



Integrated Serological Surveillance of Communicable Diseases using the Multiplex Bead Assay in the Region of the Americas

Report of the Fourth Regional Meeting

Washington, D.C., 28–30 March 2023

PAHO



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Americas

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PAHO/CDE/VT/23-0010

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1. Background

In 2016, the Pan American Health Organization (PAHO) and the United States Centers for Disease Control and Prevention (CDC) established a joint strategic initiative to strengthen the capacities of countries in the Region of the Americas in the use of integrated serosurveillance using the multiplex bead assay (MBA).

As of March 2023, two phases of the initiative have been implemented. The first phase focused on the process of transferring MBA technology to three laboratories officially designated by the authorities of each country: Institute of Epidemiological Diagnosis and Reference (InDRE), in Mexico; the Central Public Health Laboratory (LCSP), in Paraguay; and Paraná Institute of Molecular Biology (IBMP) in Brazil. In 2018 and 2019, Mexico and Paraguay conducted serological surveys in selected populations and analyzed the samples in their national laboratory. In addition, Guatemala and Guyana incorporated dried blood spot (DBS) sampling into planned surveys to assess the prevalence of neglected infectious diseases (NIDs), and these samples were analyzed by CDC.

In the first phase, three regional meetings were held with the countries and partners participating in the initiative. At the first meeting, held in Bogotá, Colombia, in July 2016, the initiative was presented to delegates from Colombia, Mexico, and Paraguay. The second meeting, held in Mexico City in July 2017, was to follow up with delegates from Brazil, Mexico, and Paraguay.

The third was to review progress and establish the main activities needed to continue the process of expanding integrated serosurveillance. The meeting was held in March 2020 in Cuernavaca, Mexico, with delegates from Brazil, Mexico, and Paraguay.

The second phase of the initiative began in 2020, with a focus on leveraging installed capacities and lessons learned in the participating countries to expand integrated serosurveillance in the Region of the Americas. Mexico and Paraguay made progress in analyzing the results of the first integrated serological surveys. In 2023, Brazil and Mexico will develop new methodological approaches using serum bank samples from previous surveys such as the National Health and Nutrition Survey (ENSANUT) in Mexico, as well as laboratory-based surveillance in Brazil.

The fourth regional meeting of the initiative was held 28–30 March 2023 in Washington, D.C. It was a hybrid meeting with the virtual participation of country delegates and PAHO offices and face-to-face participation of delegates from PAHO regional programs and CDC. This report summarizes the issues discussed during the meeting with regard to progress made, lessons learned, and key actions needed in the short and medium term to expand integrated surveillance of communicable diseases in the Region of the Americas, in order to assist programmatic decision-making in public health.

2. Purpose of the meeting

The purpose of the meeting was to analyze the progress made and lessons learned in integrated serosurveillance of communicable diseases using the MBA platform in the Region of the Americas, and to consider and agree on the actions needed to use MBA to improve programmatic decisions in public health. See Annex 1 for the meeting agenda.

3. Participants

- Delegates from the ministries of health of eight countries, including:
 - Integrated serosurveillance teams and designated laboratories in the countries participating in the initiative (Brazil, Mexico, and Paraguay) and in the countries that have incorporated serosurveillance in NID surveys (Guatemala and Guyana).
 - Guests from the ministries of health of Ecuador and Peru, where research projects are being carried out, and from Argentina, a country interested in learning about regional experiences in integrated serosurveillance.
- CDC delegates from the Division of Parasitic Diseases and Malaria and the Global Immunization Division.
- PAHO delegates: experts in vaccines and communicable diseases from the invited countries; the Neglected, Tropical and Vector-borne Diseases Unit; the Special Program for Comprehensive Immunization; and the HIV, Hepatitis, Tuberculosis and Sexually Transmitted Infections Unit.
- Researchers on integrated serosurveillance projects under development in the Region of the Americas: University of California at San Francisco (UCSF), United States of America; Universidad San Francisco de Quito (USFQ), Quito, Ecuador; and Universidad Peruana Cayetano Heredia, Lima, Peru.

See Annex 2 for the list of meeting participants.

4. Progress of integrated serosurveillance in the Region of the Americas

At the regional level, through joint work between PAHO and CDC, progress has been made since 2020 in the training of interprogrammatic teams and in the development of tools to improve skills in the analysis, interpretation, and use of integrated serosurveillance data. The following key actions have been taken:

- In April 2021, with the assistance of CDC, PAHO held a virtual regional workshop with delegates from the interprogrammatic teams of Brazil, Guatemala, Guyana, Mexico, and Paraguay. Basic concepts were reviewed, practical exercises were conducted for the analysis of serosurveillance data on groups of diseases, and data triangulation methods were applied for the interpretation of results, identifying their scope, limitations, and applications in public health.
- In September 2022, the English version of the Toolkit for Integrated Serosurveillance of Communicable Diseases in the Americas was published (1). In March 2023, the Spanish version was published.
- Technical assistance was provided to each national team in charge of analyzing data from the completed surveys in Guatemala, Guyana, Mexico, and Paraguay. Together with the program managers, reports on the integrated serological surveys were presented, analyzed, and prepared for different audiences: the teams of related programs, health authorities, other sectors (e.g., education, environment, agriculture), and communities.

