the country level.

The 2030 Neglected Tropical Diseases (NTD) Road Map 2021–2030 is a high-level document and a critical tool—evidence-supported and feasible to achieve—that sets the strategies, policies, and goals to guide the global response to NTDs over the next decade (*6*). It calls for cross-cutting approaches, intersectoral coordination, and integrated approaches. Integrated surveys are logical tools to monitor

and evaluate progress toward the targets of the NTDs Road Map by using methods to determine overlap to identify cross-cutting interprogrammatic opportunities (*7, 8*).

The Global Vaccine Action Plan 2011–2020 (GVAP) established the first global monitoring and evaluation framework for immunization (*9*) and raised awareness of the importance of accelerating innovation and increasing access to reliable data to improve program performance. As important challenges remain over this decade, the Immunization Agenda (IA2030) was built on GVAP lessons (*10*), to guide a dynamic operational phase to sustain high levels of vaccine coverage and improve epidemiologic surveillance to eliminate and eradicate vaccine-preventable diseases (VPDs) (*11*).

Serosurveillance is a tool that complements traditional public health methods for surveillance of communicable diseases (*12*), and provides valuable information on disease transmission in populations; for example, to identify gaps in immunity against VPDs (*13, 14*). Likewise, these profiles are useful for monitoring population exposure to diseases such as malaria (*15–17*), neglected infectious diseases (NID), foodborne diseases, waterborne diseases, vector-borne diseases (*18–23*), and emerging infectious diseases (*24–26*). As many infectious diseases are or have been present in populations that live in environments where various risk factors overlap, consequently, integrated serosurveillance facilitates synergies and optimizes the utilization of public health resources.

Population-wide integrated serosurveys are used to characterize patterns of infectious disease transmission and to monitor the impact of public health interventions. This information can move countries further toward the control and elimination of communicable diseases and strengthen post-elimination surveillance of already eliminated conditions. Antibodies are excellent biomarkers and are among the molecules most widely employed as biomarkers (*27, 28*), because they allow recognition of protective immunity to VPDs, measurement of past exposure to various pathogens (including bacteria, parasites, protozoa, and nematodes), and have the potential to generate information that can be used to detect increased transmission of NIDs.

National serosurveillance programs are well established in many countries worldwide. Countries such as Australia, Canada, United Kingdom, and United States of America, among others, have implemented national serological surveillance programs using various models, ranging from the implementation of periodic population-based surveys for sample collection to serum bank-based surveillance using samples submitted by public health laboratories (*29, 30*).

In 2016, PAHO, in partnership with the U.S. Centers for Disease Control and Prevention (CDC), started a joint initiative to transfer capacities to use integrated serological surveillance to complement epidemiological surveillance and intervention coverage data in populations through multiplex bead assay (MBA) to countries in the Region of the Americas. This assay uses Luminex® technology (developed in 1995), which allows the identification of IgG antibodies against multiple antigens in a single sample (serum, blood, including dried blood samples, and other body fluids). The assay offers good advantages

in comparison with other serology methods (e.g., ELISA) because it requires a small sample volume, has lower cost as several antigens can be analyzed in a single test for one person, with comparable sensitivity and specificity, among others (*31–36*).

The PAHO and CDC initiative has been a learning process, not only on the use of the MBA platform but in the cross-cutting work required to develop integrated serological surveillance of communicable diseases that are usually kept separate from a programmatic standpoint, but which in reality overlap in the same population groups and geographic areas. Integration efforts are not simple, because programs must find common interests to align strategies and share resources, based on the lessons learned in countries participating in the initiative, and understanding that the integrated serosurveillance demands the integrated and sustained work of interprogrammatic teams at the national and local levels.

This toolkit was developed to facilitate the design, implementation, analysis, interpretation, and use of results of integrated serosurveys to reinforce countries' capacities toward the elimination of communicable diseases. The first part describes the basic concepts of serosurveys and serosurveillance, its uses, benefits and challenges, ways to improve its efficiency, and its potential to contribute to decision-making in public health. Subsequently, this toolkit presents a stepwise process for the implementation of survey-based integrated serological surveillance. It includes recommendations on how to identify the need for and purpose of gathering serological information; the survey design and methodology; laboratory methods; practical considerations for survey implementation; data analysis and interpretation; and the use of findings to support decision-making.

Purpose and audience

This toolkit is focused on the design and implementation of serosurveys as a complementary tool for epidemiological surveillance and intervention coverage data at the population level. It is primarily aimed to support program managers and teams involved in the control and elimination of communicable diseases. The target audience includes, but it is not restricted to, coordinators of communicable diseases, NID, and immunization programs; epidemiological surveillance managers; public health laboratory staff; and other staffers of cabinet-level and subnational health departments or authorities who may be interested in incorporating integrated serological surveillance into the tools of their surveillance systems, as a means of gaining additional insight into population transmission of infectious diseases.

By using this toolkit, it is expected that the reader will be able to:

- Identify the uses and limitations of integrated serological surveillance and its potential applications in the prevention, control, and elimination of communicable diseases and in monitoring the impact of public health interventions.
- Describe the various epidemiological scenarios in which integrated serological surveillance would provide insights on disease transmission at the population level, which could be used to design,

implement, and evaluate the impact of interventions, as well as to detect and anticipate diseases at risk of emergence or reintroduction.

- Promote joint work among communicable diseases program managers, epidemiological surveillance groups, public health laboratories, national institutes of health, PAHO/WHO Collaborating Centers, research groups, among others involved in the control and elimination of communicable diseases in the development of serosurveys for the integrated surveillance of communicable diseases.
- Conduct each of the necessary steps and activities for planning, implementation, analysis, interpretation, and use of the results obtained through integrated serosurveys and serosurveillance.

Structure and contents

The steps to implement an integrated serosurvey are outlined in Figure 1.1. These steps are described in detail in six modules, shown in Table 1.1. For training purposes, educational material will be also developed with case studies and exercises for both the facilitator and the participant, supplemented with PowerPoint® presentations to support designing the protocol, data and sample collection, analysis, interpretation, and preparation of the reports.

FIGURE 1.1 **Steps to implement the integrated serosurvey**

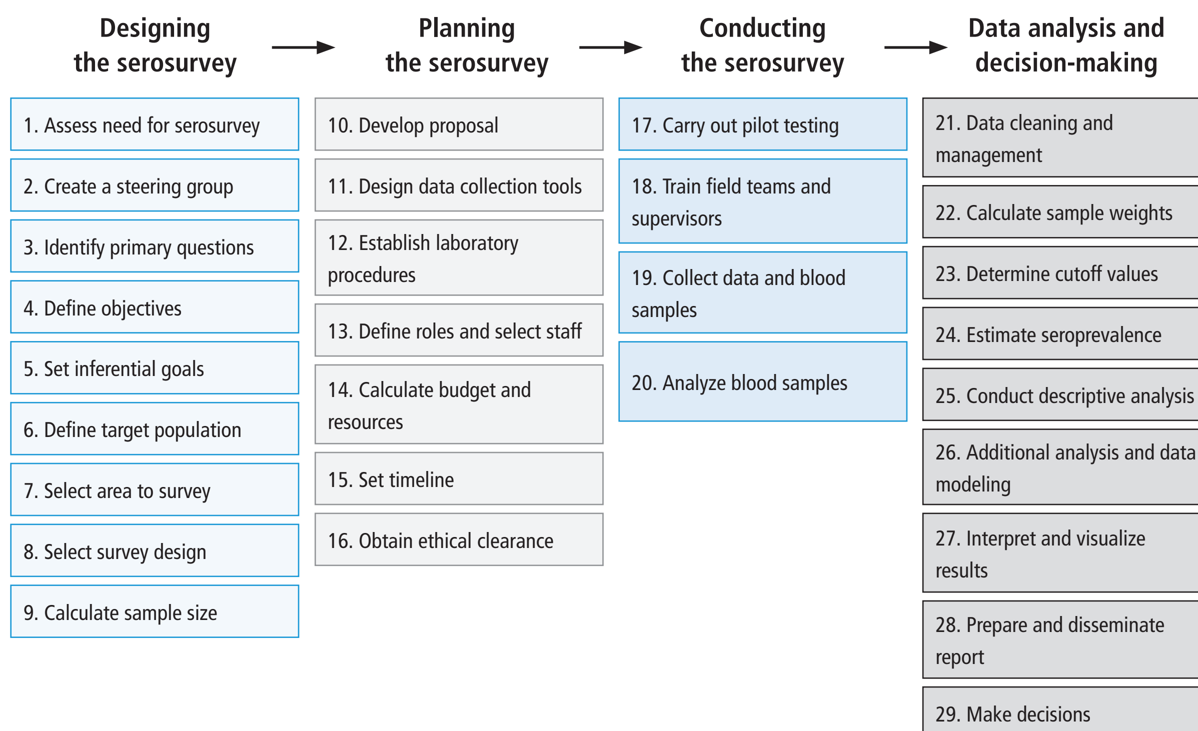


TABLE 1.1 Contents of toolkit for implementing integrated serosurveys

MODULES	CONTENTS	RESOURCES
Module 1 *Concepts, rationale, and approach*	• Provides background information about the multiplex initiative in the Americas and describes basic concepts of integrated serosurveys and serological surveillance, potential uses in different epidemiological scenarios, how to improve the efficiency of serosurveys, benefits, challenges, and future perspectives.	
Module 2 *Designing the serosurvey*	• Includes **steps 1 to 9**, starting on assessing the need of the serosurvey, how to create steering group, identify primary questions, define objectives, set inferential goals, select target population and area to survey, and how to select the survey design and calculate the sample size.	• Example of surveys into which serological sampling could be incorporated
Module 3 *Planning the serosurvey*	• Describes **steps 10 to 16** to develop the proposal, design data collection tools, define roles and select staff, estimate budget and resources, set timeline of the serosurvey, and obtain ethical clearance of the protocol. *Note*: This module briefly describes how to establish laboratory procedures, but more detailed information is included in Module 5: Laboratory methods.	• Example of protocol template • Roles and responsibilities of staff • Example of questionnaires • List of supplies to collect dried blood spots • Example of budget and timeline template • Example of informed consent and assent
Module 4 *Conducting the serosurvey*	• Develops **steps 17 to 20** about how to conduct the pilot testing and train the teams, collect data, sample collection, and analysis.	• Example of training agenda • Flow diagram of standard operating procedures for blood sample collection
Module 5 *Laboratory methods*	• Describes the type of laboratory tests used for integrated serosurveys, advantages, limitations, and criteria to select quantitative and qualitative serology tests. • Schematizes the laboratory process to run and read the MBA results and the methods used to define the cutoff value and seropositivity. • Defines sensitivity, specificity, and cross-reactivity. • Develops key concepts and considerations related to quality assurance and quality control. • Describes the main steps recommended for basic descriptive analysis when using MBA.	• Description of antigens available for integrated serological surveillance in the MBA platform, their utility in different scenarios and potential interventions • Sensitivity and specificity of validated antigens for integrated serological surveillance in the MBA
Module 6 *Data analysis and decision-making*	• Develops **steps 21 to 29**, including the procedures for data cleaning and management, sample weights calculation, estimation of seroprevalence, descriptive analysis, data modeling, how to interpret and visualize results, preparation and dissemination of the report, and making decisions based on findings.	• Recommendations for descriptive analysis of the results of a serosurvey where MBA was used • Basic structure and contents of the report

Module 1

Concepts, rationale, and approach

1.1 Integrated serological surveillance

1.1.1 Concepts

The dynamics of infectious disease transmission depend on:

- The type of pathogen (viral, bacterial, protozoan, helminth, fungal, etc.), its route of transmission, and its virulence, genotype, and serotype, among other characteristics;
- The individual characteristics including the immune status, genetic susceptibility, and previous exposures and infections; and
- The characteristics of the population including intersections between individual and population-wide immunity profiles.

Antibodies are sensitive biomarkers to inform if a person has ever been infected with a pathogen, has had multiple infections, had a recent infection or is currently infected, has been vaccinated, among others. When antibodies are measured at the population level, they can inform if a population has sufficient vaccination coverage, or if an infection is prevalent in a population, and if those measures are done periodically, changes in the time can be identified.

A ***serosurvey*** is the collection and analysis of samples from a defined population, over a specific time, to estimate the prevalence of antibodies (seroprevalence) against a given, specific infectious pathogen to better understand the disease transmission and intervention coverage data. Serosurveys provide a direct quantitative measure of population immunity derived both from natural disease exposure and public health interventions (e.g., vaccination), as well as to characterize differences by groups (age, ethnicity, migration status, etc.) and changes over time (*37*).

Serosurveillance refers to serosurveys that are conducted periodically or through the ongoing collection and testing of specimens to assess changes in seroprevalence over time.

Integrated serological surveillance or ***integrated serosurveillance*** of communicable diseases is the implementation of population-based serosurveys to collect and analyze samples and data from a defined population living in a geographic area at a given time for simultaneous estimation of prevalence of antibodies against multiple pathogens.

1.1.2 Uses of serosurveillance

When used to complement epidemiological surveillance and intervention coverage data, serosurveys provide information that can help estimate the size of the population susceptible to disease; characterize patterns of pathogen transmission; monitor changes in immunity over time due to exposure, infection, or interventions; and identify high-risk groups, among other purposes. Therefore, conducting surveys for objective quantitative measurement of biological markers provides a strong rationale and useful information that can be used to set priorities and guide policies and strategies for disease control and elimination (*38*).

The incorporation of serological surveillance into epidemiological surveillance systems can contribute to the early detection of outbreaks before the first clinically apparent case is observed; detect the reintroduction or reemergence of diseases in the post-elimination phase; and provide useful information for prediction models to understand the transmission patterns (*25, 39–42*).

Integrated serological surveillance supports monitoring and evaluation of the impact of public health interventions on disease transmission. It promotes cross-cutting efforts, strengthening surveillance systems, and generating better information to support decision-making in public health. It can contribute to innovate and develop more efficient surveillance options to improve universal health coverage and access to health care by the population, especially for groups living in vulnerable conditions. Potential uses of serological surveillance to support health situation analysis, improve epidemiological surveillance, and provide information for decision-making are listed in Box 1.1 (*43, 44*).

BOX 1.1 **Examples of potential uses of serosurveillance**

- Estimate the burden of disease
- Identify high-risk populations
- Assess the risk of outbreaks
- Determine the duration of immunity after vaccination and need to modify schedules or introduce boosters
- Monitor changes in pathogen transmission to target and evaluate control programs
- Monitor progress toward elimination goals and identify immunity gaps
- Detect the reintroduction or reemergence of diseases
- Establish theoretical herd immunity thresholds
- Investigate causes of the resurgence of diseases
- Evaluate the impact of interventions

1.1.3 Epidemiological scenarios for integrated serosurveillance

Within the framework of the joint PAHO/WHO–CDC initiative to incorporate integrated serological surveillance as a complementary tool for epidemiological surveillance and intervention coverage data in the Region of the Americas, at least three epidemiological scenarios in which this tool can be used to produce information to support public health decision-making have been identified (Table 1.2).

TABLE 1.2 **Epidemiological scenarios for integrated serosurveillance**

EPIDEMIOLOGICAL SCENARIOS	OBJECTIVES	EXAMPLES
1. Areas where epidemiological surveillance systems are fragile or in epidemiological silence	To provide complementary baseline information about disease transmission and intervention coverage data where information is lacking or unknown to support the design and implementation of interventions.	In hard-to-reach areas and populations living in areas with limited access to basic services (health, water, sanitation, education, safe housing, etc.), where information is not available and conducting studies for individual diseases or intervention coverage would be logistically complex and expensive, serosurveillance can help to identify high-risk groups for communicable diseases.

EPIDEMIOLOGICAL SCENARIOS	OBJECTIVES	EXAMPLES
2. Areas where interventions have been implemented and must be monitored to assess progress toward programmatic goals	To monitor changes in exposure (to vaccines or infectious agents) over time and ascertain whether the frequency or quality of interventions needs to be modified or strengthened.	In population groups and areas where disease control and elimination interventions have been implemented (vaccination, mass drug administration, intensified detection and treatment of cases, access to water and sanitation, integrated vector-borne diseases control management, environmental improvement interventions, improvement of personal hygiene, housing improvement, zoonoses control interventions, etc.), serosurveillance can provide information on the effect of these interventions to guide the decision-making.
3. Areas where diseases are close to elimination or have been eliminated and post-elimination surveillance is needed	To detect disease reintroduction or risk of reemergence toward monitoring achievement and sustainment of disease elimination.	In the post-elimination phase, for example for malaria, trachoma, or measles, serological surveillance can provide information on the exposure of cohorts born after elimination and provide early warning of the risk of disease reemergence. It can help anticipate risks, support more in-depth investigations, and help support initiation of actions to prevent reintroduction or reemergence of one or more diseases (e.g., those that have already been eliminated).

These scenarios can overlap within a country or in a population group in a specific geographical area depending on the epidemiological status or intervention coverage for each disease or program. For example, it can be possible that in a specific population the elimination of a disease (e.g., malaria) is on track but there are no data on the transmission of a neglected infectious disease (e.g., strongyloidiasis) although risk factors for its occurrence are present, and elimination of VPDs (e.g., measles and rubella) was reached and now they are in the post-elimination phase. In this case, the three epidemiological scenarios overlap in the same population. In this example, if an integrated serosurvey is implemented, serosurveillance can provide serological data to complement the malaria elimination process, which is important because in very low-incidence areas or areas without cases reported, the sensitivity of the surveillance system can be reduced.

Also, serosurveillance can provide baseline serological data to identify areas at risk of transmission of diseases such as strongyloidiasis that can guide public health programs to implement additional actions to understand better the magnitude and distribution of the disease to implement interventions accordingly. Serosurveillance can provide data to identify possible susceptible populations to measles and rubella in the post-elimination phase to implement vaccination interventions to avoid the reemergence of these diseases.

These three scenarios can help countries to identify diseases and interventions to be surveyed through an integrated serosurvey in a specific group of population and geographical area, and can be used to establish the serosurvey questions that are fundamental for designing the protocol and analyzing and interpreting the results, as will be described in the following modules of the toolkit.

1.1.4 Improving efficiency of serosurveys

Serosurveys for one or multiple diseases can be planned, designed, and carried out. However, it might be also possible that surveys for communicable diseases or coverage assessment are already planned, and with proper and timely coordination, serological studies can be included. This might help to use efficiently the available resources. The following are some examples:

- A collection of specimens for a single disease survey with a disease-specific study design. For example, in a vaccination coverage survey in which specimens will be collected, serologic data can be produced for other diseases. In this case, the survey was developed to assess vaccination coverage but, depending on the sample strategy, it could be used to study other diseases without any design modification.
- A collection of specimens for a single disease survey with a slightly expanded study design. For example, if a soil-transmitted helminthiasis survey will be carried out, the design of the survey can be slightly expanded to include the collection of specimens to carry out serological studies for other diseases of interest.
- A collection of specimens for a single disease survey with significantly expanded study design. For example, if a survey for a neglected infectious disease will be carried out, the study design can be expanded to include other diseases of interest for serosurveillance. In this case, planning and time to coordinate the adjustment of the study design are critical.
- Another example is the use of specimens collected in previous studies that are stored in biobanks to carry out serological studies.

In the above examples, serological studies can be integrated into already planned surveys, but there are some limitations to take into consideration:

- The sampling frame of the already planned survey might not be appropriate for serosurveillance of other diseases.
- Additional costs have to be considered.
- If stored specimens will be used, timing can be critical, and aspects such as seasonality of sample collection and if serologic data will be generated in an epidemiologically meaningful time frame should be considered.

Integration of several components within planned surveys, including the provision of health services, is important and ideal. However, careful and timely planning, coordination, and adequate design are important not only to obtain quality data but also to use resources effectively.

1.1.5 Challenges of integrated serosurveillance

Some challenges and limitations must be considered when implementing integrated serosurveillance. These include:

- **Costs and logistics.** Considering the epidemiological scenarios outlined in this document, challenges include geographic access and the operational costs of carrying out this type of surveillance. However, as surveillance is implemented for multiple diseases or events of public health interest, efficiencies will be generated in terms of the costs of investment and the return on investment (in the form of information), as several programs or strategies will benefit from a single effort. Also, studying multiple diseases in a single survey of a population of interest reduces the number of times individuals are required to participate in activities, thereby saving them time and related costs.
- **Collaboration and aligning the needs.** There are several aspects to consider when planning and implementing integrated serosurveillance. It is crucial to integrate capacities across programs, with the involvement not only of managers of individual disease control and elimination strategies but also statisticians, epidemiologists, and experts on specific diseases and topics, among others, to turn the data from integrated serological surveillance into useful information for public health decision-making. Inappropriate, incorrect, or incomplete use of results may lead to confusion among technical staff and decisionmakers and result in incorrect public health action being taken. Box 1.2 lists key elements for successfully integrated surveys (*45*).
- **Use of seroprevalence data for program decision-making.** Interpretation of serological data requires threshold values to monitor changes in transmission patterns of seroprotection (e.g., waning immunity from vaccination) and kinetics of antibody response for each disease of interest. This is very important, especially for diseases in elimination. Herd immunity threshold is a useful value to define when the percentage of a population immune to an infection, whether through vaccination or previous infections, is enough to reduce the likelihood of infection for individuals who lack immunity. Critical herd immunity thresholds are established for most VPDs (e.g., measles, rubella, mumps, polio, pertussis), but for many diseases, a seroprevalence threshold is not currently available to be used explicitly to define public health action (e.g., malaria and NIDs). However, serological data are a complementary tool to the epidemiological system and intervention coverage data.

BOX 1.2 Key elements for successful integrated serosurveys

Planning

- All key stakeholders, competing priorities, and mitigation strategies identified.
- National government buy-in and leadership at all levels and for each stage of the survey process.
- Support of national and international experts.
- Protocol developed with input from experts for all diseases included.
- Clear survey objectives.
- Development and/or strengthening of local capacity and knowledge-sharing.
- All correct equipment planned and procured.
- Well-planned logistics for all field-work activities.
- Sufficient time and budget allocated for the survey.
- All necessary approvals in place.

Training

- Sufficient planning, coordination, and funding for training.
- National and local program managers involved in the selection, training, and supervision of field team members.
- Standardized training materials.
- Training program combines theory with field practice.
- Means to assess survey task competence.
- Specific roles and responsibilities assigned to each individual.
- Field team member movements and sample handling reviewed in detail.
- Field-work pilot.

Implementation

- Good community awareness and mobilization.
- Strong supervision.
- Team leads who are technical experts in the different diseases.
- Organize and guarantee logistics for storage and transportation of samples according to the local context, capacity, and survey needs.
- Adequate selection of point-of-care (POC) diagnostic tests, well established procedures to handle multiple POC diagnostic tests and take advantage of multi-disease diagnostic platforms.
- Limit questionnaires to the minimum data needed for programmatic decision-making.
- Electronic data capture and management.
- Automated data analyses.

Source: Harding-Esch EM, Brady MA, Angeles CAC, Fleming FM, Martine DL, McPherson S, et al. Integrated survey methodologies for neglected tropical diseases. Trans R Soc Trop Med Hyg. 2020;115(2):124–6.

1.1.6 Future perspectives

Because the integrated serological surveillance initiative is still a work in progress, better collaboration across countries is ideal to expand the ability to use integrated serological surveillance, ensuring not only the use of standardized laboratory platforms but also to improve the ability to analyze, interpret, and use the results of the integrated serosurveillance to support decision-making in public health. The expanded use of integrated serosurveillance in different epidemiological scenarios will contribute to better understand and use this tool for supplemental surveillance of communicable diseases and intervention coverage data.

Likewise, the support and participation of the countries of the Region of the Americas are essential to contribute to the characterization and validation of antigens for diseases of interest such as Chagas disease, leishmaniasis, and leprosy, among others, for which currently there are no reliable serological tests. This will allow continued strengthening of laboratory platforms such as the MBA and will increase its potential for monitoring of additional pathogens of interest, thus making surveillance more cost-effective.

Module 2

Designing the integrated serosurvey

This module describes the following steps to design an integrated serosurvey.

Designing the serosurvey

1. Assess need for serosurvey
2. Create a steering group
3. Identify primary questions
4. Define objectives
5. Set inferential goals
6. Define target population
7. Select area to survey
8. Select survey design
9. Calculate sample size

2.1 Assess the need for serosurvey

Developing an integrated serosurvey requires technical discussions and coordination, as the desired survey outcomes for each disease of interest may require different sampling approaches and sample sizes. The need to conduct a population-based survey for integrated serological surveillance may be guided by the need to obtain information in one or more of the three epidemiological scenarios described in Module 1.

Key aspects must be taken into consideration to assess the need for an integrated serosurvey:

- The questions to be answered and the feasibility of carrying out a survey for several communicable diseases must be clearly defined. Aligning interests and needs of different programs can be challenging.
- To define whether serological information is required for two or more diseases of interest occurring in a given population, in a specific geographic area, and at a certain time, it is important to bear in mind the applications and scope of survey-based integrated serological surveillance, considering that IgG antibody levels are indicative of past exposure (months to years).
- It is necessary to define the most appropriate source and cost-effective process to collect the samples for the survey—whether to incorporate sample collection for serological surveillance into an already planned survey, such as a large national health survey, an NID survey, or a VPD survey, or use existing serum samples from a serum bank. Annex 2.1 includes examples of surveys into which serological sampling could be incorporated.
- Existing samples from a serum bank have great potential for the analysis of population immunity profiles against communicable diseases, although they may have some limitations, such as representativeness only of certain age groups or certain geographic areas; limited data on the demographic, social, and economic characteristics of the subjects from whom samples were obtained; and insufficient sample volumes to carry out additional studies (*46*).
- If using a serum bank is a possibility to reduce resources and time, key information is required about the sampling strategy, questionnaires used, stored specimens available, consent forms, and ethical considerations, among others.

2.2 Create a steering group

The steering group plays an essential role during the initial phases, supporting and participating so that others can provide strategic information, endorse the implementation of the integrated serological surveillance strategy and the mobilization of necessary resources, and liaise with other sectors or strategic partners that should be involved (e.g., grassroots organizations, community leaders, the education sector, other sectors).

