

# Lessons learnt from laboratory capacity building in supporting integrated disease serosurveillance using a multiplex bead assay in Nigeria



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**Background:** Serologic assays can monitor population exposure to pathogens and immunity to vaccine-preventable diseases; the multiplex bead assay (MBA) can test for multiple analytes simultaneously.

**Intervention:** Specimens collected during the Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS) were used to estimate the seroprevalence of diseases of public health importance, and specimens collected from special studies were used to estimate the seroprevalence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to improve the surveillance programmes. In 2019, Luminex MAGPIX<sup>®</sup> instruments were installed at the MBA laboratory in the National Reference Laboratory, Nigeria Centre for Disease Control and Prevention, in Abuja, Nigeria. Dried blood spot specimens from the NAIIS, stored with consent for future testing, were prioritised for testing. Between 2019 and 2022, the National Reference Laboratory MBA laboratory conducted antibody and antigen assays for an estimated 130 000 NAIIS participants, completed two rounds of coronavirus disease 2019 (COVID-19) serosurveys, and validated SARS-CoV-2 serological assays for use in Nigeria.

**Lessons learnt:** The data generated by the MBA laboratory supported funding requests for childhood immunisation and the application of the National Malaria Elimination Programme Global Fund. The MBA results strengthened the National HIV Serial Rapid Testing Algorithm. During the COVID-19 pandemic, the laboratory validated a SARS-CoV-2 MBA, which measured antibodies against three antigens. This multi-target assay was used to complete two additional rounds of the COVID-19 household serosurvey in Nigeria.

**Recommendations:** This strategy of investing in MBA laboratory capacity building could serve as a model for other nations seeking to fortify their public health infrastructure.

**What this study adds:** Nigeria's integrated disease surveillance capability was boosted through MBA technology.

**Keywords:** multi-disease; serosurveillance; multiplex bead assay; capacity building; Nigeria.

## Background

Serosurveillance can be used to monitor population exposure to HIV, malaria, enteric pathogens, many neglected tropical diseases, and emerging pathogens and disease risks. It can also identify immunisation coverage gaps or waning immunity for vaccine-preventable diseases and risk factors associated with exposure.<sup>1,2,3,4</sup> Serosurveillance measures antibody responses to or antigens from pathogens, providing information on population immunity, prior disease exposure, or recency of infection.<sup>4,5,6</sup>

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Specimens collected and archived during surveys and other studies, stored appropriately in a biorepository, with consent for future testing, are potential sources of current and past pathogen exposure and immunity levels.<sup>1,6</sup> Public health programmes can leverage these data to identify changes in the epidemiology of diseases over time, monitor antibody levels in the population, and assess recent infections.<sup>7,8,9</sup>

Serosurveillance data may be used to measure disease endemicity, making serosurveillance extremely useful for monitoring transmission interruption or recrudescence for diseases such as malaria, vaccine-preventable diseases and neglected tropical diseases in elimination settings.<sup>1,10</sup> The United States Centers for Disease Control and Prevention (US CDC) support and strengthen global health security preparedness.<sup>11</sup> The multiplex bead assay (MBA) tests simultaneously for multiple analytes using a low volume of serum or dried blood spot (DBS) specimen, and it offers cost and time advantages compared to single-analyte immunoassays.<sup>12</sup> Multiplex bead assay panels developed by the US CDC have been transferred successfully to laboratories in multiple countries, resulting in a substantial public health impact. In addition, results have been used to help inform global vaccination policy, identify immunity gaps for targeted interventions, and support validation of disease elimination.<sup>8,9</sup> The benefits of the use of the MBA as an integrated serosurveillance platform across public health programmes include assessment of intervention effectiveness, guidance for evidence-based policy and strategy, and verification of disease control and elimination goals, providing cross-cutting opportunities for partnership and cost savings across organisational structures, increased feasibility of use in developing countries, and addressing the needs of traditionally neglected disease programmes.<sup>13,14,15</sup>

The Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS) was a nationwide population-based household survey conducted in July–December 2018 with a sample size of over 200 000 participants between 0 and 64 years of age. The primary objectives were to estimate national and state-level HIV prevalence and viral load suppression in each of the 37 states in Nigeria, as well as national HIV incidence.<sup>16,17</sup> The Nigeria multi-disease serologic surveillance using stored specimens, leveraged remnant NAIIS specimens stored with consent for future testing to expand surveillance for other diseases of priority interest in Nigeria.<sup>16,17</sup> We describe the process of developing MBA capacity in Nigeria to test stored NAIIS samples and leveraging that capacity to estimate a severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) seroprevalence during the pandemic.