- The process of developing integrated regional-level serosurveillance was systematized and lessons learned in the Region of the Americas were published (2).

Key aspects of the methodological approaches used in the countries to implement integrated serosurveillance are summarized in Annex 3. The following is a description of the progress achieved as of March 2023 in the following groups of countries: 1) countries participating in the initiative, 2) countries that have included serological tests in surveys to determine the prevalence of NIDs, and 3) countries where academic institutions and researchers are carrying out serosurveillance projects applied to public health.

4.1. Countries participating in the initiative

- In 2018, **Mexico** became the first country in the initiative to implement an integrated serological survey to study seven diseases (10 antigens) in a non-probabilistic sample of children between ages 3 and 15, and adults (18 to 30 years old) from six municipalities in the states of Chiapas, Morelos, and Sinaloa. The study was coordinated by the Technical Advisory Group comprising the General Directorate of Epidemiology, the National Center for Preventive Programs and Disease Control, and the National Center for Child and Adolescent Health. This experience made it possible to transfer laboratory capacities in MBA sample analysis to InDRE; improve capacities and knowledge about the potential uses of integrated serosurveillance for communicable diseases, and capacities for the analysis and interpretation of seroprevalence data; foster interprogrammatic and inter-institutional work; and learn lessons for the scale-up of serosurveillance.

The national working group completed the first draft of a manuscript with lessons learned from the first integrated serosurvey, which is pending publication. As of 2020, the National Institute of Public Health, a leader in national surveys in Mexico, became a strategic partner to promote serosurveillance of communicable diseases through ENSANUT. A protocol was developed to analyze the samples obtained from these studies, which are carried out periodically using a probabilistic, stratified, three-stage cluster design in households throughout the country. The objective of this second phase of expansion of integrated serosurveillance in Mexico is to use the 2018 ENSANUT samples to analyze 21 antigens in order to estimate seroprevalence of exposure to causative agents related to 13 diseases: strongyloidiasis, taeniasis, cysticercosis, toxoplasmosis, malaria, trachoma, onchocerciasis, yaws, and coronavirus infection; and vaccination against measles, rubella, diphtheria, and tetanus in preschoolers, school-age children, and adults.

- **Paraguay** conducted an integrated serological survey to analyze 11 diseases (14 antigens) in a sample of 1200 schoolchildren aged 6 to 15 years in the Paraguayan Chaco region in 2019. This area was selected because it reports high levels of unsatisfied basic needs and a high proportion of Indigenous groups living in dispersed rural communities, with weak epidemiological surveillance systems and difficulties in accessing basic health and sanitation services. MBA technology was transferred to LCSP, which analyzed the samples obtained; however, since the results of the measles virus antigen differed from those obtained by CDC, and due to a shortage of reagents to repeat the standard curves to establish cut-off values, it was decided to complete the analysis of the samples at CDC.

Preliminary analysis of the results¹ shows low seroprevalence of *Chlamydia trachomatis* antigens: Pgp3 = 4.6% (95% CI [3.3, 6.3]) and Ct694 = 8.5% (95% CI [7.1, 10.0]), with even lower values in the population aged 6–9 years, indicating that trachoma does not seem to be a public health problem in the Paraguayan Chaco. Seropositivity of *Strongyloides stercoralis* (NIE) was 9.5% (95% CI [5.2, 16.6]), with higher values in rural areas and in older children. *Cryptosporidium parvum* antigens showed high seroprevalence: Cp17 = 75.82% (95% CI [70.26, 80.63]) and Cp23 = 55.51% (95% CI [47.43, 63.31]), which indicate poor access to drinking water and basic sanitation. With respect to vaccine-preventable diseases (VPDs), seroprotection (0.01 IU/mL) for tetanus and diphtheria was above 90% for both antigens, and seroprotection values showed a decreasing trend with increasing age. A draft manuscript with the results is expected to be ready for publication by the end of 2023. In order to continue expanding serosurveillance, between 2023 and 2024 Paraguay will develop a proposed protocol for a new integrated serological survey with the participation of technical teams from the relevant programs, within the framework of the country's public health priorities.

- In 2022, **Brazil** completed a protocol to determine the seroprevalence of 12 communicable diseases of public health interest using 22 antigens available in MBA. The study area comprises the states of Amazonas and Pará. These states were selected because they have populations that have been historically endemic for malaria and for NIDs such as trachoma, lymphatic filariasis, schistosomiasis, taeniasis/cysticercosis, and yaws, and are interested in measuring immunity to tetanus, diphtheria, measles, and rubella, since reported vaccination coverage has been declining in these populations. The study will analyze 1464 serum samples from people aged 0 to 19 years obtained through systematic epidemiological surveillance of communicable diseases at the Evandro Chagas Institute from 2016 to 2021. The samples will be analyzed at IBMP, which was designated by the Ministry of Health to receive the MBA transfer.