The composition of this group will depend on the characteristics and complexity of the integrated serosurvey, but, overall, it is recommended that the following professional skill sets are represented:

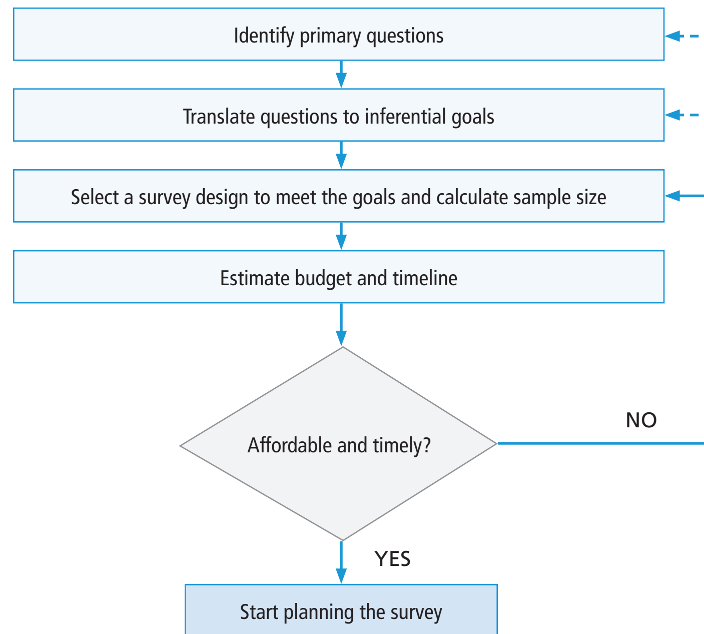
- Program managers who have adequate knowledge of the occurrence and trends of the diseases in different epidemiological scenarios, and background information on the programs and interventions that are already in place to address these diseases in the study population; who can contribute information and make decisions about the objectives of the survey; and who will participate actively in the design, implementation, analysis of results, and decision-making.
- Experts in the diseases and epidemiologists that support the determination of the objectives and help to understand the specific characteristics of the occurrence of the disease, the cycle of transmission, the immune response, etc. If serum samples of a serum bank will be used, people who have the data and information of the existing residual sera should be involved in the steering group.
- Statisticians or sampling experts, to support the definition of sample design, calculate the minimum sample size according to the degree of representativeness required, define parameters according to the set objectives, and support data management and analysis of results. It is useful to include delegates from the national statistics institute of the country because they can provide demographic data, maps, and support sampling frames and size calculations.
- Biologists, biochemical scientists, or experts in laboratory methods, who know analytical methods, sample collection and transport logistics, custody and processing of samples, quality assurance, interpretation of laboratory test results, and biosafety procedures, among other aspects.
- Additional help with data management and analysis will likely be needed in terms of advising on data collection, development of the database, management of data entry, as well as data analysis.

The steering group not only participates during the initial stage of the survey design; they must be involved in the implementation, analysis of data, interpretation of results, and decision-making stages.

2.3 Identify primary questions to be answered with the serosurvey

A series of questions that affect the survey design and sample strategy must be discussed and agreed upon before working on a protocol (Figure 2.1). It is necessary to translate those questions to inferential goals.

FIGURE 2.1 Early steps to design the serosurvey



Adapted from: World Health Organization. Vaccination Coverage Cluster Surveys: Reference Manual [Internet]. Geneva: WHO; 2018 (WHO/IVB/18.09). Available from: https://apps.who.int/iris/handle/10665/272820.

Some primary questions are:

- What are the objectives of the survey or the questions the survey is meant to answer? If multiple diseases are of interest, is there one or some that are more relevant? Why?
- In which population and geographic area is it relevant to conduct the survey? Why?
- What are the hypothesis-testing questions that will be assessed: is it a programmatic threshold or comparison between populations and geographic areas or between demographic and socioeconomic characteristics?
- What are the desired survey outcomes and levels (or strata), and which estimates of those outcomes are desired (e.g., national, state, municipality; ages 0–14 years, 5-year age group)? Are there any special populations of interest for which estimates would be helpful? Depending on the robustness of the results needed, could a non-probabilistic sample be useful to answer the primary question?
- What is the best estimate of the expected outcome (e.g., 80% seroprevalence) and precision (e.g., ±5%) required?

2.4 Define objectives

The objectives will depend directly on the needs of the epidemiological context, on the necessary level of survey representativeness—i.e., the need to obtain disaggregated information with sufficient statistical power to make inferences at different strata; e.g., by geographical level (national, subnational, regional, district, municipal, etc.), age group, sex—serology test available, and on the available funding and technical capacity (personnel, laboratory).

The **primary objectives** for the serosurvey should be defined based on the primary or most important outcomes of interest that drive the survey design and sample size. There are three types of primary questions (*37*):

- **Estimation questions** that will result in a quantitative estimate of seroprevalence.
- **Classification questions** that yield qualitative seroprevalence labels (e.g., "high," "intermediate," or "low," instead of precise quantitative estimates).
- **Comparative or hypothesis testing questions** that compare seroprevalence with an important programmatic threshold (e.g., target immunity levels for measles or level of transmission of enteric pathogens), or before and after the intervention, or between categories such as geographic strata, age, sex, education, or wealth.

Extending the geographical area or age range beyond the minimum defined may be necessary to accommodate the objectives of other diseases; however, countries should recognize that this will increase the cost and complexity of the logistics of the survey.

An alternative may be to accept that the survey is not ideally designed or of sufficient size to address the objectives of the other diseases and to consider these as **secondary objectives** where some information is obtained but usually less than would be the case if the survey was designed for this sole purpose.

2.5 Set inferential goals

An inferential goal states how much uncertainty is acceptable in the primary outcome. In general, the more certain you need the outcome of the survey to be, the larger the sample size you will need. Uncertainty and inferential goals depend on the primary survey question and objective of the serosurvey.

- When estimating a quantitative estimate of seroprevalence, the inferential goal is expressed as a confidence interval (CI). Select a sample size that balances precision (typically represented with the 95% confidence interval) with the budget and time required to survey large numbers of respondents.
- When classifying qualitative seroprevalence labels, the inferential goal is expressed using the probability of classification error (often called misclassification).
- When comparing two seroprevalence estimates using a formal hypothesis test, the inferential goal is expressed as statistical power.

Because the process of designing a survey is dynamic and iterative, if very ambitious or unfeasible objectives are defined, the proposal may need to be revised and adapted during the design stage. Conversely, if it is clear from the outset not only which goals are desirable but also relevant and possible, valuable time and resources will be saved.

2.6 Define the target population

One challenge when selecting the target population of survey-based integrated serological surveillance is that different diseases may affect different populations of interest. For example, surveillance of antibody seroprevalence in the pediatric population has been used to monitor vaccine-preventable diseases (VPDs), because young children will reflect more recent program performance (*24*) and identify at-risk populations.

However, wider age ranges will consider the additive effect of any supplementary immunization on top of routine immunization, as well as waning immunity (e.g., tetanus, diphtheria), which could be relevant for changing immunization schedules or adding booster doses. Waning immunity in adults (e.g., tetanus, diphtheria) could be relevant for assessing sex-specific risk from differential receipt of booster doses (e.g., women for maternal–neonatal transmission elimination, or males in the military).

In the majority of neglected infectious diseases (NIDs), serological surveillance of children may reflect the intensity of transmission and—more accurately—demonstrate recent exposure, which is useful for monitoring the interruption of transmission or recrudescence. For example, in some studies of antibody seroprevalence for NIDs, children have been the ideal population to assess the impact of deworming and water and sanitation interventions for the control of soil-transmitted helminthiasis. In the case of lymphatic filariasis, onchocerciasis, and schistosomiasis, children aged 6 to 15 years have been studied, while children aged 1 to 9—preferably, under 3—are the target populations for trachoma surveillance (*31*).

Another example is malaria, when estimating the persistence of *P. falciparum* transmission. If the transmission is sustained, no matter at how low a level, the parasite will still be present in the area of interest, and residents will have a greater cumulative risk of lifetime exposure as they age. Conversely, if the transmission is occurring at a moderate or high level, children aged 0 to 11 months in the area of interest will show a rapid increase in antibodies against the parasite (*36*).

When designing serosurveys to monitor several diseases at once, it is essential to determine the optimal target age group to ensure the objectives of the study are met. There may be different age groups of interest for each surveillance objective. For example, in the post-elimination phase of any NID or malaria, children under 15 (or even under 5) are ideal groups, because antibody levels in young children are a sensitive measure of recent exposure to pathogens and have been shown to fluctuate with seasonal changes in malaria transmission (*17*). However, it is also important that serological studies of malaria also survey populations other than children, such as young adults (age 20 to 29) and older adults (over

60), due to the risk of complications in these age groups. Other diseases in which surveys of the adult population can be useful include HIV, rubella, and tetanus, among other pathogens (*25*).

If representative data are required from multiple age groups of interest for the serosurvey, it is important to consider the feasibility of enrolling a large sample size in each of the different age groups. Also, seroprevalence estimates for multiple age groups will have an exponential impact on survey size if the serosurvey is meant to provide representative estimates for additional strata; for example, states or provinces, specific ethnic groups, or rural vs. urban areas.

Box 2.1 summarizes some aspects that should be considered when defining the population to be monitored by survey-based integrated serological surveillance.

BOX 2.1 **Example of criteria to define the population of interest in a serosurvey**

Selecting the population of interest for a serosurvey depends directly on the proposed objectives of surveillance, as well as on the characteristics of transmission of the pathogen, the immune response to the disease, and the intervention, prevention, control, or elimination strategy in place in the country.

When defining the objectives, other characteristics apart from age—such as the risk of contracting the illness or belonging to certain population groups of interest (ethnic minorities, migrant population, etc.)—should also be considered. For special populations of interest, a practical consideration is whether a frame exists to allow sampling of individuals in this group, or whether they live in a particular area that could be oversampled to address the objective.

It is also important to define whether two or more populations (such as two different age groups) are required, depending on the objectives of the survey.

2.7 Select area to survey

The geographic area of interest for the survey is the area or areas inhabited by populations exposed to risk factors for transmission or immunity gaps; that is, the areas where social determinants, difficulties in access to health services, and environmental or ecological conditions conducive to the transmission of the pathogen of interest are concentrated. Researching small spatial scales has also allowed high-income countries to obtain accurate information on NIDs.

Robust findings in an integrated serosurvey are associated with the delimitation of the geographic area of interest. It is necessary to determine whether national or subnational representativeness is needed, keeping in mind how the results of the survey will be used to support decision-making (conducting additional research or implementing interventions).

The availability of data from previous surveys can be used to inform which geographic areas need to be monitored. These include existing baseline data, surveillance data, morbidity and mortality studies, environmental information, and data from geospatial modeling studies that show the predictive likelihood that the prevalence of the disease of interest is exceeding a given threshold, among others (*47–49*).

In surveys that cover multiple diseases, the area of interest for each disease may be different. Therefore, the delimitation of the survey area should start by achieving a consensus among those responsible for the programs or strategies involved. As an example, if a survey is being conducted to ascertain the seroprevalence of antibodies against malaria in residual foci of transmission, the area of interest will be defined by identifying these foci (high-transmission areas), and the survey should include an analysis of *receptivity*[1] and *vulnerability*[2] in the selected area (*50, 51*).

It is necessary to understand the sociodemographic, epidemiologic, and environmental characteristics of the populations living in each geographic area. Rural areas can face limited access to basic health, education, water, and sanitation services; however, urban areas are also of interest due to the greater concentration or mobility of the population, and because cities often grow by the expansion of informal settlements.

It is important to take into consideration that, due to the different transmission dynamics inherent to different diseases, the utility of looking for several diseases will be different. As an example, dengue could be urban or rural, but most NIDs would be much more likely to be found in rural settings; or, including schistosomiasis in surveys where no one is residing near freshwater would be of limited utility because transmission is dependent on freshwater snails.

1 Receptivity: degree to which an ecosystem in a given area at a given time allows for the transmission of *Plasmodium* spp. from a human through a vector mosquito to another human.
2 Vulnerability: likelihood of malaria infection based on living conditions or behavioral risk factors, or likelihood of increased risk of severe morbidity and mortality from malaria infection.

BOX 2.2 Defining the areas to survey

Countries that decide to perform survey-based integrated serological surveillance of (selected) communicable diseases should base their decision on their epidemiological context, which may be characterized as one of the three epidemiological scenarios.

It is important that the decision be made consensually with those in charge of the national programs for control of surveillance of the diseases to be included in the survey, who, in addition, can be called upon to provide information on the epidemiological context and take part in setting objectives for the survey according to each one of the scenarios described in Module 1:

- Provide reliable information about disease transmission and intervention coverage data to support the design and implementation of interventions where information is lacking or unknown.
- Monitor and evaluate the impact of interventions and identify changes in exposure to pathogens or coverage of interventions (e.g., vaccination).
- Detect disease reintroduction or risk of reemergence toward monitoring achievement and sustainment of disease elimination.

2.8 Select survey design

Probability sampling strategies are: simple random sampling, systematic sampling, stratified random sampling, and cluster sampling. In household or school-based surveys, cluster sampling is very common because it is more cost-effective and efficient compared with simple random sampling.

The sampling strategy will depend on the objectives of the study, the geographic area of interest, and economic and logistic feasibility, among other aspects. If the team decides to use existing data and serum samples, the sampling strategy will depend on the protocol used to implement the already available survey.

The representativeness of a sample of a survey allows one to extrapolate—and, therefore, generalize—the observed results of the survey to the target population. A sample is considered representative of the target population when the distribution and value of the variables of interest in the sample can be reproduced within calculable margins of error (*52*). On the other hand, the subjects selected must also be representative of the population from which the sample was drawn, concerning the distribution of the variable of interest in the overall population (*53*). Systematic errors may arise during any phase

of the survey and create bias or systematic deviations that can overestimate or underestimate the population parameter.

2.9 Calculate sample size

In general, to estimate the sample size, parameters such as the expected prevalence of the event of interest, the desired level of confidence, precision, statistical power, design effect, and non-response rate must be considered, among others (Table 2.1). Sample size calculation in integrated serosurveys must take into account the primary objectives of the study and the expected outcome for the study population of interest (e.g., age group, specific geography) and desired level of precision. The criteria for sample size calculation should be applied to each of these, according to the necessary statistical parameters.

TABLE 2.1 **Necessary parameters for sample size calculation**

PARAMETER	DEFINITION
Expected seroprevalence rate (p)	For surveys of this type, p is defined as the expected seroprevalence for each of the pathogens of interest or, in the case of VPDs, the expected seroprotection rate. If the actual rate is unknown, a value of 50% is advised.
Level of confidence 100(1–α)%	Type I error, or alpha (α) error, also known as the level of significance of a test, is the likelihood of rejecting the null hypothesis when it is true. It is associated with the confidence interval, which will generally be 95% for sample size calculation purposes.
Precision (*d*)	Also known as relative sampling error, precision determines the accuracy of the results. The greater the precision required, the larger the sample must be, and the more accurate the results of the study. For sample size calculation, a level of 5% or 10% is usually established, but this will depend on the expected prevalence of the disease of interest.
Statistical power	Is the probability of a hypothesis test of finding an effect if there is an effect to be found. It ranges from 0 to 1. The probability of making a type II error (wrongly failing to reject the null hypothesis) decreases as statistical power increases.
Non-response rate	Non-response is defined as the impossibility of obtaining the measurement of one or more variables of interest for one or more elements (*k*) selected for the survey. For general surveys, the calculated sample size is usually increased by 10% to account for non-response; however, this should be considered within the context of the survey and the quality of the sampling frame.
DEFF	Design effect (DEFF) is the variability due to the subject selection when any sampling method other than simple random sampling is used. The variability depends on the stratification of the sample, the number of respondents per group, and the heterogeneity of this number.

It is advisable to rely on epidemiologists, statisticians, or trained sample specialists when doing these calculations and more complex ones (e.g., to calculate sample sizes for diseases whose expected

prevalence is zero or close to zero), to ensure that estimates are appropriate for the defined objectives, the population of interest, and the desired representativeness.

In surveys in which serum bank samples are used to perform retrospective analyses of seroprevalence or seroprotection, the working group must include a statistician who can calculate the sample size based on a detailed assessment and understanding of the original study in which the samples were collected (sampling frame, representativeness, etc.). Again, this will help ensure that the parameters used to calculate sample size responds to the objectives of the survey and is appropriate for the established population groups and the geographic area of interest.

There are available guidelines on the use of serosurveys of VPD (measles and rubella, hepatitis B, dengue, tetanus). The following documents provide more detailed procedures about designing and conducting serosurveys (*37, 54, 55*).

- World Health Organization Regional Office for Europe. Guidance on conducting serosurveys in support of measles and rubella elimination in the WHO European Region. Copenhagen: WHO; 2013. Available from: https://apps.who.int/iris/handle/10665/350485
- World Health Organization. Documenting the Impact of Hepatitis B Immunization: best practices for conducting a serosurvey. Geneva: WHO; 2011. Available from: https://apps.who.int/iris/bitstream/handle/10665/70808/WHO_IVB_11.08_eng.pdf
- World Health Organization. Informing vaccination programs: a guide to the design and conduct of dengue serosurveys. Geneva: WHO; 2017. Available from: https://www.who.int/publications/i/item/9789241512589
- World Health Organization. Tetanus serosurveys. Annex 2 in: Surveillance standards for vaccine-preventable diseases, second edition. Geneva: WHO; 2018. Available from: https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-annex2

Module 3

Planning the serosurvey

This module describes the following steps to plan the serosurvey.

Planning the serosurvey

10. Develop proposal
11. Design data collection tools
12. Establish laboratory procedures
13. Define roles and select staff
14. Calculate budget and resources
15. Set timeline
16. Obtain ethical clearance

3.1 Develop proposal

Each serosurvey needs a written protocol, which should cover all of the technical, ethical, and logistics aspects, as well as the budget and timetable. It must be written in a clear, simple fashion so that it can be understood by all those involved in the development and implementation of integrated serological surveillance.

The protocol serves as the basis for organizing all field operations. It defines the problem of interest for surveillance and lays out all the information needed to conduct the serosurvey. This is particularly useful when requesting funding and for peer review or review by ethics committees (*56, 57*). It should include a title, table of contents, summary, introduction, theoretical or conceptual framework, problem statement, rationale, objectives, methodology, ethical considerations, analysis plan, limitations, budget, schedule or timetable of activities, and references. Forms such as questionnaires, informed consent templates, and others can be provided as appendices. Once it is complete, the protocol must be submitted to the relevant ethics committees for approval. Annex 3.1 includes a protocol template example.

3.2 Design data collection tools

3.2.1 Define the variables

The variables of interest in serosurveys should answer the research questions and should be useful for the objectives of the study. This is particularly relevant in surveys covering multiple diseases, as there is the challenge of collecting information for each disease of interest, which creates greater complexity, increases the time needed to complete the survey, and may increase costs. Also, it poses additional challenges for the analysis of the results.

Depending on the research questions and the objectives of the study, variable categories may include:

- Demographic variables: age, sex, place of residence, ethnic group, etc.;
- Socioeconomic: occupation, educational level, income level, etc.;
- Household conditions related to water and sanitation, overcrowding, etc.;
- Vaccination history: doses administered according to routine schedule and campaigns;
- Knowledge, perceptions, and practices such as handwashing and hygiene, travel history, etc.;
- History of illness or related symptoms (e.g., lymphedema and hydrocele for suspect lymphatic filariasis, fever for specific vector-borne diseases).

When analyzing immunity to vaccine-preventable diseases (VPDs), a key aspect is to collect data of the vaccination history; this will allow estimation of the efficacy of vaccination or of waning immunity as a function of the time elapsed between vaccine administration and sample collection. This requires reliable data on the date of vaccination and age at vaccination, preferably collected from individual vaccination records and, if feasible, from nominal records generated by health facilities. These data must

be recorded correctly. Alternatively, vaccination records may be photographed to ensure accurate data entry at a later stage.

In the case of already planned surveys leveraged for serological studies, the team should assess the set of variables that will be collected and advocate for the inclusion of variables of interest in the serosurvey, if feasible. Otherwise, a consensus should be reached with the investigators in charge of the primary survey regarding the variables required for the serological study, operational aspects such as file structure, a data dictionary, and turnaround times, and any ethical aspects related to data confidentiality. If the use of serum or blood bank samples is being considered, whether a database exists and is available—and, if so, which variables it contains—should be checked with the serum bank manager. If so, the next step is to ascertain whether these variables contribute to the objectives, population groups, and geographic area of the serological study.

3.2.2 Questionnaire design

Questions to include in the questionnaire should be those directly related to the variables of the serosurvey that should answer the research questions. When designing a data collection questionnaire, it is important to devote time to define the order of the sections and questions, establishing a skip logic and filter questions, and writing detailed instructions, among other aspects. When writing, questions should be clear and precise; joining two or more questions into one should be avoided, as should the use of biased or emotionally charged terms, negative words such as "no," "none," or "nobody," and overly long answers or choices.

The potential need to translate the questionnaire into the local language should be assessed. The questions should use colloquial, familiar, or vernacular language, according to the cultural context of the study population, but care should be taken that the instructions and filter questions are written as defined by the team.

Annex 3.2 includes sample questions to assess vaccination and neglected infectious diseases (NIDs) such as soil-transmitted helminthiasis, schistosomiasis, lymphatic filariasis, and water, sanitation, and hygiene (WASH) risk factors. The questionnaire must be validated and subjected to pilot testing before or during the training of field teams. Furthermore, a need for changes may arise during training: if the paper and pencil interview (PAPI) methodology is used, there should be no rush to have the questionnaire printed; if the computer-assisted personal interview (CAPI) methodology is used, it is essential for the developer in charge of creating the application to be in constant contact with the study coordinator and data management team.

As samples collected in the field are usually only analyzed several weeks after the study, laboratory data must be linked to the sociodemographic variables collected during the interview through a unique, individual identifier for each participant. The use of identification codes is recommended to link

questionnaire data with laboratory data and variables from other forms (e.g., informed consent forms). This not only facilitates data linkage but also helps maintain anonymity.

When defining the variables, identifying the questions, and designing the questionnaire, it is essential to specify the desired analyses, table shells, and figures at an early stage of the survey, to ensure that the survey design will be adequate to meet the survey objectives. People responsible, time, and budget (if necessary) for the analysis of data produced by serosurveillance surveys should be included in the protocol and timetable.

It is essential to ensure timely availability of results for decisionmakers, participating communities and individuals, and public health programs involved in the serosurveillance. Delays or lack of results may result in missed opportunities to implement interventions, lack of trust and engagement from the population, partners, and interprogrammatic and interdisciplinary teams, jeopardizing the integrated work for future initiatives.

3.2.3 Data collection methodologies

Traditionally, the paper and pencil interview (PAPI) methodology has been used for data collection. To minimize measurement errors, field teams should be apprenticed to personnel with proven interviewing and data collection skills, who will provide proper training and supervise field work to ensure good performance. This methodology requires that data collected on paper be entered manually into a software program. In this setting, the use of double data entry is mandatory to keep processing errors to a minimum.

Computer-assisted personal interview (CAPI) can use a laptop, smartphone, or tablet for data collection instead of pen and paper, which is an excellent alternative that even allows real-time monitoring (online or offline) of data collection and quality. As CAPI systems rely on the Internet to send data to a server for storage, Internet access and electricity are required to keep the data collection devices running.

Although CAPI can be a challenge in remote rural areas without access to these services, it can improve data quality by reducing the errors on data entering during the survey, allows providing feedback to data recorders while they are in the field collecting data, and the progress on the field work by field teams can be tracked using global positioning system (GPS) data, among other advantages. There are several systems to collect data using mobile devices that can store questionnaires offline which can be uploaded to Cloud-based services once the device is connected to the Internet. Furthermore, solar-powered portable chargers can be used to keep devices running.

Although this methodology may appear complex or expensive, if good planning is ensured, including several rounds of piloting the forms, effective training, and technical support and supervision during the field work, it allows the collection of higher-quality data in a shorter time and obviates the need for double data entry; the higher initial cost is thus offset by savings during and after the field-work stage.

3.3 Establish laboratory procedures

All aspects of the laboratory testing and reporting should be thoroughly planned well in advance of starting the study. Module 5 on laboratory methods describes in detail the planning aspects related to the laboratory and should be consulted to develop the protocol.

3.4 Define roles and select staff

To implement the serosurvey, the roles and responsibilities of staff who will be involved with planning, data collection, and analysis must be clearly defined and must be carefully selected before the field work starts. Some of these persons can be hired, depending on complexity of the survey and availability of capable staff in the national institutions participating in the serosurvey.

The survey coordinator has a key role because he/she is responsible for overseeing the study implementation by ensuring compliance with the survey design. The coordinator must procure the required supplies and resources, define the best logistics to collect data, conduct appropriate and timely training and supervision, track field-work progress to ensure high-quality data collection, entry, and management, and guarantee respect for confidentiality of participants, among others. The coordinator is supported by laboratory specialists, data managers, and regional supervisors. An administrative person also must be assigned to assist on budget management, procurement, and logistics of the survey.

Supervision is critical to ensure high-quality data. Depending on the sample size and characteristics of areas to be surveyed, the national coordinator must be supported by regional supervisors assigned to one or more strata of the survey. They must assist on the preparation of materials and supplies to be used in the field, ensure that field teams are trained and carrying out their functions correctly, review the list of sample units, and organize the field-work routes according to the design of the survey. They should address any problems or contingencies that may arise during the operation. Field supervisors are also crucial to make sure that the field work is conducted according to the survey design and procedures. They are often selected during the training, based on their leadership and good performance.

The number and composition of the field teams will depend on the survey design and operational procedures for data and sample collection, but overall, each team must include an interviewer, laboratory technician(s), and a team leader, that will be in charge of coordinating the field work, contacting the community leaders, health centers, and schools—depending on the survey design—and keep in close contact with the assigned supervisor. Drivers also perform an important role, in that they ensure proper timing and safety of transportation to and from the sites and the safety of the teams. Annexes 3.3 and 3.4 describe the main responsibilities of key roles of survey staff at the national level and in the field.