## Description of the intervention

### Ethical considerations

An application for full ethical approval was made to the National Health Research Ethics Committee and ethics consent was received on 27 August 2019. The ethics approval number is NHREC/01/01/2007-27/08/2019. The Nigeria HIV/AIDS Indicator and Impact Survey and Nigeria

multi-disease serologic surveillance using stored specimens were conducted under two different protocols, reviewed and approved by the National Health Research Ethics Committee, Nigeria, the University of Maryland, Baltimore Institutional Review Board, and the US CDC. The US CDC employees did not have access to personal identifying information or contact with the clients. The SARS-CoV-2 projects also received National Health Research Ethics Committee and US CDC approvals. These activities were reviewed by the US CDC, deemed not research, and were conducted consistent with applicable federal law and CDC policy§ (see, for example, 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 USC; §241(d); 5 USC. §552a; 44 USC. §3501 et seq.)

### Intervention

The main objective of the Nigeria multi-disease serologic surveillance using stored specimens project was to support the Government of Nigeria in conducting serosurveillance to understand disease epidemiology better and to provide information for public health action, specifically to control malaria, vaccine-preventable diseases, and other diseases of public health importance.<sup>18</sup> Discussions about using remnant samples to support this objective started during NAIIS planning stages in 2017. Subsequently, the NAIIS protocol included consent for storage with future testing for diseases of public health importance; NAIIS funding included equipping and staffing the national biorepository at the National Reference Laboratory (NRL), Nigeria Centre for Disease Control and Prevention. In January 2019, the MBA laboratory of the Nigeria Centre for Disease Control and Prevention started equipment procurement with support from the US CDC, the Global Fund to Fight AIDS, Tuberculosis and Malaria, the United States President's Malaria Initiative, and the Bill and Melinda Gates Foundation, through a grant from the CDC Foundation.

### Capacity building

The International Laboratory Branch of the Division of Global HIV and TB and the Division of Parasitic Diseases and Malaria of the US CDC provided on-site training and technical assistance to MBA staff, including sample processing (Figure 1), data management, and the use and maintenance of the Luminex MAGPIX® (Luminex Corporation, Austin, Texas, United States) instruments. Three MBA management staff members were trained in project management at the US CDC, and Luminex provided advanced equipment maintenance training. Quarterly on-site mentorship was provided by a Luminex-certified engineer, as well as routine and as-needed maintenance for the MAGPIX® equipment and monthly technical assistance by subject matter experts from the US CDC Atlanta to build the capacity of MBA staff. The MBA staff developed daily, weekly, monthly, and semi-annual equipment maintenance schedules. During the coronavirus disease 2019 (COVID-19) pandemic travel restrictions, the team received virtual technical assistance from the US CDC. Additional training included virtual training on scientific writing skills and a weekly journal club, where staff reviewed and presented selected articles on serosurveillance topics.

Additional support included virtual training by the US CDC in Atlanta on inter-laboratory comparison, assessing the precision and validation of a SARS-CoV-2 antibody test. Public Health England (now United Kingdom Health Security Agency) provided biosafety and risk assessment training. In addition, four MBA staff members were trained on bead coupling for the MBA through a collaboration between the African Centre for Excellence in Genomics of Infectious Diseases and the Nigeria Centre for Disease Control and Prevention.

## Supplies and logistics

The US CDC, the Global Fund, President's Malaria Initiative, and the CDC Foundation supported the commodities used in the MBA laboratory. Sample sets were prioritised into three tranches based on priority data needs (Table 1). Testing was completed between 2019 and 2022. Supplies were received and maintained in the MBA storeroom at the NRL and in an offsite warehouse. The weekly tracking of commodities was reported to all stakeholders to avoid stock-outs and to prevent the expiration of commodities before usage.