¹ The serological results reported by Paraguay at the regional meeting are preliminary and are being reviewed for publication.

In April 2023, two IBMP professionals were trained at CDC to process the samples during the second half of 2023.

4.2. Countries that have integrated serosurveillance into surveys on neglected infectious diseases

Guatemala and Guyana integrated serological testing into surveys carried out in 2019 to assess prevalence and determine the impact of interventions on NID transmission. They presented the following progress:

- **Guatemala:** In the survey to estimate the prevalence of soil-transmitted helminthiasis in schoolchildren aged 6–14 years, samples were taken for the analysis of 18 antigens to determine the seroprevalence of 12 diseases, including waterborne and foodborne diseases, malaria, onchocerciasis, trachoma, lymphatic filariasis, and VPDs. The results² showed the following values for seroprevalence: trachoma (Ct694 and Pgp3), 3.5% (95% CI [2.4, 4.6]); malaria (PfMSP1-19 and PvMSP19), 2.1% (95% CI [0.7, 3.4]); strongyloidiasis (NIE), 2% (95% CI [0.7, 3.0]); and onchocerciasis (OV16, OV33), 0%. Seroprevalence of giardiasis (VSP3 and VSP5) was 19.0% (95% CI [15.4, 22.6]); seroprevalence of taeniasis and cysticercosis (T24H and rES33) was 10.2% (95% CI [6.1, 14.4]). Analysis of the results for VPDs showed seroprotection of 75.5% (95% CI [72.2, 78.9]) for measles and 86.6% (95% CI [83.2, 90.0]) for rubella. The proportion of children above the minimum protection value (>0.01 IU/mL) for tetanus was 98.5% (95% CI [97.4, 99.6]) compared with 92.2% seroprotection against diphtheria (95% CI [90.4, 94.4]). A report with a descriptive analysis of results was analyzed with the teams from the respective Ministry of Health programs and an official report is being prepared for dissemination by the end of 2023.
- **Guyana:**³ Serological analysis of 18 antigens was included in two surveys for the assessment of 11 diseases. Two survey methodologies were used to estimate lymphatic filariasis transmission: 1) survey of schoolchildren in endemic areas in the interior of the country; and 2) survey of populations aged 6 years and older in four sentinel communities in urban areas. The regions of Guyana that are historically endemic for malaria (I, VII, and VIII) showed the highest seroprevalence of *Plasmodium vivax* and *Plasmodium falciparum*, a finding that coincides with the data reported by epidemiological surveillance. No antibody response to *C. trachomatis* or *Treponema pallidum* was found in children under 10, an important finding for documentation of the elimination of trachoma as a public health problem and for continued progress in verifying the interruption of yaws transmission. Seroprevalence of *S. stercoralis* (NIE) was higher in some regions, particularly in region VII, which reported

² Preliminary serological results reported by Guatemala at the regional meeting are being reviewed for publication.

³ Preliminary serological results reported by Guyana at the regional meeting are being reviewed for publication.

16.5% (95% CI [13.9, 19.3]), and region I, with 10.8% (95% CI [9.4, 12.3]), followed by regions VIII (5.1%; 95% CI: [3.7, 6.5]) and IX (3.6%; 95% CI [2.6, 4.5]). Seroprotection (0.01 IU/mL) against tetanus and diphtheria was above 98% in all regions. The results have been discussed with the team in charge of the surveys and managers of the vaccination and vector control program at the Ministry of Health. A draft manuscript with the results is expected to be ready for publication by the end of 2023.

4.3. Integrated serosurveillance research projects

Research teams working on integrated serological surveillance of priority public health diseases in countries of the Americas such as Ecuador and Peru shared their experiences at the meeting, providing opportunities for collaboration with other countries and with PAHO and CDC, to expand integrated serosurveillance in the Region of the Americas.

For example, consideration was given to the possibility that MBA could include antigens of arboviral diseases (dengue, chikungunya, and Zika), influenza, and enteric infections, among others for which the participating research groups have made progress and reported data. These research groups presented serosurveillance projects that aim to expand knowledge on the evolution, epidemiology, and use of services for the diagnosis, care, and surveillance of infectious diseases through innovative methodological approaches such as the following:

- A study to analyze malaria seroepidemiology in very low transmission settings, and other pathogens in rural areas and remote communities in the Peruvian Amazon. The study is being carried out by the London School of Hygiene and Tropical Medicine, Universidad Peruana Cayetano Heredia, the Alexander von Humboldt Institute of Tropical Medicine, Health Innovation (InnovaLab), and the Bill & Melinda Gates Foundation. The methodology for sampling and selection of individuals for the survey includes the use of geospatial and stratification methods to identify key elements of health services management (e.g., availability of diagnostic tests), and provides estimates and information useful for disease surveillance and treatment.
- The Enteropathogens, Growth, Microbiome and Diarrhea (ECoMID) project is led by researchers from UCSF, the University of California, Berkeley, and USFQ, among others. This study has a community-based birth cohort design to prospectively analyze factors affecting the gut microbiome during the first years of life. The study follows children up to 2 years of age born in three locations on the North Coast of Ecuador. The rural–urban gradient design allows measurement of seroconversion rates and prevalence through PCR testing to assess how social, environmental, and dietary factors affect the relationship between transmission of various enteric pathogens and the intestinal microbiome (3). An active fever surveillance project is also being conducted in the Ecuadorean province of Esmeraldas, using a community cohort to measure the co-circulation of Zika and dengue disease in a study area with urban–rural representation. The study uses antigens to estimate the seroprevalence of both settings.