3.5 Calculate the budget

The budget for an integrated serological surveillance survey will depend on the defined objectives, survey design, and sample size. It must be realistic while providing for the highest-priority aspects. Some of the information necessary for budgeting includes the cost of sample collection, transportation, and processing; training; pilot testing; laboratory and survey supplies; field work (including daily allowances, transportation, and fuel, as appropriate); supervision of field teams; data analysis; and the selected questionnaire format (PAPI or CAPI: the former will require printing, while the latter will require specific data-collection software and data management support to collect good quality data). The working group must identify costs and funding gaps, and, with this information, establish strategies to look for funding options to close these gaps.

It is important to determine whether the budget is subject to restrictions (deadlines, types of expense, etc.). It is also necessary to check whether there are processes in place to authorize, expand, or reduce the budget, and to identify possible sources of additional funding (e.g., donors or other sources of cooperation). It is also important to monitor spending and identify funding gaps that might affect the implementation of the survey. Annex 3.5 lists the supplies and materials required to collect dried blood spots, and Annex 3.6 includes a template to calculate the budget.

Survey-based integrated serological surveillance of communicable diseases is proposed as a tool for national surveillance systems to produce supplemental information to support public health decision-making. Therefore, to ensure sustainability, it is recommended that national and subnational governments include funding for this surveillance activity in their annual budgets.

3.6 Set the timetable

One way of organizing and monitoring survey activities is to consolidate a timetable, which can be adjusted as the development of the survey progresses. The timeline will depend on the type of serosurvey being conducted. For instance, surveys that require sample collection can usually be carried out over 2 months (field work alone), but all field activities must be planned well before and during the field work, which can add up to 12–18 months. This timeline will differ if the survey is to be carried out within the framework of another already planned study. Surveys designed to use existing samples from a serum bank, in turn, can be carried out in a relatively shorter period, although the careful analysis of data available and characteristics of the samples banked should be completed ahead of time to define later the survey questions that can be answered with those samples.

Countries must recognize that the ethical approval process of survey protocols takes time, due to revisions, possible adjustments, and the likelihood that reviews by different committees (such as a national research ethics committee or one of the organizations or institutions participating in the survey) will be required.

The supply procurement process must be taken into account (e.g., some supplies may need to be brought in from overseas), while during the implementation stage, the timetable must be adjusted around any particular events in each country that might cause delays in compliance (public holidays, vacations, the school calendar; operating distances; the rainy or wet season; population movements; etc.). Seasonal variations (e.g., the local rainy or dry season) must also be considered when planning field work as weather conditions can determine the feasibility of reaching the geographic areas of interest. A sample study timetable is shown in Annex 3.7.

3.7 Obtain ethical clearance

Previous sections of this document have mentioned potential sources of samples for serological studies (primary collection of samples, samples collected for already planned studies, and serum bank samples). Different ethical aspects apply to each of these sources and must be taken into consideration. Annex 3.8 describes key elements to consider for ethical aspects.

3.7.1 Ethical aspects in surveys that include sample collection

In any epidemiological surveillance process, including those in which population surveys are conducted, participants must be properly informed and understand the objectives, procedures, scope, benefits, and risks involved in their participation; only then will they be able to make a voluntary and informed decision. An essential aspect is to guarantee the protection of the privacy and confidentiality of personal data linked to the samples, questionnaires, and results of the survey. This can be done, for instance, by assigning a unique identifier or code, thus ensuring that samples remain anonymous.

Field teams and supervisors must be trained on ethical aspects of the survey as well as on the process of getting informed consent from participants, interview techniques, and the process to explain the survey objectives and procedures in the appropriate local language used by the target population and encourage them to participate in the survey. If the serological surveillance survey requires the collection of samples from minors ("minors" will be defined according to the laws of each country but will usually be taken to mean children under 18 years), informed consent must be obtained from each minor subject's parent or guardian. In the case of children over 9, who can understand and agree to participate in the survey, it is also recommended that their informed assent be obtained (Annex 3.9). No child should be forced to participate in a survey, even if their parents or guardians have given consent.

It is recommended that participants be asked to give broad informed consent for future use of the samples. This reduces the number of times that the same population has to participate in studies that collect biosamples, allows the use of samples collected for future studies based on new protocols that should be approved by ethics committees, etc. (*58*).

3.7.2 Ethical aspects of utilization of serum bank samples

Integrated serosurvey protocols designed to use blood or serum bank samples must be approved by the respective ethics committees. An important aspect to consider is a thorough review of the

ethical procedures of the original study in which the serum and blood bank samples were obtained. Understanding the ethical aspects, scope, and limitations for new studies is an essential component of designing protocols for the use of these samples in integrated serosurveys.

Each serosurvey must include procedures to protect human subjects in accordance with the Declaration of Helsinki of the World Medical Association and must comply with all relevant local and national ethical regulations, as well as with any requirements of agencies and institutions involved in the survey.

Once the protocol has been completed, it must be reviewed and approved by a national ethics committee and by the corresponding committee or committees of any collaborating institutions that so require. In the case of studies that will receive funding or technical support from international organizations or donors, their institutional regulations and procedures must be assessed for compliance with ethical approval requirements (*37, 59, 60*).

Module 4

Conducting the serosurvey

This module describes the steps to conduct the serosurvey, as follows:

Conducting the serosurvey

- 17. Carry out pilot testing
- 18. Train field teams and supervisors
- 19. Collect data and blood samples
- 20. Analyze blood samples

4.1 Carry out pilot testing

To carry out the serosurveys, the instruments and procedures must undergo pilot testing so that any issues can be identified before running the study. Pilot testing consists of running a small study that can be used to determine the feasibility of the protocol and identify weaknesses so these can be addressed before starting the large-scale field operation.

Some relevant aspects of the pilot testing stage include evaluating the questionnaire (for consistency and comprehension of the items by the interviewers and interviewees, whether the questionnaire flows well, the time required to complete it, etc.); evaluating the data collection platform (electronic or paper and pencil); reviewing the sampling procedure, time taken in each process, and response rate; assessing the need for revisiting (second visits) in the field, etc., so that any issues can be identified and corrective action taken early.

In the case of using electronic data collection, programming must be done early to allow time to review and correct any errors, assess skip patterns, carry out data quality checks, and deal with any queries raised during the training and pilot testing (*37, 61*).

Pilot testing should be done by members of the coordinating team, alongside coordinators and supervisors. Conducting the pilot test as part of field-team training is also an effective training method that also allows performance evaluation of the roles of each team member and advance implementation of the field workflow.

4.2 Train field teams and supervisors

Training of field team members is essential to ensure the quality of the data and samples that will be collected and to obtain data that will answer the research questions the integrated serological surveillance program was designed to address. Ideally, training should take place just before the start of field operations and should be led by trainers and facilitators who are familiar with all procedures of the survey protocol in detail (epidemiologists, laboratorians, health workers, systems technicians, translators into local languages, community partners, etc.). Training must allow sufficient time to review and adjust procedures, questionnaires, and consent and assent forms. Box 4.1 describes some of the necessary elements that must be considered in the training of field teams.

BOX 4.1 **Essential elements of field-team training**

The goal is to standardize the performance of survey procedures among staff. Various teaching methodologies can be used, as long as they are suitable for training adults. The chosen training methodology should be interactive and dynamic, including hands-on practical exercises and role-playing with clear examples that allow participants to understand and practice their roles within the field team. Ideally, training should include practice under the same (or similar) conditions as those encountered during field work. For instance, if the survey will be conducted in rural schools, practical training in a school with these conditions should be part of the process.

If the survey will be carried out in remote rural communities, information about the characteristics of these populations and how to communicate (language, ethnic and cultural considerations, etc.) should be included in the training. Although practice under these special conditions may be perceived as an additional expense, it is in fact an investment to ensure the quality of the sampling procedures, which should result in quality data that respond to the objectives of the survey.

Among the topics to be addressed are the theoretical framework; methods for sample collection, storage, and transport; data collection and logistics processes for the interview; ethical and biosafety aspects; and proper management of supplies and materials, among others. Annex 4.1 contains an example of a training agenda for personnel who will participate in a survey involving sample and data collection.

Before training, the number of field teams (and their size) should be defined, as well as the structure, roles, and functions of each member. As a result of training, it may be necessary to reassign roles and functions among the members to ensure optimal flow, performance, and quality of field work.

The supervisors of field teams must also participate in the training, as they play an essential role in supporting the proper implementation of protocol procedures and taking appropriate corrective measures when needed. Those in charge of data entry and data management must also be trained, including data entry specialists (if a paper and pencil interview (PAPI) design will be used) and analysts who will run the databases and interpret the results.

Likewise, laboratory personnel must have completed proper training for analysis of the samples by the selected assay method (if samples will be analyzed in-country) and must take part in field-team training to ensure that the procedures for collecting, preserving, storing, and transporting the samples are carried out in a standardized manner.

In the case of already planned studies in which additional samples will be collected for serological analysis, it is essential to ensure that field teams are properly trained in the collection, preservation, storage, and transportation of the samples for analysis. For studies with serum bank samples, training should cover sample selection and conservation and transport of the aliquots taken for serological study. In both cases, those individuals in charge of compiling the databases and analyzing and interpreting the results will require training.

4.3 Coordination before starting the field work

In the case of school-based surveys, prior coordination with regional coordinators, school principals, and teachers must be ensured. This activity should be carried out by the survey leader and operational team and should be scheduled well ahead of the start of field operations, as more than one visit may be required. It involves close coordination with those responsible for the education sector at the national and local levels, as well as with the principals and teachers of the selected schools.

If the survey will be carried out in households, the survey leader should ensure that proper communication and coordination is done well ahead of field operations with local health authorities and leaders of the selected communities. This process involves the provision of clear information on the objective and procedures of the survey, expected dates for the arrival of the field teams, and requests for permission to visit each community. The dates for the field work must be coordinated and confirmed with local leaders and health workers. It is necessary to take into consideration special holidays, seasonal events, among other special dates in the selected communities, to ensure that the field teams will find most of the inhabitants.

This coordination includes providing clear and detailed information on the objectives and procedures of the survey, the population to be studied, benefits and risks for the participating population, the importance of the participation of schools and inhabitants when surveys are carried out in households, and liaising with parents and children. This advance coordination is essential to ensuring the participation and adherence of the population of interest. Participants should receive consent forms in advance through the regional and local coordinators of the survey to explain the purpose of the survey, procedures, benefits, and risks, and have the opportunity to ask questions and clarify any doubts or concerns.

Before venturing out into the field, each team must verify that it has all the necessary supplies and materials to collect the data and samples. The following section provides an example of field processes during a survey designed to be carried out in schools. This can be adapted if surveys are carried out in households. Annex 4.2 shows the flow of activities, divided by participants' roles in the design and implementation of an integrated serological surveillance study.

4.4 Collect data and samples

To carry out data and sample collection, several key activities are conducted:

- **Presentation to selected participants on the day of the survey** (selected children and their parents): This activity should be carried out by the team leader. The objectives of the survey, the selection methods, and the survey procedures in general should be explained in detail. This information must be adapted to the age and cultural and social context of potential participants.
- **Collection of informed consent, and assent when applicable, from each participant in the survey:** With the aid of the consent forms, each selected participant must be informed of the objectives of the survey, its procedures (and how long each one will take), how data confidentiality will be ensured, and the risks and benefits of their participation, etc. Any questions should be addressed immediately. As part of the consent process, each participant must be informed of their right to withdraw from the study, to refuse or stop the interview at any point, and to refuse to answer any questions if they so wish. If possible, depending on the selected area (e.g., hard-to-reach communities), it is recommended to visit the community before starting the survey to ensure that the local levels are informed and consent forms were properly understood.
- **Interview of each selected participant:** At this point, all participants who consented should be interviewed. The interviewer must make sure that all items in the questionnaire are answered and that the questionnaire itself (if the PAPI method is being used) is kept safe and protected from any elements that may cause damage or loss. If the interview is being collected on electronic media such as a computer or mobile device, the interviewer must follow all established procedures and ensure that the device has sufficient power or adequate mains power and is protected from damage or loss.
- **Blood sample collection:** This procedure must be carried out by trained personnel, using all the necessary supplies and sterile materials to ensure biological safety during sample collection, packaging, and transport. Samples will only be collected from participants who have given their consent and/or assent. It is essential to ensure that all biological waste generated during sample collection is managed following the standards and recommendations of each country.
- **At the end of the day**, each team must deliver the packages containing the forms and samples to the supervisor, who in turn will deliver these to the laboratory coordinator for processing and storage.

Box 4.2 presents a summary of relevant aspects to ensure the quality of the collected samples and completed forms.

BOX 4.2 **Aspects to ensure the quality of data and samples collected during a survey**

1. Supervision. During survey-based integrated serological surveillance, the coordinating team must define the number of supervisors needed to ensure that all field teams are supported and monitored. Field teams should be supervised from the outset of the data and sample collection stage, to ensure timely corrective actions, if necessary. Supervisors must verify that the operation is progressing properly, address any problems that arise, and ensure that both data and samples are being collected and stored correctly. In case the field team faces a problem regarding sample strategy (e.g., enrollment procedures for participants as written in the protocol), the supervisor must ask the national team how to proceed, not leaving it up to the discretion of the teams.

2. Quality control. Quality control should be carried out both on blood samples and on completed forms (letters and questionnaires, as applicable). The latter must be verified as complete and legible. Blood samples should be double checked against the established quality criteria, such as sample volume, labeling, drying completely (if dried blood samples are collected) and storing with desiccant for humidity control, packaging, temperature control, and proper linking samples to forms to ensure no mistakes. It is important to monitor and verify that the number of participants who agreed to take part in the survey corresponds to the number of questionnaires, the number of consent and/or assent forms, and the number of samples collected.

The WHO vaccination cluster survey manual (*62*) is recommended to review aspects of how to conduct the field work; in particular, it is recommended to review Section 4, related to household surveys. This provides useful information about how to reduce information and selection bias when interviewing and collecting data from vaccination cards and health registries.

4.5 Analyze the samples

Once the samples arrive at the laboratory where they will be analyzed, the laboratory personnel should check the samples' physical condition, labeling, volume, associated documentation (correct and complete forms and letters), and check the identification code of each sample against a database or list. If the samples are labeled with a barcode and there is a barcode reader in the laboratory, the staff can create this database in real-time as samples reach the laboratory. Correct linkage of forms to specimens must be ensured in the database. If samples are collected, the laboratory database must include a record of those participants who refused storage of their samples for future studies. This facilitates the identification of samples that must be discarded after processing.

It is important to document all activities carried out in the laboratory and identify the codes of any rejected samples (unidentified samples, those with insufficient volume, poor storage or packaging, etc.); this information must be taken into account when analyzing the results of the survey. The materials, reagents, and equipment required for analysis will depend directly on the chosen assay. Module 5, on laboratory methods, provides more detailed information about sample collection and analysis.

Module 5

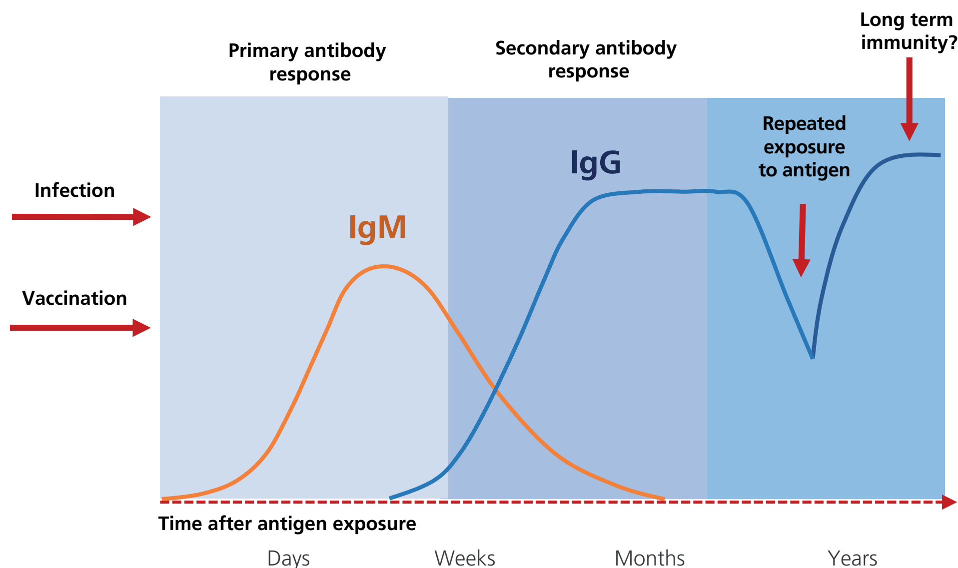
Laboratory methods

5.1 What does tracking IgG mean?

Antibodies are biomarkers useful to characterize people's immunity, derived either from vaccination or natural exposure to various pathogens (*24*). Pathogens leave behind immunological footprints in the form of antibodies that last for months or years. These can be detected from blood and, in some instances, tissues like saliva and urine. This makes antibodies useful biomarkers to characterize exposure in a population. Additionally, antibodies induced from vaccination can be used to estimate seroprotection in communities.

Monitoring immunoglobulin (Ig) G levels is useful for serosurveys due to its high abundance and longevity in plasma compared with other antibody isotypes (primarily IgM, IgA, and IgE). Figure 5.1 shows an example of IgM and IgG antibody curves and an approximate timeline from infection or vaccine-induced immunity. It graphically shows how the antibody response varies over time since the infection. IgM antibodies increase after a primary exposure to new infection and decline after days or weeks while IgG production begins. IgG antibody levels rise and stabilize over time, persisting for months or years depending on the pathogen or vaccine-specific antibody dynamics. IgG levels will rapidly increase if the person is repeatedly exposed to the same antigen and, in some cases, it can generate long-term protection.

FIGURE 5.1 **IgG antibodies mean present and past exposure to infectious pathogens or vaccine-induced immunity**



Source: Pan American Health Organization. Figure adapted for this document.

This highlights the different uses of antibodies as bloodborne biomarkers for surveillance. IgG can be used as a marker of past infection and protection against future infections, whereas IgM can be used as a marker of recent infection. Of the other antibody isotypes, which are less commonly used for surveillance, IgA reflects mucosal infection and IgE reflects specific worm infection or hypersensitivity.

While antibodies represent the potentially long-lived host response to infection, serological testing can also identify antigens derived from pathogens directly in serum as a marker of current or very recent infection; an example of this is hepatitis B virus surface antigen (*63*). There is a growing demand for rapid, accurate, and cost-effective assays for the measurement of these analytes in clinical and research settings (*33, 64*). This module is focused on laboratory aspects for the detection of IgG antibodies in blood, as these are the most commonly detected antibodies in serosurveys.

5.2 Serological assays

Several laboratory methods are currently available to detect antibodies; these can be broadly categorized as binding assays or functional assays.

Binding assays demonstrate antibody binding to antigen, such as rapid diagnostic tests (RDT) that rely on lateral flow-based systems which return dichotomous (positive or negative) results in a short time frame (normally <30 minutes), and quantitative tests, such as enzyme-linked immunosorbent assays (ELISA) or

bead-based immunoassays. Binding assays can be designed to detect specific antibody isotypes involved in this binding (e.g., IgG) and are generally performed in a lab setting; most serosurveys are based on binding assays.

Functional assays, such as neutralization assays, provide quantitative information on the ability of antibodies to neutralize a pathogen; for example, preventing a virus from entering a cell. Neutralization assays are the most time-consuming and complicated of the tests mentioned (*65*).

Multiplexed assays are a powerful technology that allows multiple analytes (e.g., antibodies for different pathogens) to be measured simultaneously in a single sample. This type of assay is useful for purposes of integrated serosurveillance of communicable diseases, because it can provide an efficient platform to monitor exposure to multiple pathogens from a single blood sample (*66*).

Serological tests, like other clinical tests, involve some degree of error. Understanding the degree to which error occurs and the effect on the individual- and population-level results are critical to using serological test results to inform public health policies and operational decision-making. The accuracy of a serological test can be directly related to the mechanism of the test itself, or it can be influenced by epidemiologic conditions, such as expected or known disease prevalence in the population.

Some aspects to take into consideration to select the serology test for serosurveys are:

- Sensitivity and specificity;
- Repeatability and reproducibility;
- Sample throughput (i.e., the use of equipment to automate antibody detection and process large numbers of samples);
- Cost (a realistic estimate of the resources required to generate the results according to the survey schedule);
- Turnaround time for results.

Furthermore, it is also important to determine the complexity of the specimen collection process, including what volume of specimen needs to be collected, and storage and transport conditions such as temperature and humidity. All these factors will have implications for the training and standardization of practices followed by field workers.

For each type of assay, clear procedures must be established for collection, storage, and transport of samples to the laboratory in which they will be analyzed. When using samples collected in surveys already planned for other disease surveillance or from serum banks, it is important to consider whether the specimens will be serum, blood, or dried blood spots (DBS), as this is important for laboratory processing purposes.

For purposes of convenience for this module on integrated serosurveillance, a multiplex bead assay (MBA) based on IgG detection will be assumed to be the assay of choice for the serosurvey.

5.3 Using multiplex bead assays for integrated serosurveillance

MBAs using Luminex technology (Luminex Corporation, Austin, TX, USA) correlate well with traditional serology methods. They have been widely used to measure antibody levels in serum samples collected in population-wide, cross-sectional, and longitudinal studies to monitor immunity profiles and help characterize the transmission of different communicable diseases (*16, 17, 20, 26, 34–36, 51, 67–73*).

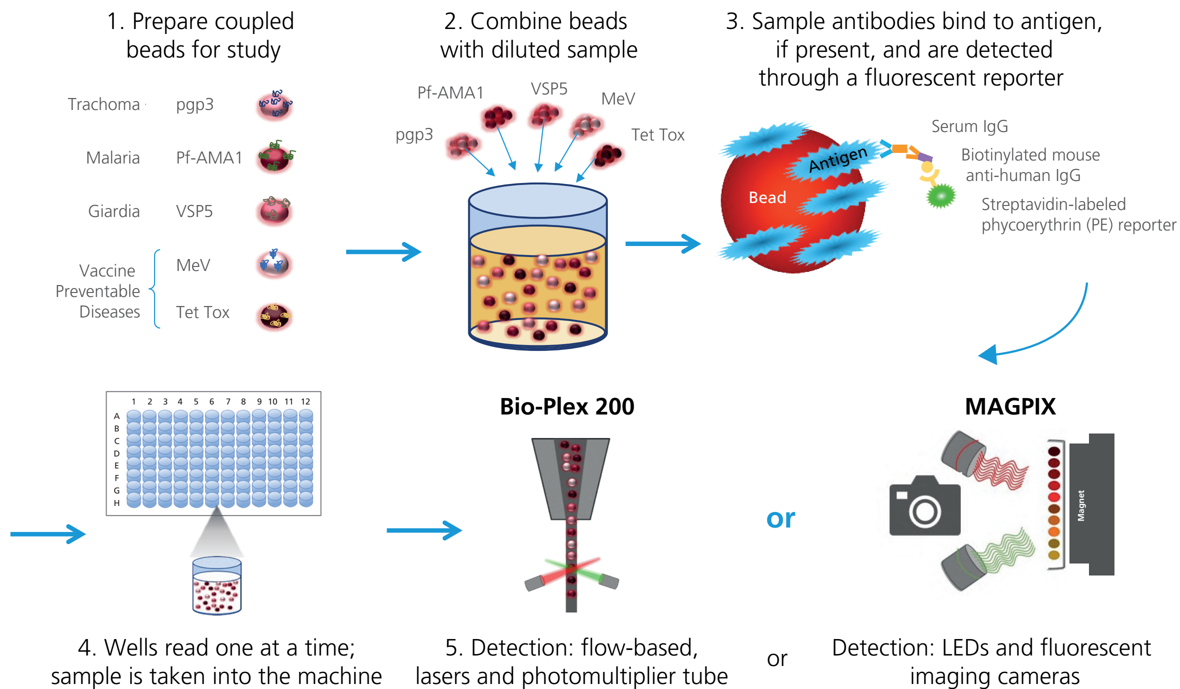
MBAs use a combination of fluorescently dyed microspheres and customized readers with software designed for these assays (Figure 5.2). MBA can simultaneously detect up to 50/100/500 antigens, depending on the instrument used, and use a very small (<1 µL) sample volume. This system allows for the creation of customized assays based on the public health needs of the programs, with a very low incremental cost of adding antigens.

Figure 5.2 schematizes the MBA process to run and read the results:

- Fluorescently dyed beads bound to selected antigens are combined in a well with a sample.
- Any antibodies in the sample that recognize any of the selected antigens will bind to that antigen.
- Beads are incubated with biotinylated anti-human antibodies—these will bind to sample antibodies that are bound to the antigens on the beads.
- Beads are then incubated with streptavidin-linked R-phycoerythrin (SA-PE) conjugate. Streptavidin binds to biotin with high affinity.
- The sample is run through the instrument (e.g., BioPlex200 or MAGPIX), which recognizes beads based on internal fluorescence and measures PE on each bead. Multiple readings from each of the beads from the same antigen are taken by the machine; these are used to calculate a median fluorescence intensity (MFI) per antigen per sample based on the median level of PE read on each bead. MFI is equivalent to the level of antibody in a sample.

Multiplexed assays offer significant advantages regarding costs and logistics, laboratory time, sample requirements, and the amount of data that can be generated (*74*), but also have limitations, as described in Table 5.1.

FIGURE 5.2 Running and reading the multiplex bead assay



Source: Centers for Disease Control and Prevention, Integrated Serosurveillance Team. Figure adapted for this document.

Several groups at the U.S. Centers for Disease Control and Prevention (CDC) and other partners have worked for over a decade on the characterization and validation of many antigens to be used in the MBA platform to support the integrated serological surveillance of neglected infectious diseases, vaccine-preventable diseases, vector-borne diseases, foodborne and waterborne diseases, among other infectious diseases. This platform is used to detect and measure IgG antibodies, as these are the most commonly detected antibodies in serosurveys.

