STRENGTHENING IMMUNIZATION SYSTEMS THROUGH SEROSURVEILLANCE: EXECUTIVE SUMMARY

OVERVIEW

The Strengthening Immunization Systems through Serosurveillance (SISS) project was implemented by the International Vaccine Access Center at the Johns Hopkins Bloomberg School of Public Health from November 2015 through June 2025 with funding from the Gates Foundation. The investment, formally titled "Assessing the feasibility of using serological data to monitor and guide immunization programs in low-income countries", was to address epidemiological, technical and operational issues critical to the generation and interpretation of valid and timely serological data to identify immunity gaps and guide routine, supplemental and targeted immunization activities.

PROJECTS

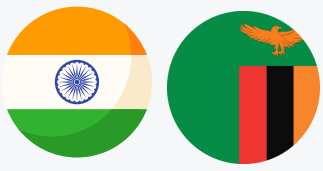
In India, we partnered with the Indian Council of Medical Research to conduct community-based measles and rubella (MR) serosurveys in four districts before and after MR vaccination campaigns that introduced rubella vaccine into the Universal Immunization Programme. In Zambia, we partnered with the National Health and Research Training Institute and Macha Research Trust to explore multiple serosurvey study designs. Through these serosurveys we addressed a wide range of epidemiologic and operational research questions.

India

- Seroprevalence before and after MR campaigns
- Use of different dried blood specimen collection devices

Zambia

- Nesting a serosurvey within a post-campaign coverage survey
- Nesting a serosurvey within a MR campaign
- Nesting a serosurvey in a Demographic Health Survey
- Leveraging the ZamPHIA biorepository of nationally representative blood specimens
- Testing fever rash surveillance specimens for measles and rubella IgG antibodies



India & Zambia

- Assess use of residual specimens to measure seroprevalence
- Estimate cost of serosurveys
- Summarize lessons learned and key recommendations

List of publications

Serosurveytools.org

Johns Hopkins Bloomberg School of Public Health

IVAC International Vaccine Access Center

KEY LESSONS LEARNED

1. Serology provides a **more accurate and complete depiction of immunity** than vaccination coverage.
2. Serology can **guide immunization activities** and evaluate program impact by:
   a. estimating disease burden
   b. identifying immunity gaps by age, space, and populations and
   c. evaluating program impact in filling immunity gaps
3. Serosurveillance is **feasible and sustainable**.
4. A serosurveillance system should consist of a **combination of sampling strategies**.

POLICY IMPLICATIONS

Seroprevalence estimates provide more confidence in the decision of **where and when to conduct targeted immunization activities** and may be **most useful where population immunity is uncertain** due to a paucity of data or contradictory data (e.g. high administrative coverage but frequent outbreaks).

Measles and rubella seroprevalence estimates provide direct measures of immunity gaps by age, space, and special populations to guide targeted immunization campaigns and activities. This is particularly important as **countries move away from nationwide, non-selective measles and rubella mass vaccination campaigns** because of limited resources.

Serosurveys can not only guide targeted immunization campaigns and activities but can be used to **measure their impact**, thus identifying persistent immunity gaps that cannot be identified in post-campaign coverage surveys (e.g., in older age groups).

RECOMMENDATIONS

Technical assistance from experienced partners and established guidelines, such as the WHO measles and rubella serology guidelines, the planned addition of serosurveys to the WHO vaccination coverage survey manual, and serosurveytools.org can guide countries planning to conduct measles and rubella serosurveys and establish integrated serosurveillance.

To generate serological data for policy makers, we need to **design and implement sustainable serosurveillance systems**. This has not yet been done, although we have the potential to do so in Zambia. **Multipathogen serosurveillance** can make serosurveillance more sustainable by including dozens of pathogens simultaneously and **leveraging resources across disease surveillance systems**.

Johns Hopkins Bloomberg School of Public Health
IVAC International Vaccine Access Center

Strengthening Immunization Systems through Serosurveillance (SISS): Final Report - Lessons Learned and Recommendations

PROJECT OVERVIEW

The Strengthening Immunization Systems through Serosurveillance (SISS) project was implemented by the International Vaccine Access Center at the Johns Hopkins Bloomberg School of Public Health from November 2015 through June 2025 with funding from the Gates Foundation. The investment, formally titled “Assessing the feasibility of using serological data to monitor and guide immunization programs in low-income countries”, was to address epidemiological, technical and operational issues critical to the generation and interpretation of valid and timely serological data to identify immunity gaps and guide routine, supplemental and targeted immunization activities. The project was conducted in India and Zambia.