5. Lessons learned

The initiative has been a process of learning about the applications and uses of serosurveillance for public health programs. Meeting participants identified the following as some of the lessons learned as of March 2023:

1. Serovigilance offers opportunities to advance the disease elimination agenda set out in the *Sustainable and Integrated Framework for the Elimination of Communicable Diseases in the Region of the Americas (4)* adopted by PAHO Member States in 2019, given that it:
 - Provides key information on exposure and immunity to diseases with elimination targets;
 - Transcends individual approaches, enabling integrated approaches to surveillance in functional epidemiological surveillance systems;
 - Takes advantage of the available health infrastructure and existing labor force in each country;
 - Promotes critical analysis of information by applying triangulation and combined data sources that consider different epidemiological contexts;
 - Facilitates synergies and interprogrammatic collaboration.

2. Serovigilance complements conventional epidemiological surveillance by providing information on exposure to pathogens that are not identified through routine surveillance and that may be circulating or could reemerge in populations. Its implementation in the countries of the Region of the Americas has made it possible to characterize the immunity profiles of communicable diseases for which no reference information was available or where the information available was not up to date; e.g., strongyloidiasis, taeniasis and cysticercosis, yaws, and trachoma, among other diseases.
3. For the VPD elimination strategy, the results of serological tests in some participating countries make it possible to characterize the level of population immunity against tetanus and diphtheria throughout the life course. Since the vaccination schedule against these two diseases requires boosters at different times in life (childhood, school age, adolescence, pregnancy, and adulthood), biomarkers have been useful in monitoring immunity profiles. To interpret observed differences in seroprotection values, it is essential to consider the characteristics of the populations studied and the context of each country when comparing results.
4. It is necessary to get beyond the idea that the use of integrated serosurveillance is limited to the transfer of laboratory technology. In addition to strengthening laboratory capacities, it is crucial to foster interprogrammatic work to properly plan and implement integrated serosurveillance as part of functional epidemiological surveillance systems, a process that requires time and coordination at different decision-making levels. It is equally important to strengthen knowledge and skills in the use of robust methods for sample design and analysis methods, in the interpretation and use of data for better programmatic decision-making, and in determining the scope and limitations of the data.
5. The questions to be answered through integrated serosurveillance should be based on the analysis of programmatic needs in the various contexts (ecological, epidemiological, social, economic, etc.) of the populations where it is to be carried out, since the goal is public health decision-making. These questions need to be well formulated in order to establish the study design (household survey, schools, biobanks, etc.), target population (children, women of childbearing age, adults, specific population groups such as Indigenous people or migrants), and study areas (urban, rural, border areas, etc.).
6. As a country approaches the goal of eliminating a communicable disease – and identifying populations that remain affected because they are underserved in terms of access to basic health, education, safe housing, and water and sanitation services – it is necessary to employ sampling designs that provide targeted estimates for risk groups at the local level in well-defined geographic areas. This should also be considered when analyzing the results of integrated serosurveillance, since information is needed to identify transmission foci, better understand the characteristics of disease transmission, and interpret potential gaps in access to basic services in order to implement better programmatic actions.

7. Analysis of serological data should be disease-specific, but it is also important to perform integrated analyses, for example, to identify overlapping exposure to various diseases or gaps in immunity to VPDs in certain populations that share risk factors associated with environmental or sociodemographic contexts that reflect inequities in access to basic services, including health services. An integrated approach makes it possible to identify synergies that facilitate interprogrammatic work and interventions aimed at improving the living conditions of populations that share these risks and face common obstacles to accessing health services.

8. It is feasible to integrate serosurveillance into surveys on other diseases or events of health interest (e.g., NID surveys, Demographic and Health Surveys, etc.) and to take samples using DBS. This makes it possible to use available resources more efficiently and to make serosurveillance of communicable diseases sustainable. It is also feasible to collect blood samples for various purposes and to obtain different types of samples for various diseases and tests in a single survey. For example, blood samples can be collected to obtain DBS for serosurveillance and for rapid testing to detect other diseases, as Guyana has done with lymphatic filariasis. Stool samples can also be obtained for serosurveillance through Kato-Katz and DBS testing, as Guatemala has done.

9. Integrated serosurveillance studies can use samples and data available in serum banks and can integrate serosurveillance into regular surveys that countries have already scheduled and funded. In countries of the Region of the Americas, national Demographic and Health Surveys, nutrition surveys, and Multiple Indicator Cluster Surveys, among others, are conducted periodically and financed by governments, and blood samples are collected, stored, and used for various studies.