Annex 5.1 provides a list of examples of available antigens at CDC for use in the MBA platform (updated in August 2021), including details about the information that each antigen can provide when it is included in population-based surveys, its utility in different epidemiological scenarios, the age group in which serological information is most useful, possible interventions to be implemented in response to seroprevalence findings, and some considerations of interest (e.g., cross-reactivity).

This information is particularly useful once a working group has decided that the MBA platform can be used for the objectives and antigens of interest of a serosurveillance survey. A list of examples of antigens that have been included in MBAs for several pathogens or diseases can also be consulted in the technical appendix of the article by Arnold et al. (*24*).

TABLE 5.1 Advantages and limitations of multiplexed assays

ASPECTS	ADVANTAGES	LIMITATIONS
Sample requirements	• Uses a relatively small sample volume (<1 µL of blood), which is easily collected by finger stick and allows the remainder of the specimen to be preserved for additional or future analyses. • Works equally well from serum sample or dried blood sample.	
Assay performance	• Detects multiple analytes from a single sample, eliminating the need for multiple single-data collection methods, such as traditional ELISA. • Relatively easy to make custom panels of antigens by covalent linkage of antigens to microspheres that can be spectrally classified by their internal fluorescent labeling. • Compares favorably to results from other assays. It has high sensitivity and specificity with "gold standards" for vaccine-preventable diseases. • Has a better signal-to-noise ratio and is more reproducible than ELISA. • Fluorescence-based detection allows for a wide dynamic range, so a single dilution can quantify a large range of responses.	• Cannot separate isotype-specific antibodies on multiplex: - IgM-specific responses need to be measured separately; - Does not differentiate specific subclasses (e.g., total IgG vs IgG4). • The concentration of specific antibodies in serum may need different dilutions: - CDC Division of Parasitic Diseases and Malaria group uses 1:400 dilutions of serum in their multiplex assay; - CDC HIV group uses 1:100 for maternal-child health testing. • Quality control is complicated by the large number of antigens included in each assay.
Costs and logistics	• Minimizes labor costs per sample: a lower cost per sample compared to the double-antigen ELISA for tetanus or an ELISA for measles and rubella, which is similar to the total cost of a 20-antigen MBA (*28*). • Data output ranges from 50 targets read in 45 minutes from 96 wells (4,800 data points) in the simplest instrument to 500 targets read from 384 well plates in 30 minutes (192,000 data points) in the most sophisticated instrument. • Requires relatively small amounts of input reagents like antigen and detection reagents. Ancillary equipment is fairly standard.	• Each new antigen takes time to standardize and validate: - Validate if it couples to a bead: weeks; - Validate if it performs well in the assay: months; - Validate how it works in different study settings and what the data mean: years. • The supply chain is challenging: - Many antigens are produced in-house at CDC; - Needs a cold chain for the transfer of reagents. • Requires extremely specialized reagents and detection instruments. • Instruments are expensive and challenging to maintain.

5.4 Sensitivity, specificity, and cross-reactivity

Sensitivity is a measure of how often a test correctly generates a positive result for people who have the condition that is being tested.

Specificity is a measure of how often a test correctly generates a negative result for people who do not have the condition that is being tested.

Most of the sensitivity data of serological assays are based on active infection, but it is important to take into consideration that a person can have IgG from exposure, current infection, or previous infection.

Specificity indicates how likely each antigen is to produce a false positive result; it reflects how well an assay performs in a group of negative individuals. Factors that affect the specificity of a test include the false positive rate of the test or cross-reactivity to antigens from other pathogens. This is often the case when two antigens have similar structural regions that the antibody recognizes.

Cross-reactivity can result from:

- Related pathogens
    - Viruses from the same phylogenic family (e.g., dengue and Zika);
    - Filarial worms (onchocerciasis and lymphatic filariasis);
    - Mites (scabies and house dust mite).
- Unrelated antigens
    - Pathogen-associated autoimmunity.

Non-specific positive responses can also impact specificity. These responses result primarily from the laboratory techniques used to purify the antigens used in the assay. Many antigens need to be produced recombinantly in bacterial or mammalian expression systems. During purification, some of the host cell molecules may contaminate the antigen, and these may also, in turn, get bound to the beads used in the MBA, and an individual may have antibodies that recognize these contaminants. The assay contains a buffer component and controls to help reduce and identify non-specific responses; these are described in Sections 5.6 and 5.7.

Annex 5.2 shows sensitivity and specificity values (95% confidence intervals) of some of the validated MBA antigens, including negative and positive classification panels used to implement the validation steps and the accepted serological reference test for each antigen, if available.

5.5 Defining cutoff value and seropositivity for the multiplex bead assay

For the MBA, the cutoff value is the MFI value above which samples are classified as seropositive. A seropositive result can be interpreted as positive for exposure to or infection with a pathogen, or a certain level of seroprotection generated by vaccination.

Different methods are used to establish cutoff values for a specific antigen (*66*). It is crucial to understand the different approaches to ensure appropriate interpretation of the data to support decision-making. The most common methodologies used to determine cutoffs for the different MBA antigens are:

- **Standard curves using International Standards or International Reference Materials.** The World Health Organization (WHO) (*75*) provides reference specimens with known antibody concentrations defined as international units for certain diseases, primarily VPDs (e.g., tetanus, diphtheria, measles, and rubella). These standards can be used to validate previously established cutoffs used in gold standard assays to performance in a new assay (*76–78*). Additionally, dilution series (i.e., standard curves) made with international standards can be used to convert raw assay output in MFI to international units for analysis.
- **Negative samples or samples from non-endemic areas.** The MFI values of samples from people assumed never to have been exposed to a disease can be used to calculate a mean plus 3 to 5 standard deviations (depending on the desired confidence level) to determine the cutoff value. This approach is predominately used for infectious diseases using samples of people living in non-endemic areas, as these can most confidently be assumed never to have been exposed. Samples collected before the introduction of a disease are also suitable (e.g., United States residents before 2020 for SARS-CoV-2).
- **Receiver operating characteristic (ROC) curves** use groups of positive samples defined by separate assays or clinical signs and negative samples from individuals presumed never to have been exposed (*79*). The MFI responses from these samples are combined, and the sensitivity and specificity are calculated at multiple possible cutoffs to identify an optimal cutoff providing the best discrimination between the true seronegative and seropositive populations (e.g., a cutoff that gives equal weight to sensitivity and specificity [*80*]). The cut-off may also be adjusted to give optimal sensitivity or specificity to better suit the study objectives. The availability of positive and sometimes negative samples is highly variable and usually a limiting factor in the use of this method.
- **Statistical methods to model study data** are used to identify a breakpoint between distributions of high and low responses within the study population data to determine positive and negatives. Examples of this are finite mixture models and expectation-maximization models (*81*).

International standards and well-validated measures of IgG cutoff values are available primarily for VPDs and a select few other pathogens. But for other communicable diseases, determining cutoffs is complicated by the lack of international standards as well as lack of clinically defined controls, and potential differences in what a "background" signal looks like in the study population compared to another population.

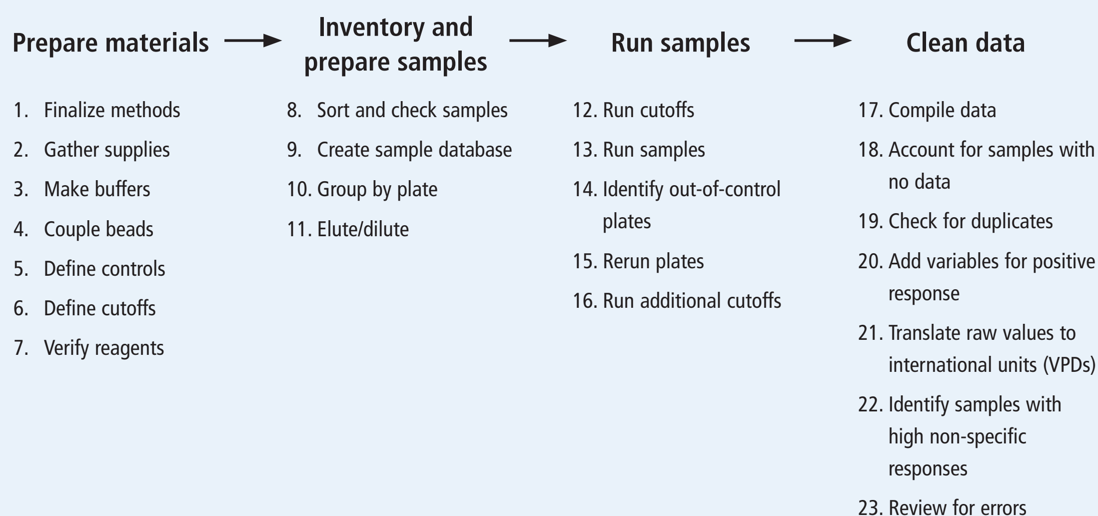
5.6 Quality assurance

Quality assurance is the art of preventing or minimizing errors before they happen. The laboratory must ensure that laboratory testing is accurate and consistent. Quality assurance measures must cover all aspects of testing, including:

- Reagents and supplies;
- Equipment maintenance;
- Staff training and competency;
- Sample collection and storage.

Planning the laboratory process is essential for ensuring quality assurance. Box 5.1 describes 23 steps to run serological tests. A workflow plan must be developed to reduce errors (especially if more than one person is working on a study).

BOX 5.1 **Laboratory processes: 23 steps to run serology tests**



Source: Centers for Disease Control and Prevention, Integrated Serosurveillance Team. Adapted for this document.

Reagents and supplies

All reagents and consumables must be procured before starting to prevent lot switches and shortages, and their performance should be verified. Changing lots of critical reagents could impact the specificity and sensitivity of the tests and complicate or confound the ability to do analysis. Reagents and consumables are grouped into three categories based on how likely changing lot (production batch) or manufacturer could change the assay signals: non-critical (consumables), semi-critical (buffers and dilutions), and critical (detection reagents, antigens, couplings).

Critical reagents for MBA include:

- Detection reagents. Anti-human IgG, IgG4, and streptavidin-linked R-phycoerythrin (SA-PE), commercial products that undergo quality control during manufacturing to meet certain specifications.
- *E. coli* lysate is a strain of *Escherichia coli* used to express recombinant proteins. It is produced in-house at CDC. Some antigens are prone to false positive responses if *E. coli* lysate is not included in the sample dilution buffer.
- Antigen-coupled beads. Antigen coupling lot can be a source of variation and some antigens are more prone to variation than others. Each lot of coupling should have a new cutoff determined. The same lot of coupling should have a new cutoff determined when changing lots of critical reagents.
- Antigen. Changing lots of antigens can introduce significant variation both in terms of the number of antigens affected and the degree of variation. New lots of antigens need to have a more rigorous verification done to reassess sensitivity and specificity with a validation panel.
- Consumables. These are often interchangeable, but key qualities should be considered when choosing substitutes, and a best practice is to verify the change of more significant items. It includes sealing foil, tubes, and assay plates where protein interactions take place, with low binding plastics being preferred.

Equipment and maintenance

Machines need continuous care to keep in good order. They require daily cleaning routines with bleach and sodium hydroxide, which are important to minimize contamination, and weekly cleaning routines including cleaning probes help prevent clogs. Also, yearly preventative maintenance must be ensured.

Staff training and competency

Technical competency must be ensured in new personnel and previously trained individuals. It is recommended to have a panel of samples with defined reactivity to a reference antigen to form the basis of post-training and yearly assessment of the laboratory technique. Proficiency Testing (PT) or External Quality Assessment (EQA) is recommended to measure the laboratory performance within a network of quality control laboratories.

A workflow of a reasonable pace should be planned according to the assays selected. For example, for dedicated staff performing MBA, eight plates per five-day workweek is a minimal starting point. More can be added by experienced staff in a well-functioning laboratory. The pace of testing should allow for time to perform machine validation and maintenance procedures, proper data management, and review of controls.

Sample collection and storage

Best practices in the collection, storage, and processing of samples are critical because objective quality indicators are not easy to define. Common issues with handling samples that could lead to errors include degradation of sera due to lack of cold chain or repeated freeze and thaw cycles, inaccurate measurement of sera due to viscosity, degradation of DBS due to contamination and/or storage in humid conditions, and under- or over-filling of DBS.

Individual DBS collected should be stored in small, tightly sealed clear bags with barcoded sample ID visible. Groups of samples are stored in a larger thick bag with a wide seal that contains desiccant and humidity indicators. DBS should be kept dry, as cool as possible, and protected from light until they can be stored in the freezer, because protection from humidity is essential for the stability of samples. Long-term storage needs should be considered early on, DBS samples must be stored at –20 °C to –80 °C.

Once the samples arrive at the laboratory where they will be analyzed, the laboratory staff should check their physical condition, labeling, volume, associated documentation (correct and complete forms and letters), and check the ID code of each sample against a database or list. It is recommended that all samples be barcoded and scanned for the creation of a laboratory sample database. Samples should be confirmed to be only from individuals having given consent for testing. The test results should be traceable and linked by the ID number of the participant. This unique code will be used throughout the entire process and will be used to link the final laboratory results to the demographic and epidemiological data collected in the field.

Once all samples have been tested and laboratory results are available, laboratory data must be compiled into a database by merging with the demographic database. The final database should be carefully reviewed to ensure that there are no repeated sample IDs and that samples with missing or excluded laboratory data have been documented, so that all samples are accounted for. Data quality control and cross-checks should be implemented to ensure the completeness and accuracy of the database.

A data dictionary defining any analysis variables should be included (e.g., if samples are flagged for having high reactions with negative controls). When applicable, the laboratory database must include a record of those participants who refused the storage of their samples for future studies. This facilitates the identification of samples that must be discarded after processing.

5.7 Quality control

Quality control (QC) is the gatekeeper of good data. To ensure that all laboratory procedures are performed at a high level of quality and thus yield reliable, accurate, and reproducible results, efficient quality control procedures must be adopted during:

- Pre-analytical phase: collection, labeling, packaging, and transportation; sample receipt and registration by trained personnel.
- Analytical phase: proper preparation, strict attention to physical/chemical parameters, equipment maintenance and calibration, quality control tests, etc.
- Post-analytical phase: proper upkeep of files and samples, disposal of samples, etc. (*82*).

QC requires standard operating procedures and thresholds to prevent, identify, and correct technical errors in the laboratory (e.g., dilution of detection reagents, photobleaching of fluorescent signals, pipetting errors, samples with non-specific responses, low bead counts, among others). QC tools used in the laboratory are:

- Controls
    - Samples or standards with known responses that are included on each plate;
    - Beads coupled to glutathione-S-transferase (GST) protein and lysate from vero cells included in each well to control for non-specific binding to these proteins involved in antigen purification.
- Bead counts
    - Defining the minimum number of beads per well required to ensure quality assay results.
- Statistical analyses
    - Statistical criteria for setting ranges and cutoff values.
- Retesting a subset of samples
    - In a second, independent lab (preferred);
    - By a second operator in the same lab (if second lab not available).

5.8 Understanding serology results

This section refers to general aspects of laboratory data analysis, given that the categorical positive and negative data from each person are being used to identify population levels and transmission trends for understanding serology results. For data analysis and interpretation of integrated serosurveys, please consult Module 6 of this toolkit.

The serology analysis plan must be disease specific. It is important to understand antibody dynamics and the natural history of each disease to interpret what responses to each antigen mean:

- Is it a current or historical infection?
- Does the response result from vaccination or natural infection?

- Do changes in antibodies in vivo occur fast enough to detect changes in transmission?
- Does response against a single antigen imply positivity or need multiple exposures?

For basic descriptive analysis of serology data when using MBA, the following steps are recommended:

- Calculate median and range in MFI for each antigen.
- Convert VPD results from MFI to IU/mL:
    - Plot standard curve and fit with regression formula (e.g., 5 parameter logistic curve fit, 5PL).
    - Standard curve points that are plateauing can be removed for better curve fit.
    - Use the formula to interpolate IU/mL from raw MFI.
    - Sample results outside the range of the curve should be truncated at the upper and lower limits of the curve, and this should be noted for analysis.
- Create binary variables for seroprevalence according to the cutoff values.
- Analyze median antibody levels and seroprevalence levels by age group, subpopulation, vaccination status, among other variables identified in the analysis plan.

Finally, it is important to take into consideration that no test is perfect, and to measure very low levels of seropositivity (<1%, <5%, <10%), large sample sizes might be needed to get accurate estimates.

Module 6

Data analysis and decision-making

This module describes the steps for data analysis, visualization, and interpretation, as well as for preparation of the final report of an integrated serological survey.

Data analysis and decision-making

21. Data cleaning and management
22. Calculate sample weights
23. Determine cutoff values
24. Estimate seroprevalence
25. Conduct descriptive analysis
26. Additional analysis and data modeling
27. Interpret and visualize results
28. Prepare and disseminate report
29. Make decisions

6.1 Data cleaning and management

A clean, scrubbed database, in which data for each variable of interest and the corresponding laboratory results are linked for each participant (i.e., merge of laboratory and survey databases by participant identification number), is essential for proper analysis. Any adjustments or modifications made to the data during the data scrubbing and cleaning process—e.g., entry corrections or exclusion—must be recorded. Issues with merging the laboratory and survey datasets can happen if proper quality control processes are not followed during the data collection and sample processing steps. These types of problems must be resolved one by one and can take a lot of time, so it is better to be careful in the first place.

The completeness and consistency of data in each variable of interest recorded in the database should be reviewed. For instance, the database should be checked for missing answers, especially for key questionnaire items related to the primary outcomes, and values requiring review and correction. Likewise, the number of records should match the sample size in the protocol if the survey was implemented correctly; check for any omitted or duplicate data points to minimize the risk of error and bias. Depending on the type of survey carried out (school-based, household, etc.), it is important to calculate the following quality indicators for the database: the number of households or schools visited compared with the number sampled; the number of individuals surveyed compared with the calculated sample size; the number of completed questionnaires (response rate); and missing data (or "don't know" answers), especially the key variables of the questionnaire.

It is also important to check the ranges and logic of the data to assess consistency. Range checks allow detection of whether values for a given variable are outside reasonable or possible limits (e.g., a pregnant male, a 250-year-old subject). Once an error is detected, it must be decided whether the information can be checked and corrected, whether the data point should be left as is, or (in the case of blatant errors) should be excluded. It is important to have standardized procedures to correct errors or missing data, such as calling the team, if it is still in the field; trying to find the records in the health facility, if that is feasible; comparing data against the paper form or photograph, if available (e.g., vaccination card), among others.

During this phase, it is also useful to label, recode, or create any new variables necessary for analysis (e.g., age groups, seroprotected status). Depending on the analysis strategy, it might be useful to analyze the variables as continuous; in this case, having absolute values in the database is essential. For continuous variables (age, amount of antibody level for each antigen, etc.), tests should be performed to obtain the minimum, maximum, median, mean, standard deviation, and standard error, as well as tests for normality (Shapiro–Wilk, Kolmogorov–Smirnov). In some cases, creating categorical variables from continuous variables or collapsing categories, in the case of many categories with small numbers, is most useful for subpopulation analysis or the primary outcomes.

Exploratory analysis usually helps identify outliers. Upon completion of this data review and exploratory analysis, it may be necessary to run a final scrub of the data to obtain raw data before proceeding to the calculation of weights and weighted results.

6.2 Calculate sample weights

For survey data, weights should be calculated based on the sampling procedure and the likelihood of an individual being selected for the survey at each stage of sampling. The sampling probability is the probability that a given individual will be drawn or included in the sample and represents a combination of the probabilities at each stage of selection (e.g., cluster, household, individual).

The calculation of sample weights includes:

- Calculating the design weight;
- Adjusting for nonresponse;
- Post-stratifying to match population totals.

Several software packages can be used to calculate weights, such as Stata, R, SAS, and SPSS. Annexes J and K (pages 186 to 189) of the WHO vaccine coverage reference manual provides guidance on the data, procedures, and software used to calculate the survey weights (*62*).

6.3 Determine cutoff values

Considering the type of assay and pathogen included in the survey, it is crucial to determine whether an antibody level in each specimen is above a postulated seropositivity cutoff value. It varies according to the sensitivity of the assay and the purpose of the analysis for each specific infectious pathogen. Based on this value, individuals are classified as seronegative or seropositive.

There are various statistical methods to determine the cutoff value in serological tests, such as those focused on optimizing sensitivity or specificity, optimizing test precision, optimizing predictive value (*76*), logistic regression analysis, receiver operating curves (ROC) (*83*), among others. All of these methods require positive and negative controls to calculate the most appropriate cutoff point. Using a logistic regression analysis and a ROC curve, it is possible to determine the best optical density cutoff value, where the linear relationship between the amount and concentration of the analyte present in the sample is seen (*84–86*).

If the multiplex bead assay (MBA) platform is used, care should be taken to examine the minimum and maximum signals for each antigen to assure that the values are reported correctly and any censoring of a signal above or below a certain critical value noted. Cutoffs for vaccine-preventable diseases (VPD) and other relevant antigens need to be calculated based on the standard curve with the respective reference serum and established cutoffs for immunological protection (*87*).

6.4 Estimate seroprevalence

The seroprevalence is the estimated proportion of people with antibody levels above a predetermined cutoff point, and it is one fundamental output of serological studies. To approximate the true value of seroprevalence, it is important to calculate not only the point prevalence but also 95% confidence intervals to increase the likelihood of including the true value within that interval. The point estimates and 95% confidence intervals should be calculated using statistical software that allows accounting for the survey design and weighting. Other primary outcomes might include the median and interquartile range of antibody values.

When calculating seroprevalence, the final results in the analysis tables should be weighted analysis accounting for survey design. Similarly, statistical comparisons of seroprevalence values between subpopulations should be calculated using these methods as well and cross-tabulated by variables of interest (age group, sex, municipality/region, urban vs. rural residence, among others).

It is important to bear in mind that, depending on the survey objectives and design, as well as on the level of stratification (e.g., locality, district, province, or rural vs urban), the sample size may be insufficient to detect statistical differences between categories with few observations in at least one group. In this case, collapsing categories of a contingency table's classification variables may be relevant if the sample size is adequate (*88, 89*).

Antibody responses to specific pathogens are described in relation to seroprotection, rates, and time intervals of infection, among others. However, for neglected infectious diseases and malaria, for instance, there are currently no established seroprevalence parameters or seroprevalence thresholds to support decision-making in control, elimination, or post-elimination scenarios. Nevertheless, seroprevalence data are useful and provide supplemental information, as explained below, for the analysis of transmission patterns in populations of interest.

6.5 Conduct descriptive analysis

Descriptive analysis allows sample characterization by geographic strata (region, district, location), demographic variables (such as rural vs. urban residence, sex, age), and other factors of interest (such as vaccination history and risk factors for transmission), depending on the disease or diseases of interest. A summary of descriptive analysis must include:

- Description of survey sample: number of administrative units, persons surveyed, number of refusals, age, sex, among others;
- For categorical variables, a frequency table including absolute numbers and percentage of dataset;
- For continuous variables, calculate minimum, maximum, median, mean, standard deviation, standard error, among others.

Annex 6.1 provides some recommendations for the descriptive analysis of data from integrated serological surveillance surveys.

6.6 Additional analyses and data modeling

Depending on the characteristics of the study, the sample size, and the variables of interest, additional complex analyses can be carried out, such as regression and correlation methods, multivariate analyses to identify the effect of various exposures or risk factors, and data modeling to determine predictors of antibody seroprevalence that explain trends and variability across zones.

Using predictive algorithms and high-resolution mapping, geostatistics allows identification of overlapping serological responses of populations living in different areas, facilitating integrated epidemiological surveillance and creating the possibility of establishing synergies between programs and interventions. Since these methodologies are more complex, their use requires the involvement of experts on specific diseases, epidemiologists, and professional statisticians—who are experts in modeling analysis.

6.7 Interpret and visualize the results

Interpretation will depend on sampling methods, type of laboratory assay, and the criteria to define immunity, in particular cutoff values for seropositivity. Analysis and interpretation of the results of the serological studies must also take into account the expected effect of the intervention strategies and targets related to elimination goals. It will also depend on the availability of threshold critical values for the various diseases. Serology results should be expressed in a way that answers (or at least facilitates the search for answers to) questions such as:

- Is the immunity profile as expected?
- Does the profile reflect any changes in immunity or transmission patterns of the disease over time?
- Is there any evidence—considering the changes in program performance and accumulation of exposure chances over time—that interventions reduced the level of disease transmission?
- Are there immunity gaps in the groups targeted by vaccination?
- Is the level of immunity that was achieved sufficient to maintain interruption of transmission of the pathogen? This is useful for VPD, because there are predetermined threshold values to assess elimination goals and for making decisions.

Since immunity profiles and antibody levels are affected by multiple factors, interpretation of the results of serosurveys in the populations of interest should incorporate other sources of information outside the survey, such as:

- Demographic, socioeconomic, and living conditions;
- Epidemiological data of the diseases of interest and characteristics by variables such as time, place, and person;
- Data on the interventions already in place, including the type of intervention, the duration of the implementation, and its coverage (e.g., historical vaccination campaign schedules and coverage

data; preventive chemotherapy coverage; mass drug administration coverage; safe water and sanitation coverage, among others);

- Surveillance and program performance variables (e.g., the quality of surveillance data for the diseases of interest and regularity of the interventions of interest, among others).

Data triangulation is essential in the analysis of immunity patterns within study populations, as it allows observation of the same object of study from different angles or time points, serving to compare different data, theories, contexts, instruments, agents, and methods and obtain the points of view of various investigators. In data triangulation, different sources of data are compared for a deeper understanding of the findings, to address limitations of any one data source and/or data collection methodology, and encourage deeper insight into the phenomena of interest through making sense of complementary information and integrating knowledge of the broader context and underlying processes (*90*). This method is very useful to detect discrepancies when analyzing data from different sources obtained by different collection methods. For example, detecting discrepancies in comparisons between administrative vaccination data disaggregated by age group and antibody seroprevalence against VPDs in the same population.