In India, we partnered with the Indian Council of Medical Research, the National Institute of Epidemiology, and the National Institute of Virology as well as local institutions. The main project was to conduct community-based measles and rubella serosurveys in four districts before and after measles and rubella vaccination campaigns that introduced rubella vaccine into the Universal Immunization Programme. Secondary projects included: 1) concurrent community-based and facility-based residual specimen collection to assess the use of residual specimens; 2) assessing the use of different dried blood specimen collection devices; 3) strategies for sharing the results of community-based serosurveys with participants; 4) measuring the cost of serosurveys; and 5) lessons learned and key recommendations.

In Zambia, we partnered with the National Health and Research Training Institute (formerly the Tropical Diseases Research Center) and Macha Research Trust to explore multiple serosurvey study designs, including 1) nesting a measles and rubella serosurvey within a post-campaign coverage survey; 2) leveraging the ZamPHIA biorepository of nationally representative blood specimens; 3) nesting a measles and rubella serosurvey within a mass measles and rubella vaccination campaign; 4) testing fever rash surveillance specimens for measles and rubella IgG antibodies; 5) concurrent community-based and facility-based residual specimen collection to assess the use of residual specimens; and 6) nesting a measles and rubella serosurvey within the 2024 Zambian Demographic and Health Survey. Through these different serosurveys we addressed a wide range of epidemiologic and operational research questions.

Strengthening Immunization Systems through Serosurveillance (SISS): Final Report - Lessons Learned and Recommendations

Key Lessons Learned

The key lessons learned from the SISS project, described in more detail below, are:

1. Serology provides a more accurate and complete depiction of immunity than vaccination coverage.
2. Serology can guide immunization activities and evaluate program impact by: 1) estimating disease burden; 2) identifying immunity gaps by age, space, and populations; and 3) evaluating program impact in filling immunity gaps.
3. Serosurveillance is feasible and sustainable.
4. A serosurveillance system should consist of a combination of sampling strategies.

Policy implications

- Measles and rubella immunity gaps are correlated with outbreak risk and are best measured by serological surveys. Administrative vaccine coverage and case surveillance data are often of poor quality and incomplete, particularly at subnational levels, across wide age ranges, and among populations with low card retention.
- Measles and rubella seroprevalence estimates provide direct measures of immunity gaps by age, space, and special populations to guide targeted immunization campaigns and activities. This is particularly important as countries move away from nationwide, non-selective measles and rubella mass vaccination campaigns because of limited resources.
- Seroprevalence estimates provide more confidence in the decision of where and when to conduct targeted immunization activities and may be most useful where population immunity is uncertain due to a paucity of data or contradictory data (e.g. high administrative coverage but frequent outbreaks).
- Serosurveys can not only guide targeted immunization campaigns and activities but can be used to measure their impact, thus identifying persistent immunity gaps that cannot be identified in post-campaign coverage surveys (e.g., in older age groups).
- Measles and rubella serosurveys can also provide measures of disease burden, particularly for congenital rubella syndrome prior to vaccine introduction. Outside of measles and rubella, multipathogen serosurveys provide a measure of cumulative exposure that can be used to assess disease burden to a wide range of pathogens as part of an integrated disease surveillance system.

Recommendations on how to conduct serosurveys

- While serosurveys provide a valuable data source for policy makers, they can be costly and logistically challenging to conduct. The value of the information generated depends upon the objectives and programmatic implications. Thus, it is important to define the use cases and policy implications prior to conducting the serosurvey and to design the serosurvey accordingly.
- Serosurveys can be conducted in a more sustainable manner using alternative study designs and specimen sources and not solely on community-based surveys. Understanding the potential biases and tradeoffs of alternative study designs and specimen sources is key to their interpretation.
- Technical assistance from experienced partners and established guidelines, such as the WHO measles and rubella serology guidelines, the planned addition of serosurveys to the WHO vaccination coverage survey manual, and serosurveytools.org can guide countries planning to conduct measles and rubella serosurveys and establish integrated serosurveillance.