This source of samples can be used for retrospective serosurveillance surveys or to integrate serosurveillance into the study design for prospective serosurveillance. This is an option for making the serosurveillance of communicable diseases sustainable and for reducing the number of times it is necessary to obtain samples from population groups. Banks of serum obtained through epidemiological surveillance of communicable diseases, such as surveillance of febrile diseases, are also a source of samples for integrated serosurveillance. Brazil and Mexico are designing surveys using samples from serum banks, and their experience will contribute to understanding the best use of existing platforms for integrated serosurveillance. It is important to recognize the advantages and limitations of using serum banks, based on the experiences of these two countries.

10. Obtaining participants' authorization (extended informed consent) to store and use samples collected on filter paper (DBS) in future studies is a good practice that also allows for efficient use of resources. However, it is necessary to find the most appropriate mechanisms in each country so that the samples are stored for as long as possible (ideally not less than 10 years), in order to have enough time to design protocols, obtain ethics approvals, and carry out studies.

11. The development of technical guidelines and practical tools for integrated serosurveillance, with training and technical assistance, has been useful in improving the formulation of questions, defining approaches that consider the various epidemiological contexts, and assisting in the analysis and interpretation of data in integrated serosurveys. This is an incipient process, making cooperation, assistance, and joint follow-up between countries and partners essential to catalyze efforts, change paradigms, and strengthen capacities in each country and at the regional level.

12. Groups of researchers are implementing integrated serosurveillance projects in some countries of the Region of the Americas. It is essential to identify common interests and for these groups of researchers to work together with ministries of health, universities, and national health institutes and laboratories in order to share not only expert knowledge from various disciplines and the experiences of public health teams in the countries, but also infrastructure, laboratory equipment, and resources for the benefit of the population.

6. Recommendations for expanding integrated serosurveillance

In light of the progress made and the challenges faced in the countries participating in integrated serosurveillance, and based on the lessons learned, the following are the main recommendations for the ministries of health and interprogrammatic groups leading the implementation of integrated serosurveillance:

1

Strengthen the political commitment of high-level government officials (ministries of health, national reference laboratories, and national public health institutes) to the adoption and sustainability of integrated serosurveillance as a complementary tool in functional surveillance systems. The technical involvement of program management teams, epidemiologists, statisticians, laboratory professionals, disease experts, academia, and research groups is also essential.

2

Strengthen interprogrammatic and inter-institutional work to design serosurveillance strategies and protocols that provide useful information to close gaps and address public health problems, and to effectively monitor field work, laboratory analyses, and the production of quality results to be interpreted and used in decision-making. The expansion of integrated serosurveillance will be effective if it is linked to programmatic decision-making in public health.

3

Identify and take opportunities to include serosurveillance in periodic surveys that already have funding and resources (NIDs, nutrition, demographics, and health, etc.). This requires partnerships between ministries of health and academic and research institutions in the countries to expand serosurveillance capabilities and innovate. The use of resources is optimized when serosurveillance is integrated into existing public health surveillance and evaluation platforms to obtain DBS samples through already planned surveys and to utilize serum banks available from periodic surveys or laboratory-based surveillance.

5

Establish partnerships with universities, academic and scientific groups, and WHO Collaborating Centers, among others, to expand the capacity to design and establish sampling methods for the implementation of serological surveys; perform data analysis, triangulation, and interpretation; and understand how to use the data to support decision-making. This includes creating spaces for program teams to undertake joint work and analysis with inter-institutional academic and scientific groups, promote critical thinking, and raise questions based on defined epidemiological contexts so that target populations, geographic areas, and methodological designs respond to a country's priorities. It is also important to facilitate the understanding and interpretation of specific aspects of serological data analysis, according to the type of laboratory test used and the analysis of triangulated data. Triangulation is essential to interpret the results of serological tests using combined sources of information on the epidemiology of a disease, interventions in populations, risk factors, and social and environmental determinants, among others, in order to seek explanations for the findings and identify actions to be taken.

4

Promote the collection of samples for various purposes (rapid tests for hepatitis B, lymphatic filariasis, syphilis, and yaws, among others) during integrated serological surveys and use extended informed consent to save and use samples in future long-term studies. In particular, it is recommended to identify opportunities to collect samples for rapid hepatitis B testing during integrated serological surveys, in order to document the elimination of mother-to-child transmission of hepatitis B virus in the Region of the Americas.

6

Innovate in the production and dissemination of messages and information on the uses and results of serosurveillance. This includes identifying and using the most appropriate means of communication, according to the roles and responsibilities of the various audiences (ministers of health, managers, technical specialists, program teams, scientists, local health teams, and the community, among others) who use serosurveillance results for decision-making.