Temporal triangulation can also be used to check data for consistency at different points in time. The data may constitute a longitudinal trend over several years or a cross-sectional analysis over a given period in a specific population. For instance, analyzing seroprevalence of an infectious disease in a population of interest and comparing this with the cases of the disease reported through routine epidemiological surveillance, in the same age group, geographic area, and period of time.

Proper interpretation of the results of survey-based integrated serological surveillance requires—from the start of the protocol design process—identification of the limitations that will have an impact on the analysis and interpretation of results. These limitations may be related to the size and representativeness of the sample, the type of study population, the risk of bias in the collected data, the sensitivity and specificity of laboratory methods, etc. Box 6.1 describes some important aspects to bear in mind regarding limitations.

BOX 6.1 **Define the methodological limitations of the study**

Countries that have decided to conduct a serosurvey for integrated surveillance should:

- Consider any possible limitations due to a failure to study a particular population group or geographic area (e.g., inaccurate source of vaccination registers, not revisiting households or schools that were unavailable during the first visit, or low response rates of selected participants).
- Assess whether any area, school, community, etc., had to be removed from the sampling frame due to insufficient sample size, accessibility, security, or other factors; any such exclusion must be recognized as a limitation and a source of possible bias.
- Take into consideration the assay validity. For example, a limitation specifically applicable to trachoma surveillance is cross-reactivity with antibodies against urogenital serotypes of chlamydia, which can occur when older, potentially sexually active age groups (>10 years) are included in the survey.
- Confirm that the representativeness of the survey responds adequately to the study objectives and that results can be applied to the study universe.

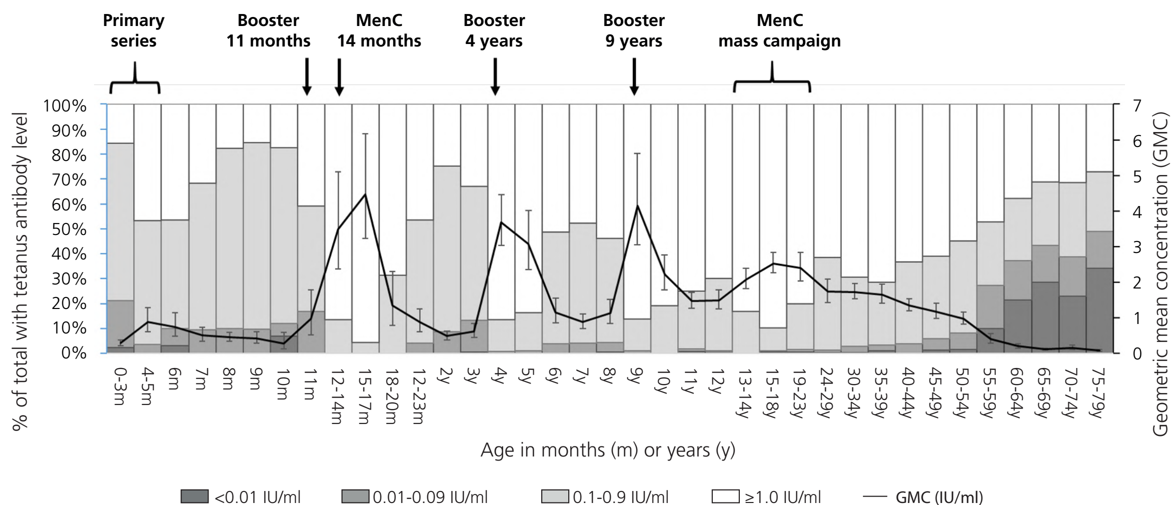
6.7.1 Analysis of immunity gaps and vaccination efficacy

Some questions of interest include:

- Are there immunity gaps in specific age groups or geographic areas, or differences in immunity according to socioeconomic level?
- Is there any mismatch between observed seroprotection levels and reported vaccination coverages? Is it consistent with observed trends in the age of recent disease cases reported to surveillance?
- Is there any evidence of waning immunity or lower-than-expected vaccine effectiveness when considering groups of age for the time elapsed since vaccine administration?
- If the survey included multiple VPDs, are the immunity gaps related to any particular vaccine, or do the results indicate that the issue is systemic, related to overall vaccination program performance?

Figure 6.1 shows sample results of antibody levels against tetanus by age group. In this study, an analysis of antibodies against tetanus toxoid was carried out on samples from a Dutch population, using the MBA. The graph includes background information about the vaccination strategies, such as the vaccination schedule for tetanus vaccines and the time of implementation of the mass meningococcal serogroup C conjugate (MenC) catch-up campaign. The stacked bar chart shows the proportion of individuals in each age group and levels of antibodies according to categories as well as the mean antibody concentration (solid black line), with confidence intervals.

FIGURE 6.1 Levels of immunity against tetanus in different age groups of the population, The Netherlands, 2006



Source: Steens A, Mollema L, Berbers GAM, van Gageldonk PGM, van der Klis FR, de Melker HE. High tetanus antitoxin antibody concentrations in the Netherlands: a seroepidemiological study. Vaccine. 2010;28(49):7803–9. https://doi.org/10.1016/j.vaccine.2010.09.036.

6.7.2 Analysis of communicable diseases in different epidemiologic scenarios

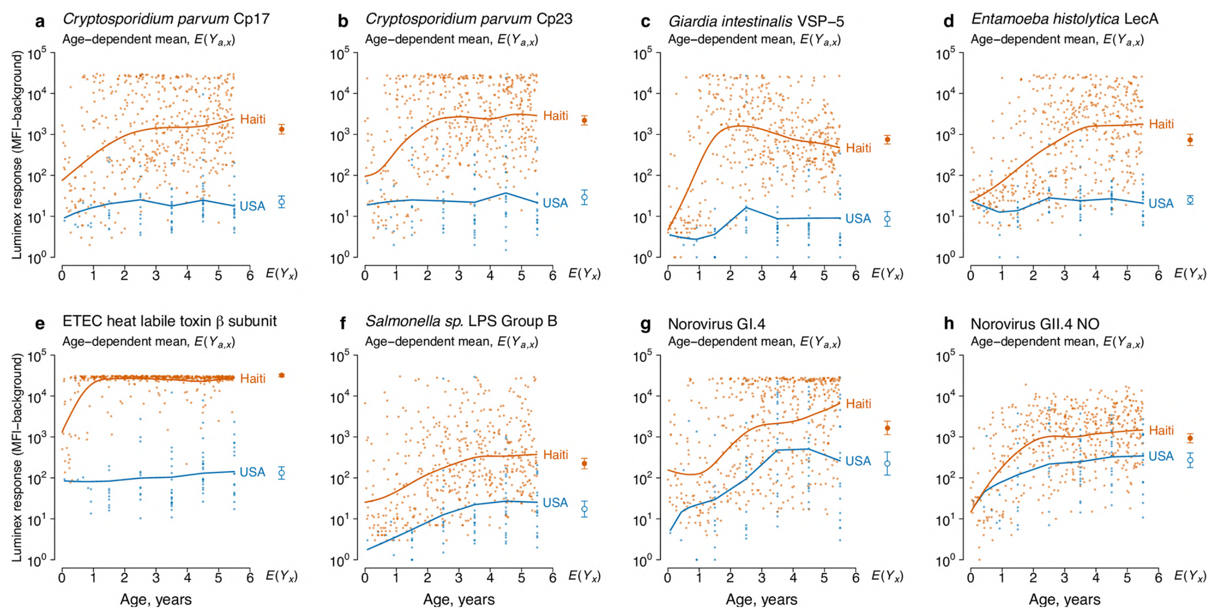
There are key questions that can guide analysis of neglected infectious diseases and malaria in different epidemiologic scenarios and social determinants, such as:

- Are there differences in seroprevalence by sociodemographic and epidemiologic variables (educational level, occupation, ethnicity, well-being quintiles, among others) and geographic areas?
- Is there any evidence—considering the changes in program performance and accumulation of exposure chances over time—that interventions reduced the level of disease transmission?
- Is the serology profile of the disease consistent when compared with transmission surveys, reported data, interventions (such as preventive chemotherapy, use of bednets, etc.), vector density, water and sanitation conditions?
- Are the levels of immunity consistent with the programmatic goals established to interrupt the transmission of the agents associated with these diseases?

Figure 6.2 presents age–antibody curves of immune response for children under 6 in two countries with different levels of income and socioeconomic development: Haiti (orange line) and the United States of America (blue line). Antibody response was measured by MBA as median fluorescence intensity (MFI), and for each enteric antibody, the authors estimated separate age–antibody curves in each country in children aged <5.5 years. Geometric means and differences between means were calculated using

statistical methods. The results showed higher levels of transmission of all enteric pathogens in Léogâne, Haiti, compared with the United States of America (*17*).

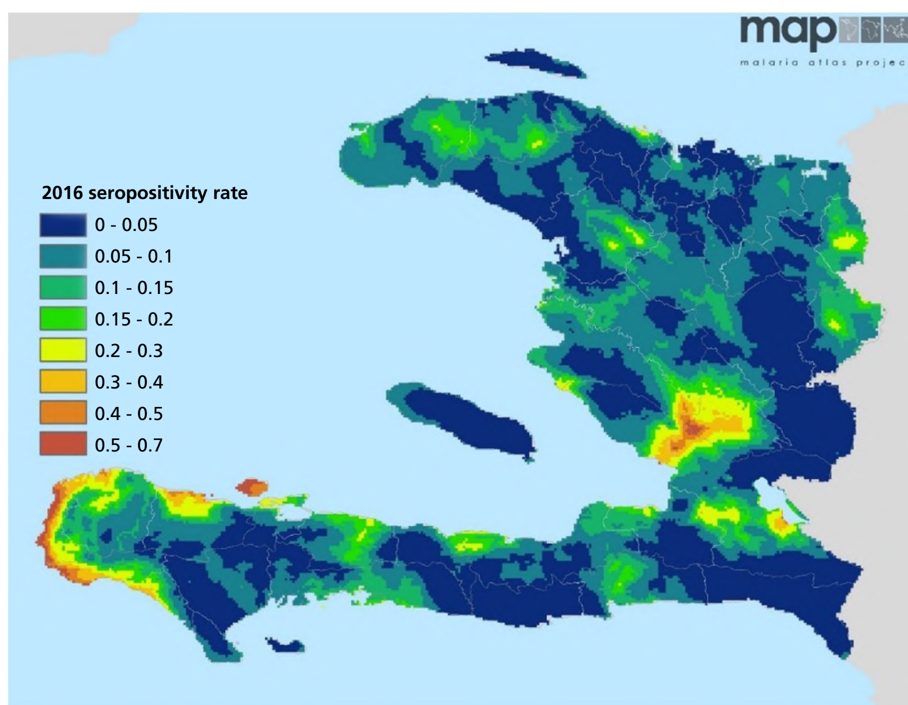
FIGURE 6.2 Differences in levels of transmission of enteric pathogens in children, stratified by age, in Léogâne, Haiti, and the United States of America



Source: Arnold BF, van de Laan MJ, Hubbard AE, Steel C, Kubofkic J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLOS Negl Trop Dis. 2017;11(5):e0005616. Available from: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005616.

Maps are very useful to visualize the results of seroprevalence studies. Figure 6.3, as an example, shows antibody seropositivity rates of malaria by geographic zones in Haiti. Due to the very low prevalence of malaria rapid diagnostic test positives in this country, serology data is a primary indicator for measuring transmission and success of interventions.

FIGURE 6.3 **Mapping malaria transmission intensity by antimalaria antibody prevalence**



Source: Rogier, E. Geospatial analysis of *Plasmodium falciparum* serological indicators: school versus community sampling in a low-transmission malaria setting. Forthcoming.

6.7.3 Assessment of the impact of interventions

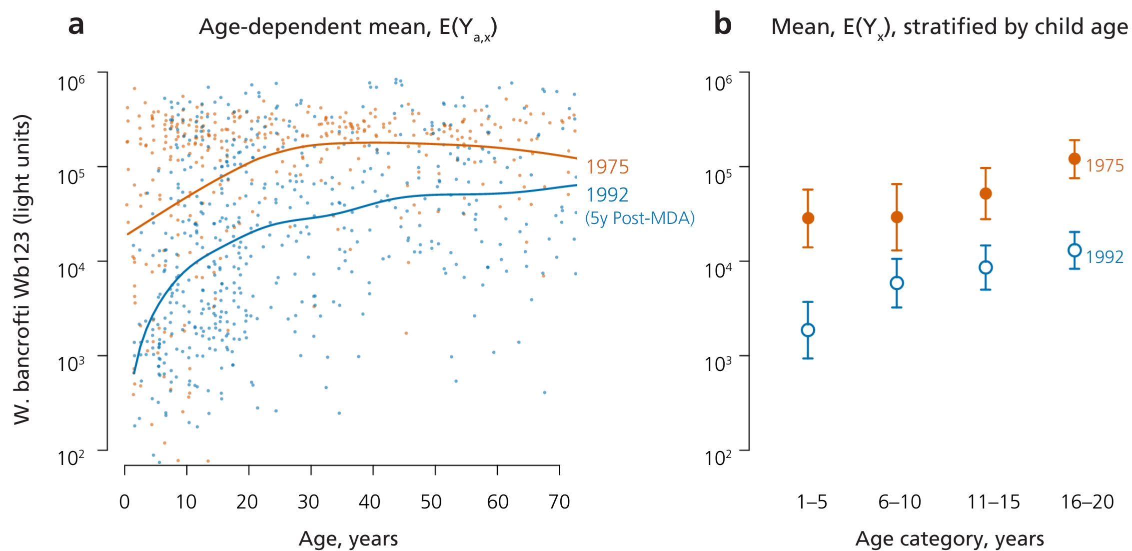
Some questions aimed at evaluating the impact of interventions include:

- What were the interventions implemented, the target populations of the intervention programs, and the reported coverage in each of the geographic areas of interest?
- When was the interruption of transmission documented and what were the post-elimination surveillance strategies?
- Was the threshold level of immunity or infection enough to maintain VPD elimination, interruption of transmission, or elimination of NTDs as a public health problem?
- Since the certification, verification, or validation of elimination of the disease of interest, have any changes occurred in the risk factors related to transmission?

Figure 6.4 shows the effect of MDA for the elimination of lymphatic filariasis on the level of transmission of this disease on Mauke Island (*91*). The IgG antibody response to the Wb123 antigen of *Wuchereria bancrofti* was measured in blood samples from residents in 1975, before MDA, and again in 1992,

five years after a single, island-wide MDA intervention with diethylcarbamazine. Figure 6.4a shows the curves of mean antibody levels by age before MDA (orange line) and after the intervention (blue line); the individual antibody responses are shown using dots (orange and blue) alongside summary curves of both surveys. Figure 6.4b shows the antibody response as the geometric mean adjusted for age, E(Yx), and 95% confidence intervals before (1975) and five years after (1992) the MDA intervention, stratified by age group (5 years). These results show that slower antibody acquisition combined with antibody loss, presumably a reflection of lowered transmission potential post-MDA, underlie the curve shift.

FIGURE 6.4 **Effect of mass drug administration (MDA) on the transmission of *Wuchereria bancrofti***



Source: Arnold BF, van de Laan MJ, Hubbard AE, Steel C, Kubofkic J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLOS Negl Trop Dis. 2017;11(5):e0005616. Available from: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005616.

6.7.4 Post-elimination surveillance

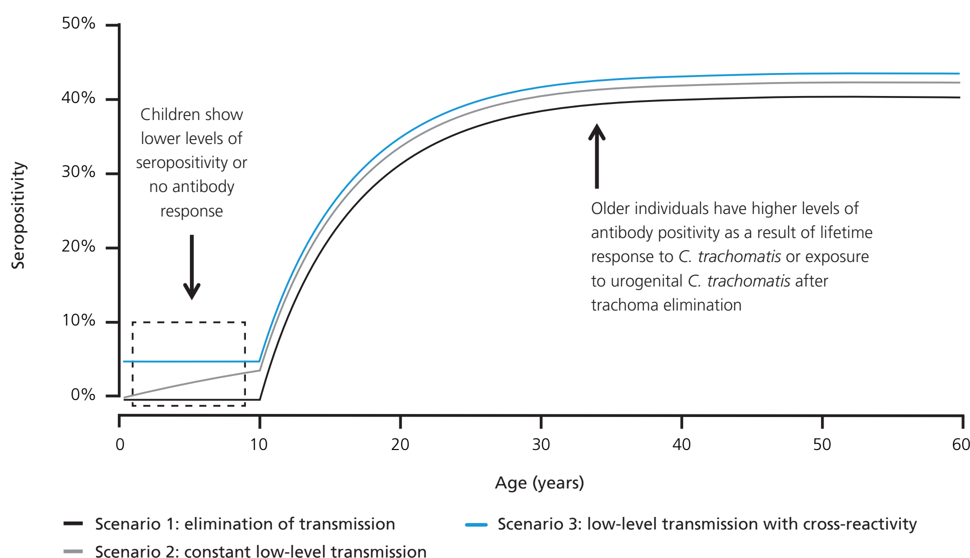
Infections with some pathogens for which elimination targets have been established may be asymptomatic or present with mild symptoms. As a result, clinical cases may only represent the "tip of the iceberg." Therefore, preventing the reintroduction of eliminated pathogens requires robust surveillance. In these cases, serology can provide additional information to anticipate the risks of reemergence or reintroduction during the post-elimination phase. Some questions that can guide this type of analysis include:

- When was the interruption of transmission documented, and which post-elimination surveillance strategies are in place?
- Since the certification, verification, or validation of elimination of the disease of interest, have any changes occurred in the risk factors for its transmission?
- Are the results of the serosurvey indicative of exposure to the pathogen or changes in the intensity of transmission across different age cohorts?

Trachoma elimination as a public health problem is an interesting example about how to use and interpret serology during the post-elimination phase. At low levels of trachoma prevalence, robust surveillance methods are needed and serological assays measuring antibody responses resulting from a single or cumulative exposure to trachoma have been used to assess changes in transmission intensity.

Figure 6.5 shows the use of serology during post-elimination surveillance of trachoma in a population aged 1 to 60 years, using data collected 10 years after the elimination of trachoma as a public health problem. The findings show that older individuals have higher levels of antibody positivity while children show lower levels of seropositivity or no antibody response whatsoever (*25*). Interpretation of trachoma serology is challenging because international standards have not been defined to determine cutoff values for seropositivity (*42*), and it is important to consider that exposure to *Chlamydia trachomatis* antigens via urogenital infection is age-dependent and can affect the serology results, especially in settings where this may be a problem.

FIGURE 6.5 **Modeling of seroprevalence curves by age for trachoma surveillance in the post-elimination phase**



Source: Pinsent A, Solomon AW, Bailey RL, Bid R, Cama A, Dean D, et al. The utility of serology for elimination surveillance of trachoma. Nat Commun. 2018;9(1):5444. https://doi.org/10.1038/s41467-018-07852-0

6.8 Prepare and disseminate the report

The draft report should be prepared as soon as possible after the completion of the survey. This version should be discussed with the steering group and presented to the national authorities. Discussion of the results will facilitate analysis of the findings, identification of potential implications for possible interventions, incorporation of new contributions to the conclusions, and the drafting of recommendations to support decision-making. Annex 6.2 describes the structure and basic contents of the report of results.

Clarify the scope and significance of the results for the participating populations. The serological surveillance of communicable diseases, as described in this document, aims to provide population-level information to supplement the data generated by epidemiological surveillance systems. The population must understand that the results of such surveys—especially those of serosurveys in which IgG antibodies are detected—do not produce data for the diagnosis of diseases at the individual level, but rather information on past exposure to diseases and interventions (e.g., vaccination) at the population level. If there is a need to share the results with the communities that participated in the survey, these should be presented in reference to the immune status of the population; for example, regarding protection against vaccine-preventable diseases or past exposure of these communities to the communicable diseases included in the survey. Communities should also be informed of any additional interventions or studies that will be conducted based on the results of the survey.

Based on the interpretation and discussion of the results, the plan of action—and how it will be incorporated into the plans of existing programs involved in integrated serological surveillance—must be defined.

6.9 Make decisions

One key factor in achieving the objectives of integrated serological surveillance is translating results into integrated interventions and incorporating these interventions into programs or plans for each disease. This incorporation should be based on synergies with existing actions, in which activities that can be improved are conceptualized or reconfigured, or ideas are generated to create new interventions. In including different programs, the plan should incorporate opportunities for improvement in the organization, training, and communication processes. Furthermore, it must create a shared awareness and leverage shared facilitators that can contribute to making processes more effective.

To formulate and implement the plan, it is necessary to prioritize actions, define a schedule or timetable, enumerate the resources required, and select the personnel responsible for implementing the plan and reporting progress and results.

The plan of action will depend on the objectives or scenarios selected for integrated serological surveillance. For example, if the goal was to obtain baseline information on the immune profiles against certain diseases or to determine the immunity of a certain population group against VPDs in

epidemiologically silent areas, the plan of action should probably be geared toward furthering in-depth studies that can explain the findings of the survey and prepare any subsequent interventions as appropriate. It should be kept in mind that serological tests detect IgG antibodies, which demonstrate past or recent history of infection, but they are not diagnostic tests. Therefore, to detect active infection in some communicable diseases, surveys usually include additional methods (e.g., filaria test strip [FTS] to detect *W. bancrofti*) to complete the characterization of transmission of the diseases of interest.

If, on the other hand, the objective of serological surveillance was to monitor the impact of interventions for the control and elimination of communicable diseases in areas with well-functioning surveillance systems and where interventions for control and elimination have been implemented robustly and with adequate coverage (e.g., vaccination, actions to improve access to water and sanitation), the plan of action will probably focus on closing any gaps that are found (e.g., susceptible populations that need catch-up vaccination, or populations that need better access to sanitation), or—if the results show that the immune profiles of the population are consistent with the goals of the implemented interventions—on reinforcing and sustaining current actions.

Whatever the objective or epidemiological scenario of integrated serological surveillance, it will always generate information that allows additional in-depth studies to be carried out for further characterization of specific aspects of one or multiple diseases of interest. Also, the plan should define what other sectors or actors (tourism, education, water/sanitation, housing, agriculture, the private sector, academia, civil society, etc.) should be involved; where the population will be easiest to reach if interventions are required; what is the optimal methodology to implement this intervention; potential limitations; and what the best communication strategy will be.

When representatives of national programs and decisionmakers are involved in integrated serological surveillance from the outset (i.e., the definition of objectives), this increases the likelihood that the results of the survey will be used in an appropriate, justified manner. To support the empowerment and to hold the interest of all those involved in implementing the plan, countries should waste no time in analyzing the results of the survey as soon as they are obtained, because the results become outdated quickly. Actions should be implemented, and the results should be published. The success of the plan of action will depend on the technical readiness and political will of each country, as well as on the commitment of the authorities of the different programs and sectors to accept responsibilities and work together.

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Glossary

Antibody

An immunoglobulin molecule having a specific amino acid sequence by virtue of which it interacts only with the antigen (or a very similar shape) that induced its synthesis in cells of the lymphoid series (especially plasma cells).[1]

Antigen

A molecule structure that elicits a specific immune response.

Baseline

An initial measurement used as a benchmark for future comparisons. In the context of this document, the baseline level of population immunity is used as a complementary surveillance tool to monitor transmission of communicable diseases and impact of interventions.

Bias

Discrepancy between the real value of the variable being studied in the population and the value obtained from the sample. The discrepancy is not the result of chance but of errors in the selection of study units, the collection of information, or other factors.

Census

The registration of each and every unit in a given population.

Communicable diseases *(also known as transmissible diseases)*

Illnesses that result from the infection, presence, and growth of pathogenic (capable of causing disease) biologic agents in an individual human or other animal host.

Cutoff value

The antibody level in a serology test above which individuals are classified as seropositive and below which they are considered seronegative.

1 National Center for Biotechnology Information [Internet]. Bethesda, MD: NCBI. MeSH - Antibodies. Available from: https://www.ncbi.nlm.nih.gov/mesh/68000906

Confidence interval

The amplitude of a range within which it is expected to find the true value of the sample with an established degree of certainty (for example, 95% or 99%). The confidence interval represents the probability of random error but not the probability of systematic error or bias.

Design effect (DEFF)

The variance associated with the selection of subjects for a survey using any method other than simple random sampling. It is the ratio of the variance in other types of sampling to the variance in simple random sampling.

Elimination (or interruption of transmission) *(applies to neglected infectious diseases)*

Reduction to zero of the incidence of infection caused by a specific pathogen in a defined geographical area, with minimal risk of reintroduction, as a result of deliberate efforts, continued actions to prevent re-establishment of transmission may be required. The process of documenting elimination of transmission is called verification.[2]

Elimination as public health problem *(applies to neglected infectious diseases)*

A term related to both infection and disease. It is defined by achievement of measurable global targets set by WHO in relation to a specific disease. When reached, continued actions are required to maintain the targets and/or to advance the interruption of transmission. The process of documenting elimination as a public health problem is called validation.

Eradication of neglected infectious diseases

Permanent reduction to zero of a specific pathogen, as a result of deliberate efforts, with no more risk of reintroduction. The process of documenting eradication is called certification.

Foodborne diseases

A set of diseases caused by the ingestion of foods contaminated by living pathogens.

Herd immunity

Occurs when a large portion of a community (the herd) becomes immune to a disease, making the spread of disease from person to person unlikely. As a result, the whole community becomes protected—not just those who are immune.

Immunoassay

A procedure for detecting or measuring macromolecules through their properties as antigens or antibodies.

2 World Health Organization. Generic Framework for Control, Elimination and Eradication of Neglected Tropical Diseases [Internet]. Geneva: WHO; 2016. (WHO/HTM/NTD/2016.6) Available from: https://apps.who.int/iris/bitstream/handle/10665/205080/WHO_HTM_NTD_2016.6_eng.pdf [accessed January 2021].