## Equipment maintenance

Initially, the MBA laboratory started with two Luminex MAGPIX® machines. Additional machines were added as resources became available. The laboratory now houses

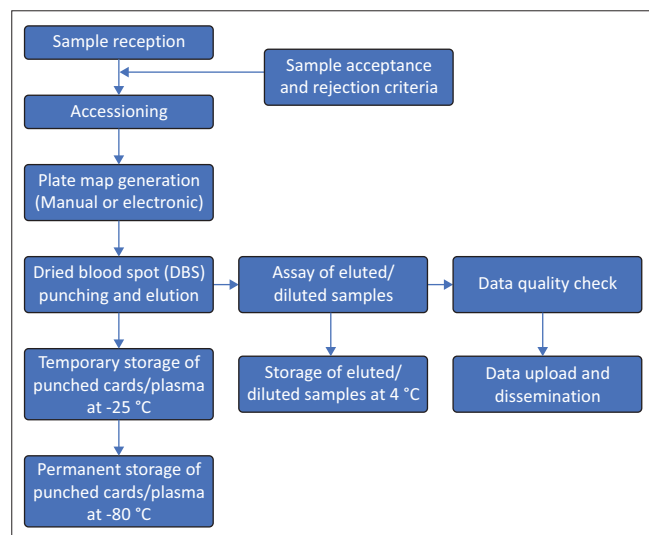


FIGURE 1: Multiplex bead assay laboratory specimen flow.

TABLE 1: Nigeria HIV/AIDS Indicator and Impact Survey (NAIS) samples† tested in the multiplex bead assay laboratory, Nigeria, 2019–2022.

NMS4 project	Sample set	Total samples tested for both the antigen and antibody assay	Priority reason	Dates of testing
Tranche 1	Priority sample sets from Nigeria HIV/AIDS Indicator and Impact Survey (NAIS) specimens: children (0–14 years, $n = 32\,023$ ) and WRA ( $n = 9915$ )	41 938	<ul style="list-style-type: none"> <li>Nigeria Measles and Rubella strategy workshop – March 2020</li> <li>Maternal and Neonatal Tetanus Elimination planning discussions – July 2020 (WRA samples)</li> </ul>	2019–2020
Tranche 2	NAIS specimens – collected from the working-age population (15–64 years) who provided consent for future testing in 11 President's Malaria Initiative-focused states in Nigeria.	51 000	The Global Fund to Fight AIDS, Tuberculosis and Malaria concept note planning for malaria grant – February 2020.	2020–2021
Tranche 3	Remnant adult samples from the NAIS specimen.	37 000	The remaining part of the adult HIV-positive and HIV-negative population	2021–2022

NMS4, Nigeria multi-disease serologic surveillance using stored specimens; NAIS, Nigeria HIV/AIDS Indicator and Impact Survey; WRA, women of reproductive age.

†, Total number of NAIS samples tested: 130 000.

seven Luminex MAGPIX® machines, one of the largest in Africa (Figure 2). The Luminex MAGPIX® Multiplexing System was chosen because of its affordability, compactness and durability. However, the equipment faced challenges such as clogging, probe jamming, XZ axis assembly malfunctioning, and communication cable malfunctioning. These were addressed through capacity-building, training the MBA staff to manage equipment challenges, including completing routine maintenance such as removing, sonicating, flushing, and replacing probes, syringes, and in-house tubing when maintenance staff could not travel to Nigeria owing to COVID-19 mitigation restrictions. In addition, the inadequate power supply to the NRL interrupted testing. However, upgrades to the NRL electrical services, including generator and rewiring upgrades, and installing appropriate uninterruptible power supplies, addressed most of the inadequate power supply problems.