Recommendations for PAHO and CDC

1. Assist in the coordination and strengthening of mechanisms that promote communication and networking with national public health laboratories that perform serosurveillance, in order to share knowledge, capacities, standard operating procedures, and mechanisms for problem-solving, among others.
2. Help countries identify mechanisms to facilitate access to reagents, instruments, and systems used in MBA, especially when these are hard to acquire in local markets.
3. Help laboratories participating in the initiative to develop standard operating procedures and a training and supervision plan, and to standardize and establish mechanisms for quality control and assurance, problem-solving, and performance evaluation.
4. Expand the availability of validated antigens for use in MBA in order to advance the analysis of priority diseases in the countries of the Region of the Americas. It is recommended to evaluate feasible options for incorporating new antigens for: surveillance of NIDs (e.g., Chagas disease, leishmaniasis, leprosy); prevention and control of arbovirosis (dengue, yellow fever, Zika, chikungunya); and vaccination programs (poliomyelitis, pertussis, hepatitis B, rotavirus infections), among others.
5. Develop and implement, with the participating countries, a methodology for countries to self-assess their installed capacity for integrated serosurveillance (analysis of the current situation, gaps, and needs). This is recommended because the COVID-19 pandemic limited progress in technology transfer and regular use of MBA, in the deployment of serological surveys, and in the analysis and interpretation of results in the countries participating in the initiative. Therefore, it is important to gather information to establish a work plan in each country to strengthen and accelerate the implementation and sustainability of integrated serosurveillance. This analysis should not be limited to the laboratory; rather, it should encompass integrated serosurveillance that includes political and technical interest in its use as a public health tool for analysis and interpretation, among other capabilities. This methodology may also be useful for countries interested in implementing serosurveillance for the first time.

7. Looking to the future

7.1. Short-term actions

- 1. Develop and apply a methodology to analyze gaps in the capacity to implement integrated serosurveillance based on the six modules of the PAHO toolkit for integrated serosurveillance:**
 - PAHO and CDC will develop the methodology and assist the countries in their self-assessment process and in the formulation of plans to strengthen integrated serosurveillance.
 - The methodology will be applied by each of the countries participating in the initiative (Brazil, Mexico, and Paraguay), as well as the countries that have implemented integrated serological surveys (Guatemala and Guyana). Countries interested in receiving technical cooperation from PAHO and CDC to implement integrated serosurveillance with MBA can use the methodology to analyze their capacities and needs for integrated serosurveillance in their functional surveillance systems.

2. Advance in the management, monitoring, and implementation of current serosurveillance projects:

- Mexico will obtain approvals from the Research Ethics Committee and the Biosafety Committee of the National Institute of Public Health to implement the survey protocol and analyze serum samples from ENSANUT 2018–2019.
- Brazil will move forward with the transfer of MBA technology in order to begin processing samples and to continue with the analysis of serological results for the diseases included in the approved protocol.
- Guyana, Mexico, and Paraguay will advance in the process of analyzing draft manuscripts on integrated serosurveillance in order to produce final versions for publication.
- In coordination with the national interprogrammatic group, Paraguay will define the questions, epidemiological contexts, methodology, and implementation plan for the protocol of the second integrated serosurveillance survey.

3. In order to strengthen the capacities of national teams in data analysis and the dissemination and use of results, PAHO, in collaboration with CDC Atlanta, will take the following action:

- Develop a modular virtual course based on the contents of the PAHO integrated serosurveillance toolkit, and produce short audiovisual materials that communicate the fundamentals and applications of integrated serosurveillance.
- Develop data analysis workshops in countries that already have serological test results in order to assist in the triangulation of results. This will involve delegates from public health programs who can make the necessary decisions and take the necessary actions.
- Implement effective ways of communicating and disseminating the results of integrated serosurveillance, identifying the most appropriate media and types of reports adapted to different audiences (e.g., health authorities, subnational levels, communities).

- 4.** Country participants will identify opportunities to work with expert groups to expand integrated serosurveillance in countries interested in incorporating it into their surveillance systems. PAHO and CDC will seek opportunities to expand the range of validated antigens for serosurveillance of priority diseases in the Region of the Americas (e.g., arboviruses, Chagas disease, among others).

7.2. Medium-term actions

Based on the analysis of capacity gaps, each country will work on an action plan aimed at incorporating serosurveillance as a functional tool in its surveillance system. Based on the countries' proposals, medium-term work will be defined, including these three basic elements:

1. New or renewed commitment by health authorities to incorporate integrated serosurveillance as a tool in the country's surveillance system.
2. Prepare action plans, indicating the serosurveillance strategies to be implemented, the persons responsible for the different components, and the available resources in each country.
3. Prepare a three-year implementation schedule for activities starting in 2024.

7.3. Long-term outlook

During the meeting, working groups were formed to discuss key areas of work and priority actions to expand serosurveillance. In summary, the participants considered integrated serosurveillance to be a complementary tool for public health surveillance – one that should be applied to the analysis, monitoring, and evaluation of achievement of the objectives for the elimination of communicable diseases. For its use to be effective and sustained, it is essential to have the political commitment of health authorities and to ensure that national public health program managers and teams are involved and work together from the planning phase, with active participation during implementation and in the analysis and use of data for decision-making.