Neglected infectious diseases *(this is the name given in the Region of the Americas to neglected tropical diseases)*

A set of infectious diseases, many of them parasitic, that primarily affect the poorest of the poor and those with the least access to health services, especially impoverished people living in remote rural areas and urban shantytowns.[3]

Non-probabilistic sample

A sampling method in which the selected individuals do not all have the same probability of being included in the sample, which means that the results cannot be generalized to the larger population being studied, as they are not fully representative.

Probabilistic sample

A sampling method in which all individuals have the same probability of being chosen, thus making it possible to determine the probability of each individual in the sample to be selected.

Random error

Deviation from the results or inferences about the truth due only to chance, without any particular pattern. Confidence intervals and *p*-values are expressions of the probability of random errors, as opposed to systematic errors (bias).

Receptivity *(to malaria)*[4]

Degree to which an ecosystem in a given area at a given time allows for the transmission of *Plasmodium* spp. from a human through a vector mosquito to another human.

Sampling error

The degree of error that the researcher is willing to accept for estimates or decisions based on the results yielded by the sample. It is also known as the *precision of error* or *margin of error*.

Sensitivity

The proportion of people with an infection or condition that are correctly identified as such by a given test (true positive rate).

Serological test

A test performed on blood or other bodily fluids to detect the presence of antibodies.

Seropositive

Detection in a specimen of an antibody level above a given cutoff value (which varies according to the sensitivity of the assay and the purpose of the analysis) for a specific infectious pathogen.

3 Pan American Health Organization [Internet]. Washington, DC: PAHO; 2022. Neglected, tropical and vector borne diseases. Available from: https://www.paho.org/en/topics/neglected-tropical-and-vector-borne-diseases

4 World Health Organization. Malaria Terminology [Internet]. Geneva: WHO; 2017. Available from: https://www.who.int/publications/i/item/9789240038400 [accessed October 2020].

Seroprevalence

Percentage of population positive for a specific antigen or antibody.

Seroprotection

Detection of antibody above a postulated immune protective cutoff value.

Serotype

A serologically distinguishable strain of a microorganism.

Serovar

A group of microorganisms characterized by a specific set of antigens within a single species of microorganism.

Specificity

Proportion of individuals without an infection or disease who are identified as negative by a given test (true negative rate).

Threshold level of infection

Proportion of infection prevalence below which transmission is likely no longer sustainable, even in the absence of control interventions.

Threshold level of herd immunity

Proportion of protected population higher than a critical value that best predict likelihood of interruption of disease or stop its spread.

Vaccine-preventable diseases (VPD)

Infectious diseases for which an effective preventive vaccine exists.

Vector-borne diseases

Human illnesses caused by parasites, viruses, and bacteria that are transmitted by vectors such as such as mosquitoes, ticks, and fleas that can transmit infectious pathogens between humans, or from animals to humans.

Vulnerability *(to malaria)*[5]

Likelihood of malaria infection based on living conditions or behavioral risk factors, or likelihood of increased risk of severe morbidity and mortality from malaria infection.

Waterborne diseases

A set of diseases caused by the ingestion of water contaminated by living pathogens or chemical agents.

5 World Health Organization. Malaria Terminology [Internet]. Geneva: WHO; 2017. Available from: https://www.who.int/publications/i/item/9789240038400 [accessed October 2020].

Annexes

Annex 2.1
Example of surveys into which serological sampling could be incorporated

SURVEY	STUDY POPULATION	METHODOLOGY	SETTING
Malaria	• At-risk populations, taking into account vulnerability and receptivity analysis of the area to be sampled	National or subnational representativeness Blood sample collection	Household
Neglected infectious diseases (*soil-transmitted helminthiasis, lymphatic filariasis, trachoma, onchocerciasis, Chagas disease, etc.*)	• At-risk populations (children aged 1 to 14, children aged 1 to 9, etc.)	National or subnational representativeness Blood samples and other types of samples (stool, eye swabs, etc.)	Schools, community
Demographic and Health Surveys (DHS)	• Large population samples including children, adolescents, and adults to evaluate indicators in the areas of population, health, and nutrition	Typically, are conducted about every 5 years, to allow comparisons over time. National or subnational representativeness Blood sample collection	Household
Nationwide nutritional health/micronutrients survey	• Women of childbearing age, preschoolers, schoolchildren, or children of all ages; adult men	National or subnational representativeness Blood sample collection	Household
Noncommunicable diseases	• All adults aged 18 to 69	National representativeness Blood sample collection (for blood chemistry tests)	Household
Multiple Indicator Cluster Surveys (MICS)	• Women and children under 5 (preschoolers)	National or subnational representativeness Blood sample collection (for anemia, HIV, and malaria)	Household

SURVEY	STUDY POPULATION	METHODOLOGY	SETTING
Vaccine coverage survey	• Children aged 12–23 months, if the final primary vaccination is at 9 months of age • Children aged 24–35 months, if the age recommended for the vaccination is between 12 and 23 months of age • Women who gave birth in the last 12 months • Girls aged 15 years (and not yet 16), if evaluating human papillomavirus (HPV) vaccine in a country	National or subnational representativeness	Household

Annex 3.1
Example of protocol template

A. Introduction[1]

- 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 and elimination of diseases to be included in the serosurvey 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 explain the research question. References to support the justification for the survey should be provided in this section.

B. Methods

1. Objectives

- The primary objective, clearly stating the survey aims 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.

2. Survey population

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

3. Study design

- Describe the inferential goals of the 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.

4. Operational definitions

- Define the criteria that will be used for key exposures and outcomes, and how this will be measured; for example, vaccination coverage by card. Define other critical operational aspects such as how seropositive, equivocal, and seronegative results will be determined (i.e., which cutoff will be used). Cite references to any methodological guidelines used.

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1 Adapted from template included in: World Health Organization. Guidelines on the Use of Serosurveys in Support of Measles and Rubella Elimination. Annexes [Internet]. Geneva: WHO; 2019.

5. Population sampling procedure

- Describe the type of sampling 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.

6. 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.

7. Data collection

- 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.
- Explain who will collect the data and the methods used. Describe the instruments that will be used to collect information and provide details of these instruments in an annex.
- Describe the methods to be used for biological specimen collection, transport, and analysis.
- Describe any other methods you plan to use to collect data and provide references as applicable.

8. Data analysis

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

- Recording 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 to be used;
- Key shell tables and figures added to an appendix; and
- Describe any modeling envisaged and collaborations established to do that modeling.

9. Training and piloting

Describe the procedures that will be used for:

- Training the survey teams and supervisors, including agenda and training materials. It should include not only presentations but also practical exercises about how to conduct the interview, data entering, and taking the blood sample.
- Before starting the field work, a pilot test should be conducted to serve as training to the field workers and supervisors.

10. Quality assurance

Describe the quality assurance procedures that will be used for:

- Field procedures;
- Data collection methods (e.g., pilot testing, training of field workers, translations, field supervision, cross-checking);
- Assays selection and laboratory methods (e.g., assay validation, standard operating procedures [SOP] training, external quality assurance system [EQAS], running controls);
- Data analysis;
- Supervisory methods including numbers of supervisors per field team, number 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 samples).

11. 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); and
- Steps taken to minimize the impact of the bias and/or limitation on the study.

12. Ethical clearance

- *Populations living in vulnerable conditions.* Note whether a group of the population living in vulnerable conditions will be studied. Such populations may include hard-to-reach communities, 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 to. 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 participant, and if vaccination or treatment will be offered to individuals.
- *Confidentiality.* Describe the practical steps taken to protect the confidentiality of survey subjects, such as the 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., program evaluation). If an 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 accessing these? Define the public sharing of the dataset (e.g., by sharing with WHO).

13. Practical considerations

- *Field work.* Describe practical arrangements for the field work (e.g., logistics).
- *Timeline.* Provide a timeline with the key milestone, best presented as a Gantt chart.

14. Communication of results

- The protocol should describe what steps will be taken to communicate results to the different stakeholders, including sensitization and coordination with communities before starting and during the survey.
- Describe the different types of reports: 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.

C. Budget

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

D. 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.

E. References

- List all references to support the background information, methods, and key aspects of the protocol.

Annex 3.2
Example of questionnaire

This questionnaire includes generic questions than can be adapted to the study populations and country context to assess seroprevalence and risk factors related to communicable vaccine-preventable diseases.

Name of locality:	Block and house number:
Name of child (if survey includes children):	Name of parent/guardian:
Name of interviewer:	Date of interview: __ __/ __ __/ __ __ __ __

On arriving at the house, greet the person who answers the door and tell him/her the purpose of the visit:

GOOD MORNING. WE ARE FROM THE MINISTRY OF HEALTH AND WE ARE CHECKING TO SEE IF THE CHILDREN BETWEEN ____ AND _____ YEARS OLD IN THIS COMMUNITY HAVE BEEN VACCINATED AND IF THE CHILDREN WHO NEED TREATMENT FOR PARASITES HAVE RECEIVED IT. IS THERE A CHILD IN THIS AGE GROUP LIVING HERE?

If the answer is "Yes," continue with the interview. If not, thank the person and leave.

SINCE THERE ARE CHILDREN IN THIS AGE GROUP LIVING HERE, I WOULD LIKE TO TALK WITH YOU AND ASK YOU TO ANSWER A FEW QUESTIONS. THE INTERVIEW WILL LAST APPROXIMATELY ***10*** MINUTES. ALL THE INFORMATION YOU GIVE US WILL BE HELD IN STRICT CONFIDENCE.

CAN WE BEGIN NOW?

☐ Yes. If permission is granted to start the interview.

☐ No. If permission is not granted. Fill the following form and discuss the result with your supervisor.

Result of the interview		
	Acceptable house 1	
	House closed 2	
	Children in the age group do not live there 3	
	Refused to participate in the interview 4	
	Other (specify) ____________ 5	

DEMOGRAPHIC AND SOCIOECONOMIC DATA		SPACE FOR CODES
HH1. Number of persons living in the household	____________	
HH2. Ages of the household members:	1. Children under 5: ____________	
	2. 5 to 14 years old: ____________	
	3. 15 to 64 years old: ____________	
	4. 65 years and over: ____________	

DEMOGRAPHIC AND SOCIOECONOMIC DATA		SPACE FOR CODES
HH3. Occupation of the father	____	
HH4. Occupation of the mother	____	
HH5. Educational level of father	1. Primary not completed: ____________________ 2. Primary completed: ______________________ 3. High school not completed:_________________ 4. High school completed: ____________________ 5. Technical school: _________________________ 6. University: _____________________________	
HH6. Educational level of mother	1. Primary not completed: ____________________ 2. Primary completed: ______________________ 3. High school not completed:_________________ 4. High school completed: ____________________ 5. Technical school: _________________________ 6. University: _____________________________	
HH7. How old was the child on his/her last birthday?	Age (in years)	________________
HH8. What is his/her date of birth?	Day/month/year	__/__/____
HH9. Sex	M ___ F___	________________
HH10. Has he/she always resided in this community?	Yes: ______ No: ________	________________
HH11. If the answer is "NO," give the name of the place where he/she lived before:		
HH12. If answer to Question HH10 is NO, indicate the approximate date when he/she moved to this community	Month/year	__/____

VACCINATION		SPACE FOR CODES
IM1. DO YOU HAVE A CARD SHOWING THE VACCINES THE CHILD HAS RECEIVED? (*If the answer is "Yes," ask:* MAY I SEE IT PLEASE?) *If the vaccination card is available, copy the dates for each type of vaccine in the box below.* *Enter a '9' if the card indicates that the vaccine was given but no date is specified.*	Yes, seen ....1 *Go to Question IM3* Yes, not seen ....2 *Go to Question IM5* Does not have card 3	

VACCINATION					SPACE FOR CODES
IM2. DID YOU EVER HAVE A VACCINATION CARD FOR (name)?		Yes ....1 *Go to Question IM5* No ....2 *Go to Question IM5*			
IM3. Vaccines (*must be adapted to national immunization schedule of the country*)		**Vaccination date**			**Space for codes**
		Day	**Month**	**Year**	
BCG/TUBERCULOSIS	BCG				
POLIO 1	OPV OR IPV 1				
POLIO 2	OPV OR IPV 2				
POLIO 3	OPV OR IPV 3				
POLIO I BOOSTER	OPV 1R				
DIPHTHERIA/WHOOPING COUGH/TETANUS 1	DPT 1				
DIPHTHERIA/WHOOPING COUGH/TETANUS 2	DPT 2				
DIPHTHERIA/WHOOPING COUGH/TETANUS 3	DPT 3				
DIPHTHERIA/WHOOPING COUGH/TETANUS I BOOSTER	DPT 1R				
DIPHTHERIA/WHOOPING COUGH/TETANUS II BOOSTER	DPT 2R				
HEPATITIS B	HBV 1				
HEPATITIS B	HBV 2				
HEPATITIS B	HBV 3				
ROTAVIRUS 1	RV 1				
ROTAVIRUS 2	RV 2				
PNEUMOCOCCUS 1	PCV 1				
PNEUMOCOCCUS 2	PCV 2				
PNEUMOCOCCUS 3	PCV 3				
MEASLES/RUBELLA/MUMPS 1	MMR 1				
MEASLES/RUBELLA/MUMPS 2	MMR 2				
HAEMOPHILUS INFLUENZAE TYPE b	Hib 1				
HAEMOPHILUS INFLUENZAE TYPE b	Hib 2				
HAEMOPHILUS INFLUENZAE TYPE b	Hib 3				
HAEMOPHILUS INFLUENZAE TYPE b	Hib 4				

VACCINATION			SPACE FOR CODES
TRIVALENT INFLUENZA	TIV		
IM4. IN ADDITION TO THE VACCINES RECORDED ON THIS CARD, HAS THE CHILD RECEIVED ANY OTHERS? FOR EXAMPLE, VACCINES RECEIVED DURING IMMUNIZATION CAMPAIGNS OR VACCINATION DAYS? ‘		Yes 1 No 2	
IM5. DID THE CHILD EVER RECEIVE THE BCG VACCINE AGAINST TUBERCULOSIS? THIS IS AN INJECTION IN THE ARM OR THE SHOULDER THAT USUALLY LEAVES A SCAR.		Yes ....1 No ....2 Doesn’t know 8	
IM6. DID THE CHILD EVER RECEIVE THE ORAL POLIO VACCINE? THIS IS A VACCINE THAT IS GIVEN IN DROPS TO PROTECT THE CHILD AGAINST POLIOMYELITIS.		Yes ....1 No ....2 Doesn’t know 8	
IM7. HOW MANY TIMES DID THE CHILD RECEIVE THE POLIO VACCINE?		Note the number of times	
IM8. DID THE CHILD EVER RECEIVE INJECTIONS IN THE THIGH TO PREVENT TETANUS, WHOOPING COUGH, AND DIPHTHERIA (DTP)? *Point out that the DTP vaccine is sometimes given along with the polio vaccine.*		Yes ....1 No ....2 Doesn’t know 8	
IM9. HOW MANY TIMES WAS THE DTP VACCINE GIVEN?		Note the number of times	
IM10. DID THE CHILD EVER RECEIVE AN INJECTION AGAINST HEPATITIS B? THIS INJECTION IS USUALLY GIVEN IN THE THIGH. *Point out that the hepatitis B vaccine is sometimes given along with the polio and DPT vaccines.*		Yes ....1 No ....2 Doesn’t know 8	
IM11. WAS THE FIRST HEPATITIS B VACCINE GIVEN WITHIN 24 HOURS AFTER BIRTH, OR LATER?		Within the first 24 hours .... 1 After the first 24 hours 2	
IM12. HOW MANY TIMES DID THE CHILD RECEIVE THE HEPATITIS B VACCINE?		Note the number of times	
IM13. DID THE CHILD EVER RECEIVE INJECTIONS TO PREVENT MEASLES OR RUBELLA (MMR)? *Point out that this injection is given in the arm, almost always starting at 1 year of age to prevent these diseases.*		Yes ....1 No ....2 Doesn’t know 8	
IM14. HOW MANY TIMES DID THE CHILD RECEIVE THE MEASLES VACCINE (OR MMR)?		Note the number of times	
IM15. HAVE YOU EVER RECEIVED THE MENINGITIS VACCINE?		Yes ....1 No ....2 Doesn’t know 8	
IM16. HOW MANY TIMES DID YOU RECEIVE THE MENINGITIS VACCINE?		Note the number of times	

VACCINATION		SPACE FOR CODES
IM17. IF THE CHILD HAS NOT RECEIVED THE COMPLETE VACCINATION SERIES, WHAT IS THE MAIN REASON FOR THE DELAY?	1. Did not know these vaccines are required 2. Did not know where to take child to get the vaccination 3. Did not have time 4. Refuses to vaccinate the child 5. Child was sick 6. Child has some contraindications 7. Health workers refused to vaccinate child 8. Child was taken to health unit but it was closed 9. Child was taken to health unit but they did not have the vaccine 10. Other (specify)_________________	

WATER, SANITATION, AND HYGIENE (WASH)		SPACE FOR CODES
WS1. What is the main source of drinking water for household members? IF THE ANSWER IS UNCLEAR, ASK TO BE SHOWN WHERE HOUSEHOLD MEMBERS USUALLY GET THEIR DRINKING WATER (COLLECTION POINT). CHECK THE ONE MOST FREQUENTLY USED	a) Piped water supply ___________________________ b) Protected well/spring _________________________ c) Unprotected well/spring ______________________ d) Rainwater __________________________________ e) Packages bottled water ______________________ f) Tanker-truck or cart ___________________________ g) Surface water (lake, river, stream) _____________ h) No source of water __________________________ i) Other ____ Specify ___________________________	
WS2. At any time during the last month did your household lack sufficient drinking water?	a) Yes, at least once: ____________________________ b) No, there was always enough: ________________ c) Don’t know: _______________________________	
WS3. Does anyone in the home treat the water in some way to make it safer to drink?	a) Yes: ____ b) No: ____ c) If yes, explain how it is treated: ________________	

WATER, SANITATION, AND HYGIENE (WASH)		SPACE FOR CODES
WS4. What is the main source of water used for bathing in your household? *(If the answer is unclear, ask to be shown where household members usually take the bath). Check the one most frequently used.*	a) Piped water supply ___________________________ b) Water from the river _________________________ c) Water from the well/spring ___________________ d) Other ____ Specify ___________________________	
WS5. How is excreta waste handled in your household? *If the answer is unclear, ask permission to look at the installation. Check the one most frequently used.*	a) Flush/pour-flush toilets _______________________ b) Pit latrines with slab _________________________ c) Composting toilets __________________________ d) Pit latrines without a slab ___________________ e) Hanging latrines ____________________________ f) Bucket latrines ______________________________ g) No toilets or latrines (open air) _______________ h) Other ____ Specify ___________________________	
WS6. We would like to know where household members wash their hands. Please show me where they wash their hands most often. *Record the answers and any observations.*	Observed: a) Installation observed inside the home: __________ b) Installation observed outside the home: ________ Not observed:_________________________________ a) There is no place for hand-washing: ___________ b) No place for hand-washing was seen on the property: ____________________________________ c) Permission to observe was denied: _____________ Other (specify): _______________________________	
WS7. Is soap, detergent, or ashes/clay/sand provided at the place where hands are washed?	Yes: _______ No: _______	

DEWORMING		SPACE FOR CODES
DW1. Has the person participated in any of the following deworming campaigns? *In referring to the campaigns, verify the date and type of health campaign (vaccination, vitamin A, deworming pills, etc.) that was carried out.* CAMPAIGN A (DATE _______, TYPE____________) CAMPAIGN B (DATE _______, TYPE____________) CAMPAIGN C (DATE _______, TYPE____________)	Yes…1 No….2 Doesn't know….8 Campaign A … … … … … … ... 1 2 8 Campaign B … … … … … … ... 1 2 8 Campaign C … … … … … … ... 1 2 8	
DW2. IN THE LAST YEAR, DID THE CHILD RECEIVE TREATMENT TO ELIMINATE WORMS OR INTESTINAL PARASITES? *Show the common types of tablets used for antiparasitic treatment.*	Yes … … … …. …. .1 No … … … … … .. 2 *Go to question DW4* Doesn't know … 8	
DW3. WHEN WAS THE CHILD LAST TREATED FOR WORMS?	Note the date. If the respondent does not remember it exactly, ask how many months.	__/__/____ _______ MONTHS
DW4. WHY WASN'T THE CHILD TREATED FOR WORMS LAST YEAR?	1. Did not know that treatment was necessary 2. Did not know where to get treatment 3. Did not have time 4. Refuses treatment 5. Child was sick 6. Child has some contraindications 7. Health workers refused to give the treatment 8. Child was taken to health unit but it was closed 9. Child was taken to health unit but they did not have the treatment 10. Other (specify)__________________	

MALARIA (IF ADULTS WILL BE SCREENED, THE FORM NEEDS TO BE ADAPTED ACCORDINGLY)		SPACE FOR CODES
M1. WAS THE PERSON SICK WITH FEVER AND CHILLS AT ANY TIME IN THE LAST TWO WEEKS?	Yes.. 1 No .... 2 Doesn't know 8	

MALARIA (IF ADULTS WILL BE SCREENED, THE FORM NEEDS TO BE ADAPTED ACCORDINGLY)		SPACE FOR CODES
M2. AT ANY TIME DURING THIS ILLNESS, WERE BLOOD SAMPLES TAKEN FROM THE PERSON'S FINGER OR HEEL TO DIAGNOSE MALARIA?	Yes.......... 1 No .... 2 Doesn't know 8	
M3. WAS THE PERSON GIVEN A DRUG FOR FEVER OR MALARIA IN THE HEALTH UNIT? *If the answer is "Yes," go to Question M4. Otherwise, go to Question M5.*	Yes.......... 1 No .... 2 Doesn't know 8	
M4. WHAT WAS THE NAME OF THE DRUG THAT WAS GIVEN TO THE CHILD? Write the name of the drug if the name is given. ____________________ *(Name of drug)*	Antimalarials *Chloroquine* .... 1 *Primaquine* .... 2 Antibiotic .... 3 Analgesics and antipyretics *Acetaminophen* .... 4 *Aspirin* .... 5 *Ibuprofen/Motrin* .... 6 Other (specify) .... 7 Doesn't know 8	
M5. DO YOU HAVE A MOSQUITO NET AT HOME THAT IS USED WHEN THE PERSON SLEEPS? *Conclude the interview and thank the person for his/her time.*	Yes 1 No 2	

SCHISTOSOMIASIS RISK FACTORS		SPACE FOR CODES
SC1. Is there a river near this household?	Yes: ____ No: _____	
SC2. Do you often swim, fish, or go to the river for recreation?	Yes: ____ No:_____	

Annex 3.3
Roles and responsibilities of staff

ROLES	RESPONSIBILITIES
Survey coordinator	• Support sensitization and communication with other sectors or organizations that should be involved (e.g., community leaders, national, subnational, and local authorities, among others) from the planning process, before starting and during the field work. • Ensure compliance with the survey design established in the survey protocol. • Request and verify that all supplies, resources, and logistics necessary for the field operation have been purchased and are available. • Ensure that all personnel are trained following the approved protocol and standard operating procedures for sampling. • Make sure that the survey sampling design is followed (list of selected sample units, response rate, etc.). • Coordinate implementation activities in the areas where the survey will be conducted, to ensure that the population is informed and is expected to be present according to the plan of visits and sampling route. • Monitor the data collection, ensuring high-quality data, tracking of field-work progress, secure management of data, and respect for the confidentiality of persons selected to be included in the sample. • Ensure timely supervision as well as adherence to the budget and the efficient utilization of resources. • Prepare written reports on the progress of survey implementation.
Laboratory coordinator	• Support request, purchasing, and verification of supplies for sample collection, transport, and storage, including supplies needed for training of field-work teams. • Ensure the quality implementation of sample collection, transport, and storage procedures, including biosafety according to national regulations. • Train field staff in sample collection, transport, and storage. • Support the supervision and monitoring of field teams. • Detect and solve problems arising in the laboratory. • Verify quality control of the samples. • Support the preparation of reports, specifically progress reports on the implementation of the laboratory portion of the survey. • Review and prepare all the paperwork needed for the courier, such as import permit, commercial invoice, packing list, etc., to send samples to the international laboratory in charge of multiplex analysis. In addition, in countries where the national laboratory will be in charge of testing samples, the multiplex laboratory coordinator and his/her staff should also be responsible to: • Assess laboratory capabilities before starting the serosurvey; • Select, validate, and approve assays and sample types as required; • Ensure appropriate quality assurance and laboratory performance; • Support training and guarantee competency of laboratory staff; • Ensure that laboratory testing and reporting is carried out according to the protocol; • Review laboratory results to detect errors and check missing values in the database before analysis; • Refer specimens for further testing according to procedures and storage for later use; • Ensure safety and security of laboratory procedures and staff.

ROLES	RESPONSIBILITIES
Data manager	• Provide technical support to develop the data collection tools of the survey. • Conduct daily monitoring and supervision of collected data, helping to ensure that sampling strategies are implemented as established in the protocol and operational procedures. • Ensure data quality, integrity of analysis, tracking of field-work progress, and secure data management. • Take notes on problems with implementation issues and challenges that deviate from the survey protocol and alert the national team as soon as possible. • Conduct data analysis of the preliminary results and support drafting of the baseline reports. • Prepare written reports with feedback regarding the progress of the survey implementation.
Regional supervisors	• Support the preparation of materials and supplies to be used in the field. • Check that field teams are trained and carrying out their functions correctly, and, if necessary, provide feedback or refresher training. • Review the list of selected sampling units and assign them to each of the corresponding field teams. • Organize field-work road maps according to the design of the survey. • Monitor the progress and quality of data and sample collection. • Address any problems or contingencies that may arise during the operation. • Maintain close communication with field teams and national coordinators.