## Biosafety and biosecurity

Working with guidance provided by the US CDC, the Nigeria Centre for Disease Control and Prevention adopted and implemented a safety plan for the MBA laboratory at the NRL. Multiplex bead assay laboratory staff members were trained on the appropriate handling and biosafety considerations of specimens (DBS and plasma samples). A safety team was established and a safety plan was created. Safety operational practices were adopted, including bi-annual risk assessments, standard waste management protocols, and refresher training. During the COVID-19 pandemic, a shift in laboratory staff operations, with longer hours, allowed activities to continue during lock-downs, with the implementation of social distancing and administrative controls.

## Data management

Each MBA run included a standard set of controls for quality control assessment. The assay was validated for detecting a range of antibodies and antigens. Laboratory data were downloaded from the MAGPIX® instrument, and results were exported using secure file transfer portals into a data file. Each record was identified by the unique sample identification, which did not include personally identifiable information. A comma-separated values data backup system was instituted using an external storage device, managed and protected to avoid data loss and unauthorised access.



Source: Photo taken by Dr Nnaemeka C. Iriemenam, United States Centers for Disease Control and Prevention, Abuja, Nigeria, 2022. Used with permission. No unauthorised duplication allowed.  
**FIGURE 2:** A cross-section of the multiplex bead assay laboratory with six of the seven MAGPIX<sup>®</sup> instruments at the National Reference Laboratory, Abuja, Nigeria, 2022.

### Quality assurance and quality control

A quality assurance programme was implemented to provide credibility and to increase confidence in the MBA laboratory results. External testing at the US CDC in Atlanta was used to ensure accurate laboratory results and early problem identification. Generally, there was good inter-laboratory reproducibility.<sup>18</sup>

### Implementation of ISO 17025 standards

It was recommended that the MBA laboratory adopt the ISO 17025 standard for calibration and testing laboratories. Here, we outline the experience of implementing the two measures and the lessons learnt. The NRL management had enrolled the MBA laboratory earlier in a mentorship programme towards achieving accreditation, the Strengthening Laboratory Management Toward Accreditation, based on the ISO 15189:2012 standard for medical laboratories to develop high-quality management systems. ISO 15189:2022 is the international standard of choice for exhibiting competence and quality in clinical diagnostic laboratories.<sup>19,20,21</sup> This necessitated the migration to the ISO 17025 standard, similar to ISO 15189. ISO 17025 is suited for surveillance laboratories performing testing and calibration.<sup>22,23</sup>

### Impact

The MBA laboratory met critical malaria, measles and tetanus data call deadlines. The information from the generated data helped to strengthen the capacity to monitor and evaluate public health interventions, provided opportunities to measure changes and trends over time and, most importantly, provided opportunities to address cross-cutting issues that are difficult with vertical programmes. Among these were the Global Fund to Fight AIDS, Tuberculosis and Malaria concept note planning for the malaria grant in February 2020, the Nigeria Measles and Rubella Strategy Workshop in March 2020, and the Maternal and Neonatal Tetanus Elimination planning discussions in July 2020 (women of reproductive age samples). Seroprevalence estimates and

modelling were used to assess disease exposure, the impact of public health interventions, supplementary immunisation activities, and routine vaccination coverage among children and women of reproductive age. The routine monitoring of this population allows one to ascertain the cumulative population immunity. Using serologic testing to understand trends in the proportion of women who were exposed to COVID-19 over time was helpful in determining the proportion of infected participants and in providing a valuable surveillance platform for women attending their antenatal care clinic.

Since the inception of the MBA in June 2019, about 130 000 samples have been tested for antibodies across multiple diseases and malaria antigens using stored NAIIS specimens. The NAIIS DBS specimens allow estimation of the prevalence of malaria, vaccine-preventable diseases, and neglected tropical diseases across the age ranges 0–64 years. As a result of the testing performed by the MBA team in Nigeria, 13 articles have been published in peer-reviewed journals. The team has also presented abstracts at the American Society of Tropical Medicine and Hygiene annual conference. Additional publications are also expected from this work.