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Annex 2. Meeting agenda

Day 1 (10:00 a.m. - 1:30 p.m.)

Time	Topic	Speaker or facilitator
Introductory session		
10:00-10:20 a.m.	<ul style="list-style-type: none"> – Logistics – Welcoming remarks (PAHO and CDC) – Purpose and objectives 	PAHO
10:20-10:50 a.m.	<ul style="list-style-type: none"> – Regional perspective on the progress of the Multiplex Initiative 	PAHO and CDC
10:50-11:10 a.m.	<ul style="list-style-type: none"> – Questions and discussion 	Plenary
11:10-11:20 a.m.	Break	
Progress, lessons learned, and country plans		
11:20 a.m. to 1:20 p.m.	Countries participating in the Multiplex Initiative <ul style="list-style-type: none"> – Brazil, Mexico, and Paraguay – Questions and discussion 	Country delegates
1:20-1:30 p.m.	Closing remarks (day 1)	PAHO

Day 2 (10:00 a.m. - 1:30 p.m.)

Time	Topic	Speaker or facilitator
Progress, lessons learned, and country plans (continued)		
10:00-10:05 a.m.	Logistics	
10:05-11:25 a.m.	Countries integrating serosurveillance into NID surveys <ul style="list-style-type: none"> – Guatemala and Guyana – Questions and discussion 	Country delegates
11:25-11:35 a.m.	Break	
11:35 a.m. to 12:55 p.m.	<ul style="list-style-type: none"> – Seroepidemiology of malaria and other pathogens in rural and remote communities in the Peruvian Amazon. – Project in Ecuador – Questions and discussion 	Gabriel Carrasco Benjamin Arnold
12:25-1:20 p.m.	Three key actions in each country for the use of integrated serosurveillance <ul style="list-style-type: none"> – Instructions – Working groups by country – Comments on key actions in each country 	All participants
Key actions within countries		
12:55-1:25 p.m.	<ul style="list-style-type: none"> – What is needed in countries to use integrated serosurveillance for programmatic decision-making in public health? Brainstorming 	Plenary
1:25-1:30 p.m.	Closing remarks (day 2)	PAHO

Day 3 (10:00 a.m. - 1:30 p.m.)

Time	Topic	Speaker or facilitator
10:00-10:10 a.m.	Logistics	PAHO
10:10-10:30 a.m.	<ul style="list-style-type: none"> – Key points of the past two days – Discussion 	PAHO
Looking to the future		
10:30-11:15 a.m.	Key areas of work (working groups: 45 minutes each, simultaneously) <ul style="list-style-type: none"> – What is needed to create a regional network of MBA laboratories? – What is needed to accelerate data analysis, dissemination, and use of integrated serosurveillance data for decision-making? – What is needed for countries to use integrated serosurveillance as a tool for functional epidemiological surveillance systems? 	All participants
11:15-11:25 a.m.	Break	
11:25 a.m. to 12:25 p.m.	<ul style="list-style-type: none"> – Summary of each working group – Questions and discussion 	
12:25-13:20 p.m.	Three key actions in each country for the use of integrated serosurveillance <ul style="list-style-type: none"> – Instructions – Working groups by country – Comments on key actions in each country 	All participants
13:20-13:30 p.m.	Closing remarks	PAHO and CDC

Annex 3. Approaches and progress of integrated serosurveillance in countries of the Region of the Americas

Countries	Sample design	Study population	Geographic areas	Epidemiological context	Diseases and antigens	Progress to March 2023
Mexico	Cross-sectional study with convenience sampling in schools using two-stage cluster sampling	Children from 3 to 15 years old (<i>n</i> = 1012) Adults 18 to 30 years old (<i>n</i> = 220)	Six municipalities in three states (Chiapas, Morelos, and Sinaloa)	Areas with low vaccination coverage, a history of malaria and trachoma, or no baseline information on neglected infectious diseases.	Malaria, trachoma, taeniasis, cysticercosis, measles, rubella, and diphtheria. (10 antigens)	Survey completed in 2018. Descriptive report and draft article ¹
	ENSANUT serum bank	Preschoolers, school children, and adults	National, representative by age groups, states, and regions (north, central, Mexico City, and south)	National survey that includes the 32 states of Mexico: each of the diseases will be analyzed according to the epidemiological context.	Malaria, trachoma, onchocerciasis, yaws, strongyloidiasis, taeniasis, cysticercosis, toxoplasmosis, coronavirus infections, measles, rubella, diphtheria, and tetanus. (21 antigens)	Protocol developed. Survey to be implemented in 2023–2024
Paraguay	Cross-sectional study with two-stage cluster sampling: schools and children	Children from 6 to 15 years old (<i>n</i> = 1200)	Western region of the country: Paraguayan Chaco	Areas with a high number of unsatisfied basic needs, Indigenous ethnic groups living in dispersed rural communities, with weak epidemiological surveillance systems and difficulties in accessing health and basic sanitation services.	Trachoma, taeniasis, cysticercosis, strongyloidiasis, giardiasis, cryptosporidiosis, toxoplasmosis, measles, rubella, diphtheria, and tetanus. (14 antigens)	Survey completed in 2019. Descriptive report and draft article