Annex 3.4
Roles and responsibilities of field teams

TEAM MEMBERS	RESPONSIBILITIES
Field supervisor (who should ideally be familiar with the geographic area of interest and speak the local language, if necessary)	• Coordinate field-work logistics and oversee the activity in the field. • Contact and coordinate with community leaders and health centers, depending on the survey sampling units. • Implement the local field-work plan according to the road map. • Monitor the field-work road map and the progress of data collection. • Ensure that all selected participants, regardless of age, have signed an informed consent or assent form as appropriate. • Check that each consent form is complete and signed. • Ensure that all participants trust the study procedures and understand what expanded consent is all about (storage of samples for future studies). • Identify and assign a workspace (desk, chair, waste disposal, access to water). • Maintain good communications with the regional supervisor. • Detect, warn of, and address any problems that could arise during field work.
Laboratory technicians	• Assist in identification of the workspace, ensuring it is adequate for sample collection. • Check that the participant identification code is correct (correct participant, correct forms, correct sample dish or test tube). • Check that the consent forms are complete (signed) and ensure that all participants are calm before sample collection. • Correctly perform standard operating procedures for sample collection, storage, and transportation. • Ensure that biosafety measures are followed correctly during the operation (waste disposal, workplace cleaning, etc.).
Interviewers	• Ensure that the subject identification codes on the consent form and on the questionnaire match. • Collect data from the subject or informant in the corresponding forms (PAPI or CAPI). • Ensure that the interview is conducted confidentially. • Check that information for all necessary variables is completed. • Provide support in various processes (e.g., obtaining informed consent, workplace cleaning).

Annex 3.5
List of laboratory supplies to collect DBS

This is a list of supplies and materials to collect dried blood spot (DBS) samples.

DESCRIPTION	UNIT	QUANTITY REQUIRED
Contact activated lancets (needle depending on target population age of the survey)	1 box / 200	
Filter paper cards for DBS collection	1 package / 400	
Humidity indicator cards (1 for each large bag of DBS supplies)	1 package / 125	
1 g silica gel packets gel in tyvek 1 g (3 per large bag)	can / 1,000	
Sharp object disposable containers	Each	
Stereophon for drying filter paper or polystyrene sheet	Each	
Bar-coded labels	One bar-code label to stick on filter paper card and other types of samples and forms depending on the survey	
Biological material disposable bags	Unit	
Small zip-lock plastic bags (8 x 12 cm)	1 unit	
Large zip-lock plastic bags (2 gal or 33 x 38.1 cm)	1 unit	
Coolers for transport of materials	Each	
Disposable gloves (sizes 7, 7½, 8)	1 box / 1,000	
Cotton balls	1 ball	
Alcohol 90%	1 liter	
Liquid soap	1 liter bottle	
Absorbent paper	Roll	
Absorbent underpads	1 case / 100	
Markers	1 pack / 12	

Annex 3.6
Survey budget template

CATEGORY	UNIT COST (USD)	QUANTITY	TOTAL (USD)
Human resources			
National survey coordinator	For each type of staff, define: Salary level: x per x months at x Per diem: per x days		
Supervisors			
Field workers			
Data entry clerk			
Statistician for data analysis and report			
Training			
Training venue			
Refreshments/lunch			
Equipment rental			
Per diem			
Supplies and consumables			
Laboratory supplies	See Annex 3.5		
Field materials (pens, pencils, plastic bags to keep forms, folders, envelopes for forms, etc.)			
Internet access			
Printer and photocopies			
Development of maps			
Phone cards			
Mobile devices			
Transportation			
Travel (air fares)			
Land transportation			
Survey report			
Development of report			
Printing final report			
Dissemination			
Meeting venue			
Media release			
Social mobilization			
Total			

Annex 3.7
Example of survey timetable

ACTIVITY OR TASK	YEAR 1												YEAR 2					
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
Survey protocol																		
Background documentation																		
Survey design and sampling strategy																		
Define variables and questionnaires																		
Elaborate informed consent form																		
Prepare timeline and budget																		
Meetings and discussions of protocol with experts																		
Update survey protocol																		
Elaborate standard operating procedures																		
Obtain ethical clearance																		
Develop the questionnaires, data entry program (mobile platform?) for data management																		
Reproduction of consent forms and printed materials																		
Logistics and coordination																		
Procure diagnostic tests and consumables																		
Hire personnel if needed																		
Define structure and organization of field teams																		
Coordination with education sector and other partners																		
Advocacy process, communication, and social mobilization																		
Training																		
Organize field training																		
Train the national team and supervisors																		
Train the field teams																		
Pilot the protocol																		
Data collection and analysis																		
Conduct the field work to collect data																		
Data entry/management																		
Data analysis																		
Decision-making and dissemination																		
Elaborate survey report																		
Discussion of results and decision-making																		
Dissemination of results																		

Annex 3.8
Example of informed consent form

General indications: The informed consent form, as letter of assent, and expanded consent form must contain the same information. This template should be adjusted as necessary according to the study design and the comprehension level of potential participants (language, maturity, educational attainment, etc.).

<table>
<tr><th>STRUCTURE</th><th>EXAMPLE</th></tr>
<tr><td>Title</td><td>Integrated serological survey of [diseases to be monitored] in [population], [geographic area of interest]</td></tr>
<tr><td>Purpose</td><td>To estimate the seroprevalence of antibodies against [pathogens to be tested] in [target population] in [geographic area]</td></tr>
<tr><td>Description</td><td>Good morning (afternoon), my name is (name of the person obtaining consent) and I work for (name of organization(s)).

The objective of this study is (mention the objective).

Note: The name of all the diseases of interest must also be mentioned. Use terms that are consistent and understandable for the population and local context. If future studies will be included, mention what other diseases might be studied.

The results of the survey will allow us to (mention how this survey will benefit the health of the community or population; for instance, to ascertain whether the population has been exposed to various diseases with a single, small blood sample; know whether vaccination levels/protection against infectious diseases are adequate, etc.). If future studies will be included, mention why it is necessary to store the sample.

The reason for our visit is: (explain the reason, e.g., the household or school was randomly selected, and the participant was also selected to participate). We kindly ask you to agree to participate.

It is very important that you understand what this survey is about:

• Explain the procedures (interview, sampling).
• Mention how long it will take and the volume of blood to be drawn.
• Mention what the survey will consist of, if the study involves children and their parents.
• Explain how the confidentiality of data will be ensured.
• Mention the process of shipping, storing, and analyzing the samples (include information on whether the sample will be analyzed in an overseas laboratory).
• Specify whether participants will be notified of the results and whether any intervention will be carried out (the survey may cover a disease where individual results will be obtained; however, in most cases, serosurveys only provide results at the population level, so any interventions will be implemented at the community level).
• In case of future studies, explain the storage time and how the sample and data will be protected.
• Explain the risks and benefits of participation.

If you agree to participate, you must (explain that the participant must sign the form or place their thumbprint).</td></tr>
</table>

STRUCTURE	EXAMPLE
	The lab test will be free of charge (describe if there is any compensation or cost to participate), but we cannot pay you for your participation. The results of this test will help us get data on past or present history of the diseases included in this study, but it is not meant to find out whether you are sick. Therefore, we will get back results on the level of antibodies against diseases in the community, but not on an individual level. This means we will not deliver a test result to you, but we will obtain information that will benefit your community now and in future. (Describe the potential benefits of participating and the risks, and how those risks would be avoided.)
	We will not be able to identify you at any point in the survey. All names will be changed to number codes. (Mention how the confidentiality of the data will be ensured and explain that potential participants are free to refuse or withdraw from the project at any time if they so desire). Participation in this study is voluntary. Whether or not you take part is entirely up to you. In addition, if you wish, you can leave the survey at any time. You will not lose any benefits, access to your health center, or anything of the sort. If there is anything you do not understand, ask for clarification before giving your consent.
Contact information	If you have any questions, contact (name and contact information of the primary investigator).
Declaration of consent	The study staff has explained the objective of this study to me. I understand what it is about, and I know that I am free to leave the study at any time if I no longer wish to take part. I know that not participating in this study will not affect the care I get at my local health center in any way. I have been explained and read the consent form, any doubts I had were addressed to my satisfaction, and I therefore give my voluntary consent for participation. Signatures and/or fingerprints: ___________________________ Note: in the case of expanded consent, include an option to have the sample anonymized and the opportunity to refuse certain tests (such as genetic tests).

Annex 3.9
Example of child assent form

STRUCTURE	EXAMPLE
Title	Integrated serological survey of [diseases to be monitored] in [population], [geographic area of interest]
Purpose	To estimate the seroprevalence of antibodies against [pathogens to be tested] in [target population] in [geographic area]
Description	Good morning (afternoon), my name is (name of the person obtaining consent) and I work for (name of organization(s)). The objective of this study is (mention the objective). I am going to give you information and invite you to participate in this study. You can choose whether you want to participate. We have discussed this study with your parent(s) or guardian, and they know that we are also asking you for your agreement. They have already agreed to have you participate. If you do not wish to take part in the study, you do not have to, even if your parents have agreed. It is your choice. If you decide not to participate, nothing will happen to you. Even if you say 'Yes' now, you can change your mind later and it will still be okay. You may discuss anything in this form with your parents or anyone else you feel comfortable talking to. There may be some words you do not understand or things that you want me to explain more because you are interested or concerned. Please ask me to stop at any time, and I will take time to explain. If you agree to participate, you must (explain that the participant must sign the form or place their thumbprint). You will have a prick on a finger from the least-used hand; a small amount of blood will be taken on one filter paper to detect if you have been infected by (name the disease or diseases that will be tested). This blood drop will be stored because other diseases could be found from it. Taking a sample of blood from one of your fingers may hurt a little, but it will pass quickly. You will be given cotton with alcohol after to hold on the finger. The sample collection will be done by a trained staff and will not cause any risk to your health.
Declaration of assent	I have been explained and read the assent form, any doubts I had were addressed to may satisfaction, and I therefore give my voluntary consent for participation. Signatures and /or fingerprints: ___________________________

Annex 4.1
Example of training agenda

General indications:

- Indicate the objective of the training workshop and explain the objective of each topic included in the training agenda based on the protocol.
- Training must follow a sequential logic. All participants must be trained in data and sample collection procedures using exercises and practices.
- A 20-minute mid-morning break is recommended. Depending on the cultural context of each country, a 1-hour lunch break is also advised.
- At the end of the training session, participants should complete an evaluation of the objectives and procedures of the survey.
- The agenda should include aspects of the makeup of each team and the roles of each member. Table A4.1 presents an example of a four-day field-team training agenda. This can be adapted to the needs and the procedures of the proposed survey.

TABLE A4.1 **Example of training agenda for an integrated serological surveillance survey**

DAY	TOPIC
Day 1	Participant registration
	Welcome session
	Training objectives and agenda
	Participant presentation
	Objectives and expected impact of the survey
	Sample design (selection of clusters and subjects)
	Ethical aspects
	Questionnaire design
	Practicum: Interview and questionnaire administration (in its actual format)
Day 2	Overview of the first day
	Methods for collecting, storing, and shipping blood samples
	Field biosafety procedures

DAY	TOPIC
	Practicum: Blood sample collection
	Roles, responsibilities, and flow of field operations
	Practicum: Formation and implementation of field teams
	Instructions and logistics for the pilot test implementation
Day 3	Feedback
	Delivery of materials and transport to the practice area
	Practicum: Field work
	Drafting of field-work reports by field teams
Day 4	Presentation of field-work report
	Summary of lessons learned and aspects requiring improvement
	Q&A session
	Evaluation of participants and workshop
	Organization of field teams: formation and assignment to geographic areas
	Agreements and timetable of activities

Annex 4.2
Flow diagram of standard operating procedures for specimen collection

Survey steering group
- Start
- Final budget
- List of supplies
- Requests quotes and makes purchases
- Checks that all supplies are available and have been delivered
- Identifies key staff for the project
- Hires personnel
- Trains and supervises personnel
- Finish

Survey coordinator
- Requests and tracks authorization processes and support from the sectors and levels having authority
- Checks and approves the list of all supplies
- Reviews the list and sends to procurement
- Receives and distributes supplies
- Receives contact information, establishes first contact, and sends information
- Reviews and delivers samples and data for processing or shipping to international lab
- Verifies compliance with daily goals at each level and nonresponse rate
- Maintains communication with supervisors and field teams

Laboratory coordinator
- Prepares list of lab supplies
- Assembles and distributes routes and supplies packages

Regional supervisor
- Maintains communication with key stakeholders and field teams at the local level
- Supervises operations to ensure compliance with the protocol
- Checks that forms are complete and samples are correct

Field supervisor
- Receives and distributes packages
- Communicates with community leaders and coordinates field-work logistics
- Monitors field work and supervises the team
- Reviews and delivers data and samples

Interviewer
- Ensures correct ID and complete consent & assent form
- Conducts the interview
- Enters data in questionnaires
- Checks data are correct and completed
- Submits forms to field supervisor

Laboratory technician
- Uses supplies in an efficient manner
- Checks participant ID and that consent forms are complete
- Takes and stores blood samples
- Follows biosafety measures
- Delivers data and samples

Annex 5.1

Antigens available for integrated serological surveillance in the multiplex bead assay (MBA) platform, their utility in different scenarios, and potential interventions

VECTOR-BORNE DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
Malaria								
Plasmodium falciparum	• Antibodies against malaria are generally used as exposure markers. • Specific antibodies directed against MSP and AMA have a longer half-life than CSP or LSA antibodies (years versus months, respectively). • The multi-antigen IgG assay provides information on the immune profile and intensity of the immune response. • Depending on the age group surveyed, the immune response can be interpreted differently. • In young populations, the absence of antibodies is indicative of the absence of transmission chains starting in that age group (in that cohort).	• Serological surveillance of malaria must be addressed based on the epidemiological scenario and needs. • In low-transmission areas, antibodies with long half-lives (such as MSP1 and AMA1) are important. • In multi-endemic settings, the inclusion of MSP1 for each of the four types of malaria (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*) will be useful, but especially so in low-transmission settings. • Since non-*P. falciparum* infections can often be subclinical, the inclusion of non-*P. falciparum* MSP1 antibodies may uncover residual transmission in the country. • In areas of active transmission, it is interesting to obtain information on the serological profile of long-term and short-term antibody responses. • Panels with different antigens can be constructed to provide information for each epidemiological scenario and should be discussed for each setting in a country.					• For subjects exhibiting an intense immune response, additional tests such as PCR or parasite antigen detection can be performed to determine whether the subject is recovering from a recent infection at the time of sampling or if the response corresponds to a previously resolved infection from previous exposure. • Depending on the epidemiological scenario, if groups of children or adults are found to exhibit a particularly intense immune response, malaria programs should define the interventions to be implemented. • Defining geographic areas with high antibody levels in children or with a high proportion of people positive for short-term antibodies may better guide the prioritization of interventions. It can also provide evidence of priority areas or actions that should be strengthened or implemented.	• Malaria serology increases the time window for detection of exposure by antibody testing, which is much broader than that of PCR, RDT, and other malaria diagnostic tests. • Serological parameters offer a theoretical advantage over parasite prevalence as a measure of endemicity, as antibodies can persist for months or years after infection, thus blunting the effects of seasonal or unstable transmission of malaria. • Serological markers have been suggested as indicators of malaria transmission dynamics, and age-adjusted immune response acquisition rates have been used to estimate the strength of infection, suggesting that immunological markers may provide a useful tool for a rapid assessment of the intensity of malaria transmission. • Current studies suggest that seroprevalence reflects cumulative exposure over time and may, in combination with parasite prevalence data, be used to infer changes in malaria transmission over time and between seasons.
Pf MSP1-19			++	++	++	++		
Pf CSP			++	++	++	++		
Pf LSA1			++	++	++	++		
Pf AMA1			++	++	++	++		
Plasmodium malariae								
Pm MSP1-19			++	++	++	++		
Plasmodium ovale								
Po MSP1-19			++	++	++	++		
Plasmodium vivax								
Pv MSP1-19			++	++	++	++		

NEGLECTED INFECTIOUS DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
Trachoma *Chlamydia trachomatis*								
Pgp3	• Provides information on infection, exposure, cumulative infection, etc.	• Establishing seroprevalence by age is not 100% specific: children are exposed to genital *C. trachomatis* transmitted during childbirth (may cross-react). • There is no established antibody seroprevalence threshold to define resurgence of the risk of trachoma during post-elimination surveillance to detect recurrence.	1–2 +++, <1 = 0	+++	+++	0	• There are no recommendations on interventions to be implemented at the individual or community level based on serology results. • Measurement of these antigens will help to better characterize the use of serological profile data in post-elimination environments.	• Use of both antigens is advised in non-endemic areas to compare serology profiles to those of the endemic areas and thus contribute to characterizing the utility of serology profiles for trachoma. • The Ct694 antigen is only available in the MBA assay, while ELISA and lateral flow tests are available for Pgp3.
Ct694			1–2 +++, <1 = 0	+++	+++	0	• Ct694 is under evaluation, as is the Pgp3 antigen, but the latter has been studied more extensively and more data are available.	
Yaws *Treponema pallidum*								
r-p17	• Current infection or previous exposure to *T. pallidum* subsp. *pallidum* (syphilis) or *T. pallidum* subsp. *pertenue* (yaws). • Serves as a marker of historical infection.	• Marker of exposure to treponemal antigens: the epidemiological context and age will determine whether the exposure was to yaws or syphilis.	0	0	+++	0	• The current WHO yaws program suggests that seroprevalence <1%, together with the historical absence of reporting of cases and no evidence of current infection, indicates a lack of transmission Positivity to r-p17 alone (not to TmPA) may indicate past exposure.	
TmPA	• Marker of current infection with *T. pallidum* subsp. *pallidum* (syphilis) or *T. pallidum* subsp. *pertenue* (yaws). • The immune response decreases after treatment.		0	0	+++	0		

NEGLECTED INFECTIOUS DISEASES

Antigen	What does the antigen measure?	Utility in different epidemiological scenarios	Utility by age group				Potential interventions based on survey findings (individual or community level)	Other considerations
			<2	2–4	5–14	≥15		
Schistosomiasis *Schistosoma mansoni*								
Sm25	• Measures antibodies against adult *S. mansoni* parasites. No significant cross-reactivity with *S. haematobium* is expected.	• Both Sm25 and SEA are good markers of historical infection. However, no distinction can be made between active and past infection, and antibody levels do not decrease over time after cure. • This antigen is useful for baseline mapping (at all ages) and to monitor progress toward elimination of transmission. • SEA antibodies may be useful in younger age groups, as seroconversion is earlier due to being a crude antigen mix.	+	++	+++	+++	• There are no recommendations on which interventions to implement. • Measurement of these antigens will help to better characterize the use of serological profile data.	• For persons living in non-endemic or low-transmission areas, serological tests can be helpful in demonstrating exposure to infection and the need for comprehensive examination, laboratory diagnosis, treatment, and follow-up.
SEA	• Measures antibodies against schistosome eggs. It is a cell lysate so is a complex mixture of antigens. It is more sensitive but less specific than Sm25. • There is evidence that high levels of antibody in children correlate with increased burden of infection as measured by eggs found in stool.		+	++	+++	+++		

NEGLECTED INFECTIOUS DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
Lymphatic filariasis (*1*) *Wuchereria bancrofti* and *Brugia malayi*								
Wb123	• Wb123 is specific for *W. bancrofti* and is highly expressed by the larval stage (L3), which is the infectious life stage transmitted by mosquitoes. Therefore, it may be a specific marker of ongoing transmission. • Antibodies can take years to develop even in high-transmission areas, and the duration of antibody persistence after cure is unknown. • There is evidence that the community load of antibodies will decrease over time after mass drug administration (MDA)	• Wb123 is being used in operational research to evaluate its utility as a marker of continuous transmission. • There is evidence in some settings that elevated levels of antibodies to Wb123 correlate with circulating filarial – antigen and may therefore be useful as markers for monitoring the efficacy of MDA (*2*). • Bm14 is also widely used and is being investigated as a potential sensitive marker for evaluation of transmission and long-term surveillance after validation of interruption of transmission. • Bm33 is highly immunodominant but is only used in conjunction with the other two antigens. • Bm14 and Bm33 are cross-reactive with *B. malayi*, *Onchocerca volvulus*, *Loa* spp., and *Mansonella* spp.; therefore, these are not useful in certain African and Asian settings. • Different epidemiological settings with different age groups may yield different information. • The absence of positive responses in children and adults is good evidence of low or absent transmission. • Positive responses in children born after MDA are possible evidence of continuous transmission, but there are no formal thresholds available to indicate which levels indicate an exposure high enough to lead to recurrence.	0	+/++	++	+	• There are no formally defined thresholds for the antigen that could be used to prompt interventions. • Provisional thresholds can be determined by subject matter experts for specific settings based on the cumulative evidence of ongoing operational research being conducted. • For communities with many individuals exhibiting an intense immune response, further follow-up studies may be required.	• The multiplex assay has not yet been accepted for programmatic evaluations; however, with additional analyses and mounting experience, it could represent a cost-effective option.
Bm14	• Measures antibodies against a highly conserved and highly immunogenic filarial antigen. Has been identified in *B. malayi* but is cross-reactive with other filarial species, especially *W. bancrofti*. • Is a relatively sensitive marker of historical infection or exposure to *W. bancrofti*. • Seroconversion can take years, even in an area of ongoing high transmission and there is evidence that the antibodies are long-lasting, but titers will eventually decrease after cure.		0	+/++	++	+		
Bm33	• Same as Bm14 • There is some evidence that, in highly endemic settings, seroconversion for Bm33 occurs earlier than for Bm14 or Wb123.		0	+/++	++	+		

NEGLECTED INFECTIOUS DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
River blindness (onchocerciasis) *Onchocerca volvulus*								
OV-16	• Current or past infection with *O. volvulus*. • Antibody responses take at least 15 months to develop; therefore, it is not an immediate marker of infection. • Antibody responses will be detectable for several years after infections have been cleared.	• Not for clinical diagnosis, only for programmatic evaluations: mapping of areas to be treated with ivermectin, monitoring, and evaluations to cease ivermectin MDA. • Also can be used to verify the elimination of transmission at the end of the post-treatment surveillance period. • Potential utility for post-elimination surveillance.	0	++/+++	++/+++	Mapping	• For standard ELISA tests, positive mapping evaluations may prompt initiation of ivermectin MDA; positive tests on evaluations meant to ascertain whether MDA can be discontinued mean the area failed evaluation and, therefore, that MDA must continue. According to WHO criteria, once there are <2% positive samples per 2,000 children, MDA can be discontinued.	• The multiplex assay has not yet been accepted for programmatic evaluations. Additional evaluations will be needed to incorporate the results of the Luminex OV-16 assay into activities to support important decisions regarding onchocerciasis elimination programs.
Toxocariasis *Toxocara canis*								
CTL-1	• Antibodies are markers of exposure or infection. Unable to distinguish between *T. canis* and *T. cati*.	• No information is available on its use in the field. • Potential utility only for mapping and baseline studies.	?	++?	++?	++?	• No recommendations on its use.	
Strongyloidiasis *Strongyloides stercoralis*								
NIE	• High titers may be indicative of current chronic infection if the individual has never been treated. IgG4 antibodies generally decrease significantly within 6 months of successful treatment; however, some patients may remain seropositive after treatment. • This antigen is well characterized as a serological tool by many groups in many settings. NIE stands out among the soil-transmitted helminth antigens because it has good specificity and does not cross-react with other common parasitic infections.	• Mapping and evaluation of the impact of interventions, both at the community and individual level.	?	++?	++?	++?	• Individuals may be treated if there are no contraindications and no history of treatment. • There are no defined thresholds, but for communities in which many individuals exhibit an intense immune response, more detailed follow-up studies are necessary.	• Infection is associated with greater risk in immunocompromised individuals.

NEGLECTED INFECTIOUS DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
Fascioliasis *Fasciola hepatica*								
FhSAP2	• Antibodies are markers of exposure.	• No information is available on its use in the field. • Potential utility only for mapping and baseline studies.	?	++?	+++?	++?	• There are no defined thresholds, but for communities in which many individuals exhibit an intense immune response, more detailed follow-up studies are necessary.	
Cysticercosis (neurocysticercosis) *Taenia solium*								
T24H	• Antibodies are markers of exposure to cysts. Sensitivity has only been characterized in patients with evidence of cysts in imaging studies.	• Potential utility only for mapping and baseline studies.	0	+?	++?	+++?	• T24H seropositivity in the multiplex bead assay should not require individual intervention unless the individual who tested positive also showed clinical symptoms of neurocysticercosis (e.g., epileptic seizures, severe headache). • Low-level, transient seropositivity for this antigen is not indicative of clinical disease. • There are no defined thresholds, but for communities in which many individuals exhibit an intense immune response, more detailed follow-up studies are necessary.	• Recommended for joint use with rES33.
Taeniasis *Taenia solium*								
rES33	• Antibodies are markers of exposure to adult tapeworm.	• Potential utility only for mapping and baseline studies.	0	+?	++?	+++?	• Antibody positivity for rES33 (taeniasis) should be followed in individuals, confirmed by another test (such as ELISA, fecal antigen, or stool microscopy), and then clinically treated if confirmed positive. • Treating positives is important because these individuals are likely contributing to the transmission cycle. • A <10% positivity rate is expected based on previous studies. If positivity exceeds 10%, the cutoff method may need to be evaluated before proceeding with the intervention. • There are no defined thresholds, but for communities in which many individuals exhibit an intense immune response, more detailed follow-up studies are necessary.	• Recommended for joint use with T24H.

FOODBORNE AND WATERBORNE DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
Cryptosporidiosis *Cryptosporidium parvum*								
Cp17	• Previous infection	• The prevalence of antibodies against these antigens is very high in areas without access to clean water and good environmental sanitation. • These antigens could be useful in combination with other water quality indicators when comparing different communities with unknown sanitation conditions.	+++	++	+	0	• Water, sanitation, and hygiene (WASH) interventions	
Cp23								
Giardiasis *Giardia lamblia*								
VSP3	• Previous infection	• The prevalence of giardia infections is expected to be high in areas without access to clean water and good environmental sanitation. • Giardia infections can become chronic when left untreated. - There is evidence that chronic infection leads to immunotolerance and a decrease in detectable IgG levels; i.e., seroprevalence goes down in highly endemic areas after the first 1–5 years (*3, 4*). Thus, it is critical to look at young age groups to get the most useful information for these antigens. • These antigens could be useful in combination with other water quality indicators when comparing different communities with unknown sanitation conditions.	+++	++	+	0	• Water, sanitation, and hygiene (WASH) interventions	• Studies may be run with VSP5 alone without a deleterious loss of sensitivity.
VSP5								
Toxoplasmosis *Toxoplasma gondii*								
Sag2A	• Current (lifetime) infection	• Mapping and community surveillance.	++	++	++	+++	• Assess community risk for high-risk populations such as pregnant women.	