Looking forward and next steps

- There is expressed interest from immunization programs to include serological data into programmatic decision-making. However, more work needs to be done to not only assist Ministries of Health design and conduct serosurveys but to translate the findings to policy makers and inform immunization strategies. To assist with the design and conduct of serosurveys, we are working with WHO to incorporate serosurveillance into the WHO vaccine coverage survey manual by including sections in each corresponding chapter on how to include serology when conducting a coverage survey. We are also working to refine the use cases and, most importantly, assist policy makers in using the findings from multipathogen serosurveillance through the Seroanalytics Hub funded by the Gates Foundation. However, further work is needed to engender examples of how serological data have guided policy decisions.
- To generate serological data for policy makers, we need to design and implement sustainable serosurveillance systems. This has not yet been done, although we have the potential to do so in Zambia. Multipathogen serosurveillance can make serosurveillance more sustainable by including dozens of pathogens simultaneously and leveraging resources across disease surveillance systems. Such a sustainable system could consist of multipathogen bead-based assays using specimens collected through different study designs and sampling strategies. Periodic nationally representative serosurveys could provide a snapshot of seroprevalence estimates across diseases at the provincial or district

levels, and anchor future estimates. More spatially targeted, disease specific, convenience serosurveys using residual specimens could be used to monitor trends at lower cost between the nationally representative serosurveys.

- We are well positioned to collaborate with the National Public Health Institute to create a sustainable, multipathogen serosurveillance system in Zambia as a pathfinder country, exploring the integration of different sampling strategies and translating findings to policy makers to ensure they are used to guide immunization and disease control programs.

Contents

SISS Publications

India

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survey within a post-campaign coverage evaluation survey. *Vaccine*. 2019;37(17):2387-93.
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Assay development

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Work leveraging findings from SISS

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Future publications

The following manuscripts from the SISS project are under development and will be submitted to journals in late 2025 or early 2026 (Table 1).

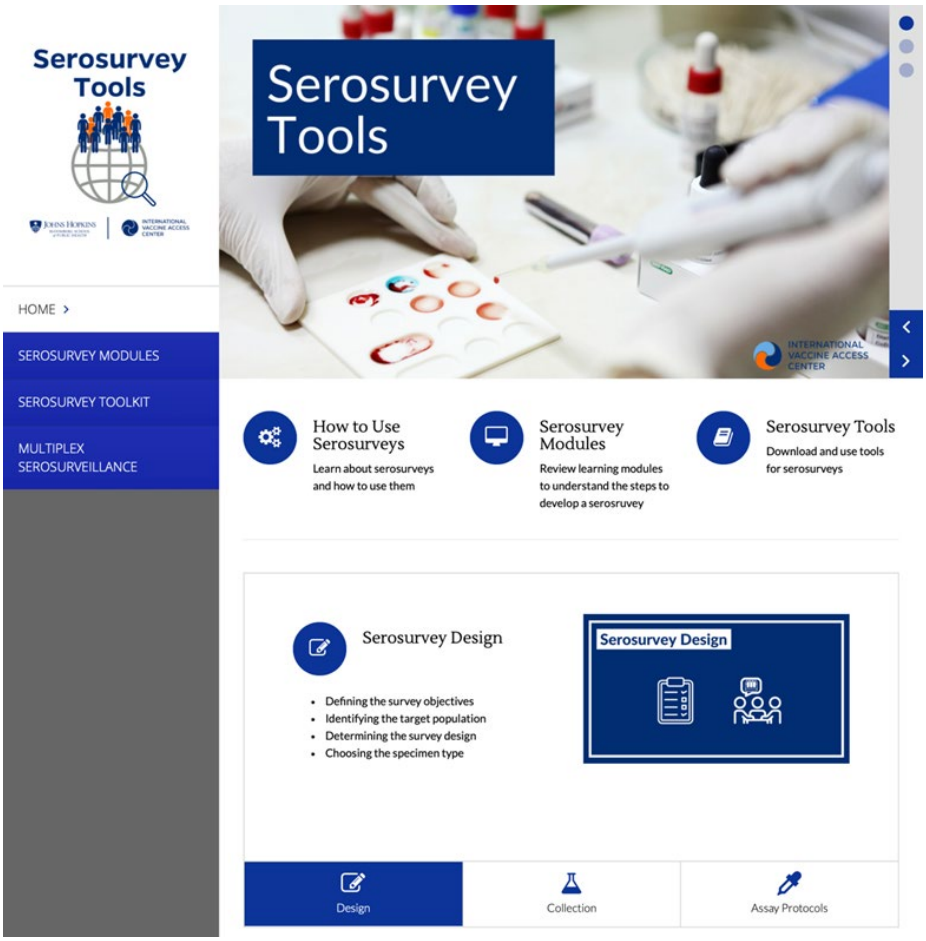
Table 1. Forthcoming publications from the SISS project