Countries	Sample design	Study population	Geographic areas	Epidemiological context	Diseases and antigens	Progress to March 2023
Brazil	Randomized selection of samples from a serum bank for surveillance of communicable diseases. Discarded samples with diagnoses related to the diseases to be monitored	Individuals aged 0 to 19 years whose serum samples were obtained from 2016 to 2021. (n = 1464)	States of Amazonas and Pará	Areas that have been historically endemic for malaria and neglected infectious diseases such as trachoma, lymphatic filariasis, schistosomiasis, taeniasis and cysticercosis, and yaws, and where exposure values for tetanus, diphtheria, measles, and rubella are of interest considering that reported vaccination coverage has been declining.	Malaria, trachoma, lymphatic filariasis, schistosomiasis, taeniasis, cysticercosis, yaws, tetanus, diphtheria, measles, and rubella. (22 antigens)	Protocol developed. Survey to be implemented in 2023
Guatemala	Cross-sectional study with two-stage cluster sampling of schoolchildren enrolled in official primary schools in the country.	Children from 6 to 14 years old (n = 1500)	Nationally representative sample	Nationally representative survey to estimate the prevalence of soil-transmitted helminthiasis in school-aged children.	Malaria, onchocerciasis, lymphatic filariasis, strongyloidiasis, trachoma, giardiasis, taeniasis, cysticercosis, measles, rubella, diphtheria, and tetanus. (18 antigens)	Survey completed in 2019. Descriptive report to be published by the Ministry of Health
Guyana	Cross-sectional study with selection of 100% of the schools in 33 evaluation units and systematic sampling or census of children in each school.	Children from 6 to 14 years old (n = 7200)	Six regions (I, II, VI, VI, VII, VIII, IX)	Areas in the interior of the country that have historically shown focal transmission of lymphatic filariasis and have been endemic for malaria.	Malaria, lymphatic filariasis, trachoma, yaws, strongyloidiasis, taeniasis, cysticercosis, measles, rubella, diphtheria, and tetanus. (18 antigens)	Survey completed in 2018–2019. Descriptive report and draft article
	Communities selected for their risk of lymphatic filariasis transmission (sentinel sites in disease foci)	Children, adolescents, and adults 6 years of age and older (n = 300 per sentinel site)	Four regions (III, IV, V, and X)	Urban communities that historically showed high levels of lymphatic filariasis infection and where mass drug administration was implemented.	Malaria, lymphatic filariasis, trachoma, yaws, strongyloidiasis, taeniasis, cysticercosis, measles, rubella, diphtheria, and tetanus. (18 antigens)	

¹ The antigens used to test the diseases selected by the countries participating in the integrated serosurveillance initiative in the Region of the Americas: *Wuchereria bancrofti* (Wb123, Bm14, and Bm33), malaria (PvMSP1-19, PfMSP1-19, PfCSP, PflSA1, PfAMA1, PoMSP1-19, and PmMSP1-19), *Strongyloides stercoralis* (NIE), *Chlamydia trachomatis* (Pgp3 and Ct694), *Cryptosporidium parvum* (Cp17 and Cp23), *Giardia lamblia* (VSP3 and VSP5), *Toxoplasma gondii* (SAG2A), *Taenia solium* (rES33 and T24h), *Treponema pallidum* (rp17 and TmpA), *Onchocerca volvulus* (OV16 and OV33), *Schistosoma mansoni* (Sm25 and SEA), whole measles virus and rubella virus, diphtheria toxoid (*Corynebacterium diphtheriae*), tetanus toxoid (*Clostridium tetani*), and SARS-CoV-2 (S, RBD-541, RBD-591, and N).

This document presents a summary of the topics discussed at the Fourth Regional Meeting on Integrated Serological Surveillance of Communicable Diseases using the Multiplex Bead Assay (MBA) in the Region of the Americas. This initiative, which began in 2016, is a partnership between countries in the Region, the United States Centers for Disease Control and Prevention (CDC), and the Pan American Health Organization for the use of integrated serosurveillance as a tool to complement epidemiological surveillance systems.

Serovigilance complements conventional epidemiological surveillance by providing information on exposure to pathogens that are not identified through routine surveillance and that may be circulating or reemerging in populations. Its implementation in the countries of the Region of the Americas has made it possible to characterize the immunity profiles of communicable diseases for which no reference information was available or where the available information was not updated (e.g., for diseases such as strongyloidiasis, taeniasis and cysticercosis, and yaws). MBA allows up to 50, 100, or 500 antigens to be detected simultaneously, depending on the instrument used, with a very small sample volume (<1 µL). This system allows the creation of assays tailored to the public health needs of each program, with a very low incremental cost for adding antigens.

The fourth meeting, held in March 2023, discussed progress and lessons learned in the use of integrated serosurveillance of communicable diseases using the MBA platform in the Region of the Americas. The meeting also reviewed and agreed on actions to employ MBA to improve programmatic public health decisions.

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