VACCINE-PREVENTABLE DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
Tetanus *Clostridium tetani*								
Tetanus toxoid	• Vaccine-acquired immunity (only)	• Provide evidence of the impact of vaccination programs (good marker of routine immunization program because natural immunity is not relevant). • Assess immunity gaps in subpopulations (age groups, regions). • Monitoring the achievement and maintenance of maternal and neonatal tetanus elimination in women of reproductive age.	+++	++	++	+++ (especially in women)	• Specific correction of immunity gaps (catch-up immunization). • Directed strengthening of the immunization program and/or surveillance system. • Close immunity gaps and improve maternal and child health. • Optimization of immunization programs and/or introduction of booster doses.	• Good marker of routine immunization programs because natural immunity due to infection is not long lasting. • Waning immunity after vaccination is important in tetanus (six doses are required: three primary doses in childhood and three boosters to provide life-long immunity). • Other relevant information to interpret the seroprotection observed includes historical vaccination coverage by birth cohorts; historical vaccination schedules; vaccination campaigns for women of childbearing age; cold chain management (especially freezing); and outreach ability to reach remote rural areas.
Diphtheria *Corynebacterium diphtheriae*								
Diphtheria toxoid	• Immunity in the target population	• Provide evidence of the impact of vaccination programs. • Assess immunity gaps in subpopulations (age groups, regions). • Supporting evidence for the achievement of elimination goals.	++	++	++	++	• Specific correction of immunity gaps (catch-up immunization). • Directed strengthening of the immunization program and/or surveillance system. • Optimization of immunization programs and/or introduction of booster doses.	• Waning immunity after vaccination is common in diphtheria. • Other important information to interpret the seroprotection observed includes historical vaccination coverage by birth cohort; historical vaccination schedules; coverage achieved in vaccination campaigns; cold chain management (especially freezing); ability to reach remote rural areas; vaccine efficacy; cases or outbreaks of the disease in the study population.

VACCINE-PREVENTABLE DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
Measles Measles virus								
Whole virus	• Vaccine-acquired immunity or natural infection	• Provide evidence of the impact of vaccination programs. • Assess immunity gaps in subpopulations (age groups, regions). • Evaluate the impact of national campaigns. • Supporting evidence for the achievement and maintenance of elimination.	+++	+++	++	++	• Specific correction of immunity gaps (catch-up immunization). • Specific strengthening of the immunization program. • Optimization of immunization schedules.	• For measles, serological surveillance studies can provide good estimates of immunity at the community level. • Proper interpretation of the results of measles immunity profiles must include analysis of: historical vaccination coverage by birth cohort; historical vaccination schedules; coverage achieved in vaccination campaigns; cold chain management; ability to reach remote rural areas; vaccine efficacy; cases or outbreaks of the disease in the target population.
Rubella Rubella virus								
Whole virus	• Acquired immunity or natural infection	• Provide evidence of the impact of vaccination programs. • Assess immunity gaps in subpopulations. • Adjust disease transmission dynamics and provide evidence for vaccine introduction (when rubella vaccine is not used in routine immunization or has been recently introduced). • Evaluate the impact of national campaigns. • Supporting evidence for the achievement and maintenance of elimination.	+++	+++	++	+++ (especially in women)	• Specific correction of immunity gaps (catch-up immunization). • Specific strengthening of the immunization program and/or surveillance system. • Optimization of immunization schedules.	• Proper interpretation of the results of rubella immunity profiles must include analysis of the following information, which is relevant to the expected level of immunity: historical vaccination coverage by birth cohort; historical vaccination schedules; coverage achieved in vaccination campaigns; cold chain management; ability to reach remote rural areas; vaccine efficacy; cases or outbreaks of the disease in the target population.

VACCINE-PREVENTABLE DISEASES								
Antigen	**What does the antigen measure?**	**Utility in different epidemiological scenarios**	**Utility by age group**				**Potential interventions based on survey findings (individual or community level)**	**Other considerations**
			<2	**2–4**	**5–14**	**≥15**		
COVID-19 SARS-CoV-2								
S	• Antibodies against any part of the spike trimer. Antibodies could be from vaccination or natural infection.	• Estimate the seroprevalence of antibodies to SARS-CoV-2 in the general population and how this may change over time. • Estimate the fraction of asymptomatic, pre-symptomatic, or subclinical infections in the population. • Determine risk factors for infection by comparing the exposures and other characteristics (demographics, underlying medical conditions, etc.) of infected and non-infected individuals. • Estimate the case fatality ratio. • Answer questions about antibody kinetics following SARS-CoV-2 infection	0	TBD	TBD	***	• These antigens are only for gaining community level information and not for diagnostic purposes.	• Assay sensitivity greatly increases 21 days post symptom onset. A proportion of mild infections may fall below the detection threshold several months post exposure. • It is recommended to assess sensitivity and specificity using study-specific samples (e.g., 75 pre-pandemic samples from appropriate country/age group and 25 PCR positives from same age group post-outbreak). Especially important for N protein, which may have greater risk of false positives than S or RBD due to seasonal coronaviruses. Varying levels of false positives against spike/RBD protein has also been seen in certain populations but the root cause is unknown. • Antigens are from the D614G variant circulating early in the pandemic. Effect of variants on sensitivity and specificity are currently unknown.
RBD-541	• Antibodies against a portion of the receptor binding domain of the spike protein. Antibodies could be from vaccination or natural infection. Some antibodies binding to this region are neutralizing.		0	TBD	TBD	***		
RBD-591	• Antibodies against a portion of the receptor binding domain of the spike protein. Antibodies could be from vaccination or natural infection. Some antibodies binding to this region are neutralizing.		0	TBD	TBD	***		
N	• Antibodies against the nucleocapsid protein acquired from natural infection.		0	TBD	TBD	***		

Note: Table updated to June 2021.

References Annex 5.1

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Annex 5.2
Sensitivity and specificity of validated antigens for integrated serological surveillance in the multiplex bead assay (MBA)

Disease and pathogen*	Antigen	Sensitivity validation		Specificity validation		Serological reference tests/gold standard	Known cross-reactive species
		Positive panel classification	Sensitivity (95% CI)	Negative panel classification	Specificity (95% CI)		
VECTOR-BORNE DISEASES							
Malaria (*1–3*)							
Plasmodium falciparum	Pf MSP1-19	Blood smear + (active infection)	75% (59–87)	Non-endemic population	100% (96–100)	None	MSP1 isoforms have potential for cross-reaction
	Pf CSP	No data	No data	No data	No data	None	
	Pf LSA1	No data	No data	No data	No data	None	
	Pf AMA1	No data	No data	No data	No data	None	
Plasmodium malariae	Pm MSP1-19	No data	No data	No data	No data	None	MSP1 isoforms have potential for cross-reaction
Plasmodium ovale	Po MSP1-19	No data	No data	No data	No data	None	MSP1 isoforms have potential for cross-reaction
Plasmodium vivax	Pv MSP1-19	Blood smear + (active infection)	94% (81–98)	Non-endemic population	99% (94–100)	None	MSP1 isoforms have potential for cross-reaction

Disease and pathogen*	Antigen	Sensitivity validation		Specificity validation		Serological reference tests/gold standard	Known cross-reactive species
		Positive panel classification	Sensitivity (95% CI)	Negative panel classification	Specificity (95% CI)		
NEGLECTED INFECTIOUS DISEASES							
Trachoma (*4*)							
Chlamydia trachomatis	Pgp3	Amplicor PCR (active infection)	91% (62–98)	Non-endemic population	98% (93–99)	Chlamydia MIF IgG (Focus Diagnostics)	*Chlamydia pneumoniae*; genital chlamydia
	Ct694	Amplicor PCR (active infection)	91% (62–98)	Non-endemic population	98% (93–99)	Chlamydia MIF IgG (Focus Diagnostics)	*Chlamydia pneumoniae*; genital chlamydia
Yaws							
Treponema pallidum (5)	rp17	TPP(H)A+, RPR+ (active infection)	100% (97–100)	Non-endemic population	100% (98–100)	TPPA (Fujirebio Diagnostics) RPR (Alere Wampole)	*Borrelia burgdorferi* (Lyme disease); *Leptospira* spp (leptospirosis)
	TmPA	TPP(H)A+, RPR+ (active infection)	97% (92–99)	Non-endemic population	100% (98–100)	TPPA (Fujirebio Diagnostics) RPR (Alere Wampole)	*Borrelia burgdorferi* (Lyme disease); *Leptospira* spp (leptospirosis)
Schistosomiasis							
Schistosoma mansoni (6)	Sm25	Stool positive (active infection)	98% (87–100)	Non-endemic population	100% (97–100)		
	SEA	Stool positive (active infection)	94% (82–98)	Non-endemic population	97% (92–99)		Other *Schistosoma* spp

Disease and pathogen*	Antigen	Sensitivity validation		Specificity validation		Serological reference tests/gold standard	Known cross-reactive species
		Positive panel classification	Sensitivity (95% CI)	Negative panel classification	Specificity (95% CI)		
NEGLECTED INFECTIOUS DISEASES							
Lymphatic filariasis							
Wuchereria bancrofti (*7*)	Wb123	Blood smear microfilaria + (active infection)	82% (71–90)	Non-endemic population	100% (96–100)	Lymphatic Filariasis Bm14 Antibody CELISA, Cellabs	
	Bm14	Blood smear microfilaria + (active infection)	95% (87–98)	Non-endemic population	93% (86–98)	Filaria DetectTM IgG4 ELISA-RUO	*Onchocerca volvulus, Brugia malayi, Loa, Mansonella*
	Bm33	Blood smear microfilaria + (active infection)	94% (85–97)	Non-endemic population	98% (92–99)	None	*Onchocerca volvulus, Brugia malayi, Loa, Mansonella*
Onchocerciasis (river blindness)							
Onchocerca volvulus	Ov16	Skin snip microfilaria + (active infection)	95% (93–99) IgG 96% (93–99) IgG4	Non-endemic, non-endemic + for other pathogens	99% (96–100) IgG 100% (99–100) IgG4	IgG4 anti-OV16 ELISA	*Wuchereria bancrofti*
	Ov33	Skin snip microfilaria + (active infection)	91% (87–99) IgG 96% (94–100) IgG4	Non-endemic, non-endemic + for other pathogens	97% (87–98) IgG 99% (96–100) IgG4		*Wuchereria bancrofti*
Toxocariasis							
Toxocara canis (*8*)	CTL-1	Visceral larva migrans Ocular larva migrans	90% (85–94) 54% (39–68)	Normal human sera Cross reactivity panel	99% (97–100)		None relevant for surveillance (cannot distinguish between *Toxocara canis* and *Toxocara cati*)

Disease and pathogen*	Antigen	Sensitivity validation		Specificity validation		Serological reference tests/gold standard	Known cross-reactive species
		Positive panel classification	Sensitivity (95% CI)	Negative panel classification	Specificity (95% CI)		
NEGLECTED INFECTIOUS DISEASES							
Strongyloidiasis							
Strongyloides stercoralis (*9*)	NIE	Larvae positive stool or sputum (active infection)	93% (88–96) IgG4 90% (77–96) IgG	Non-endemic population Cross reactivity panel	95% (93–97) IgG4 94% (89–97) IgG		None
Fascioliasis							
Fasciola hepatica	FhSAP2	Egg positive stool (active infection)	94% (82–100) IgG 100% (80–100) IgG4	Non-endemic population Cross reactivity panel	97% (93–100) IgG 99% (96–100) IgG4	Western blot	None
Cysticercosis (neurocysticercosis)							
Taenia solium (*10*)	T24H	2 or more viable cysts single viable cyst non-viable cyst	96% (89–99) 58% (39–74) 37% (25–51)	Cyst negative and population	97% (93–98)		None
Taeniasis							
Taenia solium	rES33	rES33	Active tapeworm infection	91%	Non-endemic population		None

Disease and pathogen*	Antigen	Sensitivity validation		Specificity validation		Serological reference tests/gold standard	Known cross-reactive species
		Positive panel classification	Sensitivity (95% CI)	Negative panel classification	Specificity (95% CI)		
			FOODBORNE AND WATERBORNE DISEASES				
Cryptosporidium							
Cryptosporidium parvum (11)	Cp17	Western blot positive	91%	Western blot negative	87%	Western blot with oocyst lysate	*Cryptosporidium* spp
	Cp23	Western blot positive	95%	Western blot negative	100%	Western blot with oocyst lysate	*Cryptosporidium* spp
Giardiasis							
Giardia lamblia (11, 12)	VSP3	Stool +, outbreak	65%	Not available	Unknown		Assemblage A and B
	VSP5	Stool +, outbreak	65%	Not available	Unknown		Assemblage A and B
Toxoplasmosis							
Toxoplasma gondii (12)	Sag2A	1998 Toxoplasma Human Serum Panel	100%	1998 Toxoplasma Human Serum Panel	100%	Sabin–Feldman dye test/IgG IFA	Unknown

Disease and pathogen*	Antigen	Sensitivity validation		Specificity validation		Serological reference tests/gold standard	Known cross-reactive species
		Positive panel classification	Sensitivity (95% CI)	Negative panel classification	Specificity (95% CI)		
			VACCINE-PREVENTABLE DISEASES				
Tetanus							
Clostridium tetani (13, 14)	Tetanus toxoid	1862 DAE positive	99%	288 DAE negative	92%	Double antigen ELISA (SSI, Denmark)**	None
Diphtheria							
Corynebacterium diphtheriae	Diphtheria toxoid	974 TNT positive	95%	326 TNT negative	83%	Vero cell toxin neutralization assay (DRL, UK)**	None

Disease and pathogen*	Antigen	Sensitivity validation		Specificity validation		Serological reference tests/gold standard	Known cross-reactive species
		Positive panel classification	**Sensitivity (95% CI)**	**Negative panel classification**	**Specificity (95% CI)**		
VACCINE-PREVENTABLE DISEASES							
Measles							
Measles virus *(15)*	Whole virus	516 sera from multiple sources at a cutoff of 153 mIU/mL	98%	516 sera from multiple sources at a cutoff of 153 mIU/mL	83%	Plaque reduction neutralization test (PRNT)	Not significant
Rubella							
Rubella virus	Whole virus	160 pre and post measles–rubella vaccination in a Bangladesh cohort at a cutoff of 9.36 IU/mL	99%	160 pre and post measles–rubella vaccination in a Bangladesh cohort at a cutoff of 9.36 IU/mL	100%	Zeus ELISA positive	Not significant
COVID-19							
SARS-CoV-2	S Spike full length trimer	87 plasma collected in Mar–June 2020, RT-PCR positive	96.6% (90.3–99.3)	99 plasma collected prior to Nov 2019; 19 RT-PCR negative, Mar–June 2020	99.2% (95.3–100)	SARS-CoV-2 RT-PCR	Not known broadly; minimal in US but could be higher in other countries
	RBD-541 Receptor binding domain (GenBank MN908947, residues 319–541)	87 plasma collected in Mar–June 2020, RT-PCR positive	95.4% (88.6–98.7)	99 plasma collected prior to Nov 2019; 19 RT-PCR negative, Mar–June 2020	97.4% (92.7–99.5)	SARS-CoV-2 RT-PCR	Not known broadly; minimal in US but could be higher in other countries
	RBD-591 Receptor binding domain (GenBank MN908947, residues 319–591)	87 plasma collected in Mar–June 2020, RT-PCR positive	95.4% (88.6–98.7)	99 plasma collected prior to Nov 2019; 19 RT-PCR negative, Mar–June 2020	100% (96.9–100)	SARS-CoV-2 RT-PCR	Not known broadly; minimal in US but could be higher in other countries
	N Nucleocapsid protein	87 plasma collected in Mar–June 2020, RT-PCR positive	96.5% (90.3–99.3)	99 plasma collected prior to Nov 2019; 19 RT-PCR negative, Mar–June 2020	98.3% (94.0–99.8)	SARS-CoV-2 RT-PCR	Seasonal coronaviruses

Notes:

* Table updated to October 2021.

** Performance has been compared to standard ELISA.

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Annex 6.1
Recommendations for descriptive analysis of serosurveys where MBA was used

RELEVANT ASPECTS FOR ANALYSIS	
General aspects to consider when analyzing serology results	Seropositivity in communicable diseases can indicate who has been exposed to pathogens or vaccines (but is not infected), who is currently infected, and who has been infected or vaccinated in the past but may have been cured.
	Cutoff points of antibody levels for vaccine-preventable diseases allow estimation of levels of individual and community protection.
	For antigens of many protozoan and some bacterial diseases, the extent to which antibodies persist after exposure or cure is uncertain or unknown.
	Crude median fluorescence intensity (MFI) readings are translated to IU/mL using international reference standards.
	Antibody responses can be expressed as percent seroprevalence, median antibody levels, or antibody titer categories (for tetanus and diphtheria).
	Although there are currently no guidelines or recommendations on the interpretation of serology results for neglected, vector-, and water-borne diseases, these can be extrapolated from established approaches for vaccine-preventable diseases. Analyses can also be done using point estimates to identify areas or subpopulations of interest.
	These responses are evaluated in populations of interest to estimate immunity and identify populations with gaps or waning immunity in different demographic groups.
	The analysis of serology results in this group of diseases follows guidelines and recommendations published by WHO, such as the guide for the serological surveillance of tetanus. In other cases, other serological surveillance experiences can be drawn upon, such as the guide for measles and rubella surveillance in Europe.
EXPLORATORY ANALYSIS OF SEROSURVEY DATABASES	
Review the data collected in the survey, the denominators for the different levels of analysis, and examine the limitations	Sample size and number of clusters (if a cluster sampling strategy was used) by stratum or subpopulation for which inferences are expected (municipalities, localities, etc.).
	The average size of each cluster, including the number of clusters with no responses or participants, as applicable. Check, for example, if the desired proportion of participants per sampling unit was achieved.
	The proportion of missing data for each variable.
Describe how sampling was done	Describe how schools were selected exactly as done in the field. Note whether any schools originally selected for sampling were replaced; if so, which ones, why, how, etc.
	Describe how children were selected within schools (e.g., by simple random or convenience sampling). Note whether any participants were replaced; if so, how the replacements were done and whether they were properly documented; describe rejections and analyze the demographics of the rejected participants.
Calculate the design effect	Taking into account that the interviewees have different selection probabilities at each selection step (school and child) due to the study design, the analysis must be weighted to take into account this unequal selection probability.

Calculate post-stratification weights	The use of post-stratification weighting consists of comparing the distribution of the weighted sample (using the design effect described above) according to a given characteristic with the distribution according to the same characteristic as obtained by another source of information. The other source may be census data or a population projection. Before making this type of adjustment, the statistician must assess whether the other source of information is expected to be truly superior to the survey that has been conducted. This calculation is important if, for example, there is interest in finding out whether males were underrepresented or overrepresented in the sample.
DATA SCRUBBING AND PRE-ANALYSIS PREPARATION	
Demographic data	Review the responses to the different variables (both demographic and disease-related factors, as well as questionnaire items).
	Create new variables by recategorization or pooling of variables of interest. For example, if an analysis by age group is required, a new variable can be created from the original age ranges (under 5, 5 to 14, and over 14 years).
Laboratory data	Calculate the median fluorescence intensity (MFI) and its ranges for each antigen.
	In some antigens—for example, for parasitic diseases using recombinant proteins made in bacteria—the background reactivity of the negative control protein (GST) should be taken into account, which could eliminate nonspecific positives.
Convert the MFI results of the sample to international units per milliliter (IU/mL)	For most vaccine-preventable diseases, the results of MFI readings should be converted to IU/mL. This can be done as follows: • Plot a standard curve using international standards and fit it to a regression model. • Outliers can be excluded for a better fit. • Apply the model used to convert the MFI of the sample to IU/mL. • Outliers should be censored at the last valid value and this value is taken into account for the analysis. *Note:* This procedure does not apply for diseases that do not have defined international units standards (e.g., trachoma).
Create dichotomous seroprevalence variables	For instance: ≥10 IU/mL = protected against rubella.
ESTIMATE SEROPREVALENCE	
Point estimates of seroprevalence	Calculated with Wilson (or logit) 95% confidence intervals using Taylor series linearization to account for the cluster design (school-based survey).
	Point seroprevalence can be calculated taking into account (or not) the design effect to review the impact of variable weighting.
	These calculations can be done using specialty statistical software, such as STATA or SPSS.
Comparisons of seroprevalence	• Seroprevalence can be compared by epidemiological history (e.g., symptoms, occurrence of outbreaks, history of endemicity) or exposure to interventions. • For vaccine-preventable diseases, the proportion of seroprevalence can be calculated by state or vaccination history (number of doses received). The source of information (vaccination record, verbal report, or both) must be taken into account.
Calculate the proportion of seroprevalence in subpopulations	By age group, municipality, area (urban vs. rural), etc.
Statistical comparisons of differences in seroprevalence between subpopulations	Compare seroprevalences (e.g., between municipalities) and use appropriate statistical tests to ascertain whether the differences are significant. It is important to note that the sample size must be sufficiently powered to detect true differences between subpopulations.

Calculate the median antibody level	The median antibody level can be calculated by epidemiological history, exposure to interventions, and subpopulation. For vaccine-preventable diseases, it can be calculated by vaccination status or history and subpopulation.
Interpretation of seroprevalence	For tetanus and diphtheria, calculate the proportion of antibody levels by category, taking into account: • Higher antibody levels generally correlate with greater likelihood and duration of protection. In this analysis, no qualitative assessments of the duration of protection should be made, such as "short" or "long"; instead, the ranges of numerical seroprotection categories should be reported; e.g., <0.01 IU/mL; 0.01 to 0.09 IU/mL; 0.1 to 0.9 IU/mL; and ≥1.0 IU/mL.
	Results for each antigen must be interpreted taking into account: • The context and epidemiological scenario of the area and population studied; • The knowledge, utility, and limitations of each antigen in seroprevalence surveys.
Data visualization	Suggestions include: • Stacked bar graphs of antibody level category proportions by subpopulations; • If geographic strata are included, color-coded maps can be used to present levels of seroprotection or seroprevalence by area (state, municipality, etc.).

Annex 6.2
Basic structure and contents of the report

- **Title.** Find a title that clearly describes the location, purpose, and time period covered by the survey.
- **Summary.** Summarize the methods, key results, and implications for decision-making. The summary should contain enough information about the survey methods and any limitations for the results to be interpreted correctly, even if the document is not read in full.
- **Introduction.** Provide brief information on the country and the setting where the survey was conducted, the strategies and goals of existing programs targeting the events or diseases of interest, the interventions that have been implemented to prevent or eliminate the diseases of interest, the rationale for the survey, and its objectives.
- **Methodology.** Describe the sampling strategy and design, parameters and procedures used to calculate the sample size, weights, and methods used to analyze the data and control for errors and bias. Describe the variables and procedures for data and blood sample collection, the serological techniques and characteristics of the antigens employed, and the cutoff points used to establish immunity levels and calculate seroprevalence.
- **Results.** Use tables, charts, graphs, and maps as well as text to explain the main findings.
- **Discussion.** Discuss the main results of the survey and their implications for action, as well as the limitations of the survey and how they may affect interpretation of the results. Analyze potential sources of uncertainty, bias, and error, and how they were controlled to minimize their effect. Every study has limitations, which must be recognized before making any recommendations.
- **Recommendations.** Make recommendations that are feasible to implement and can lead to effective action. Depending on the findings of the survey, additional research can be recommended to bridge any knowledge gaps identified by the study. These additional investigations may include analysis of the determinants of disease transmission, factors associated with access gaps and service coverage, etc.
- **Acknowledgments.** The support (technical, financial, and operational) of any organizations that played a relevant role in the conduct of the survey should be acknowledged in this section.
- **Annexes.** Include any documents that may be useful to understand the methodology and procedures used in the survey, such as the data collection forms, informed consent forms, detailed descriptions of the sample and sampling frame, a list of sampled clusters, and a list of the personnel involved. Include tables listing the criteria and data used to calculate the observed design effect and weights.

Serosurveillance is a tool that complements traditional public health methods for surveillance of communicable diseases and provides valuable information on disease transmission in populations; for example, to identify gaps in immunity against vaccine-preventable diseases. This information is useful for monitoring population exposure to diseases such as malaria, neglected infectious diseases, foodborne diseases, waterborne diseases, vector-borne diseases, and emerging infectious diseases. As many infectious diseases are or have been present in populations that live in environments where various risk factors overlap, consequently, integrated serosurveillance facilitates synergies and optimizes the utilization of public health resources.

This toolkit was developed to facilitate the design, implementation, analysis, interpretation, and use of results of integrated serosurveys to reinforce countries' capacities toward the elimination of communicable diseases. The first part describes the basic concepts of serosurveys and serosurveillance, its uses, benefits and challenges, ways to improve its efficiency, and its potential to contribute to decision-making in public health. Subsequently, this toolkit presents a stepwise process for the implementation of survey-based integrated serological surveillance. It includes recommendations on how to identify the need for and purpose of gathering serological information; the survey design and methodology; laboratory methods; practical considerations for survey implementation; data analysis and interpretation; and the use of findings to support decision-making.

It is primarily aimed to support program managers and teams involved in the control and elimination of communicable diseases. The target audience includes, but it is not restricted to, coordinators of communicable diseases, neglected infectious diseases, and immunization programs; epidemiological surveillance managers; public health laboratory staff; and other staffers of cabinet-level and subnational health departments or authorities who may be interested in incorporating integrated serosurveillance into the tools of their surveillance systems, as a means of gaining additional insight into population transmission of infectious diseases.

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