### Severe acute respiratory syndrome coronavirus 2 multiplex bead assay projects

Verification and evaluation was conducted of cross-reactivity of immunoglobulin G assay for SARS-CoV-2 in Nigeria. The study verified the immunoglobulin G assay for SARS-CoV-2 with specimens collected from the Nigerian population. Previous independent verifications showed lower sensitivity and specificity of SARS-CoV-2 immunoglobulin G assays than reports and initial validations by manufacturers. Further testing revealed a significant level of cross-reactivity for two commercial enzyme-linked immunosorbent assays for SARS-CoV-2, both of which targeted only the nucleocapsid protein. In contrast, the CDC in-house beads and the Luminex xMAP SARS-CoV-2 multi-antigen assay, targeting the spike, receptor-binding domain and nucleocapsid, performed

better with less cross-reactivity, demonstrating the need to evaluate assays before implementation. The sample size included 200 Nigerian Institute of Medical Research specimens (100 SARS-CoV-2 positive and 100 SARS-CoV-2 pre-COVID-19 pandemic).<sup>24,25,26</sup>

A cross-sectional survey was also conducted among healthcare workers across three states (Rivers, Plateau, and Oyo) in Nigeria on knowledge, attitude and practice. The seroprevalence of SARS-CoV-2 infection among medical laboratory scientists, doctors, pharmacists and nurses was assessed using a CDC-developed multi-antigen SARS-CoV-2 immunoglobulin G assay in a total sample size of 1271 individuals.

Serologic surveillance results of SARS-CoV-2 and other diseases of public health importance using DBS specimens from women attending antenatal clinics for their first visit in U.S. President's Emergency Plan For AIDS Relief-supported states in Nigeria were also analysed for COVID-19 seroprevalence. The sample size in this study was 14 400.

A cross-sectional surveillance project was conducted using remnant viral load specimens from people living with HIV to assess the seroprevalence of SARS-CoV-2 among people living with HIV. A total of 37 400 viral load remnant plasma or DBS specimens from people living with HIV were tested from U.S. President's Emergency Plan For AIDS Relief-supported laboratories in Nigeria.

## Lessons learnt

Frequent quarterly supervisory visits by the CDC Atlanta team ensured adherence to the protocol which, in turn, led to quality results. International and regional partnership fostered supply chain logistics and assay standardisation significantly. Periodic competency assessments for laboratory personnel reduced errors and improved the reliability of assay results. Investments in training, mentorship and funding built a sustainable laboratory surveillance capacity for Nigeria. This success has attracted two additional multi-pathogen surveillance grants, and an uninterrupted supply of laboratory commodities through local vendors during emergencies, such as the COVID-19 pandemic, has sustained optimal laboratory operations.

## Recommendations

Based on the success of the Nigeria multi-disease serologic surveillance using stored specimens project, we recommend the implementation of a robust laboratory-based integrated disease surveillance in other African countries using MBA technology. To create a serosurveillance hub in these countries, centralised expertise is highly recommended.

Training of bioengineers in MAGPIX<sup>®</sup> maintenance is recommended for the sustainability of in-country MBA services. Advanced capacity building on local bead coupling,

data management and the establishment of multi-pathogen MBA proficiency testing programmes are recommended for country ownership and sustainability of the MBA technology.

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## Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

## Authors' contributions

A.G.A. and M.O. contributed equally to this work. A.G.A., O.O., S.G., M.O., N.C.I., and C.I. conceived and designed the concept. A.G.A., O.O.A., T.A.O., O.A.A., and N.C.I. wrote the initial draft of the manuscript. E.Y., M.C., N.P., D.M., and N.C.I. provided mentorship and technical support. A.G.A., M.O., S.G., O.O., M.O., N.C.I., and C.I. participated in administrative support of the project. A.G.A., M.O., O.O.A., A.T., T.A.O., S.G., S.A.A., F.N., B.U., I.O., J.D., L.A., M.E., A.D., O.A.A., F.V., I.I., O.O., W.N., J.A., O.B., N.P., S.G., M.A., M.S., N.M., E.Y., M.C., D.M., M.O., N.C.I., and C.I. participated in the critical revision of the manuscript for intellectual content. N.C.I. supervised this work. All authors approved the final version of the manuscript for publication.

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## Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its references.

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