Study	Description	Status
Residual specimen collection (Zambia)	Utility of residual specimens in Zambia in estimating district-level seroprevalence	Analysis complete; being drafted
Residual specimen collection (Zambia)	Feasibility of establishing a high quality, sustainable, and flexible serosurveillance system in Zambia	Reviewed by coauthors; formatting for journal
Residual specimen collection (Zambia)	Tetanus seroprevalence among children in Ndola and Choma Districts, Zambia, 2022	Drafted; under review by coauthors
Residual specimen collection (Zambia)	Cost Savings and Bias in the Use of Residual Versus Community-Based Household Serosurvey Specimens to Measure Measles and Rubella Immunity in Zambia	Drafted; under review by coauthors
Community-based serosurvey (Zambia)	Vaccination timeliness and coverage analysis in Choma and Ndola Districts in Zambia: A Secondary Analysis	Drafted; under review by coauthors
Measles serosurvey nested in Zambia Demographic Health Survey	Leveraging a national demographic health survey in Zambia to assess measles immunity gaps across space	Analysis to be completed in late 2025/early 2026
Cross-cutting	A Unified Analytic Framework for Seroprevalence Estimation from Residual and Population-Based Studies in Zambia	Analysis to be completed in late 2025/early 2026

Cross-cutting	Utility of serological data for understanding susceptibility and evaluating vaccination performance	Analysis to be completed in late 2025/early 2026
Cross-cutting	Use of calibration studies and analytic techniques to adjust EIA serosurvey output	Analysis to be completed in late 2025/early 2026
IMRVI (India)	Costs of conducting district-level serosurveys	Analysis complete; Finalizing draft

SerosurveyTools and Advocacy Briefs

The major resource we developed through the SISS project to assist others in the design, implementation, analysis, and interpretation of serological surveys on the website Serosurvey Tools (https://serosurveytools.org).



This website provides serosurvey modules, a serosurvey toolkit, and guidance on designing, conducting, and analyzing multiplex serosurveillance data.
The serosurvey modules include:

1. An introduction to serosurveys
2. Objectives of serosurveys
3. Serosurvey design
4. Specimen collection
5. Developing and running assay protocols
6. Quality assurance for assays and interpretation of results
7. Processing, storage, and shipment of specimens
8. Serosurveys using residual specimens from a biorepository or health facility
9. Multiplex serosurveillance

The toolkit contains sample protocols, data collection tools, monitoring and evaluation tools, and data analytic tools.

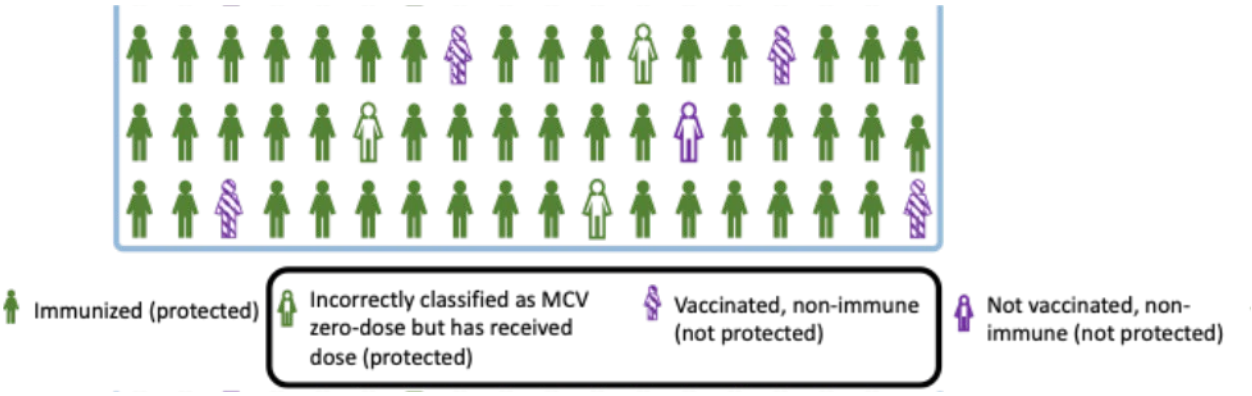
Our team is also developing two one-page advocacy briefs on: 1) the value of conducting serosurveys for vaccine-preventable disease; and 2) logistical considerations for conducting serosurveys, building upon the lessons learned from the SISS project and directing readers to resources on serosurveytools.org.

Lessons learned from the SISS Project

Lesson #1: Serology provides a more accurate and complete depiction of immunity than vaccination coverage

Administrative vaccine coverage data and case surveillance data are commonly used but flawed metrics to identify immunity gaps (Winter AK et al, 2018). Subnational administrative coverage data is often inaccurate with wide uncertainty bounds and case surveillance is often incomplete. Even with high quality vaccine coverage and case surveillance data, individual immune and susceptible status will be misclassified as shown in Figure 1.

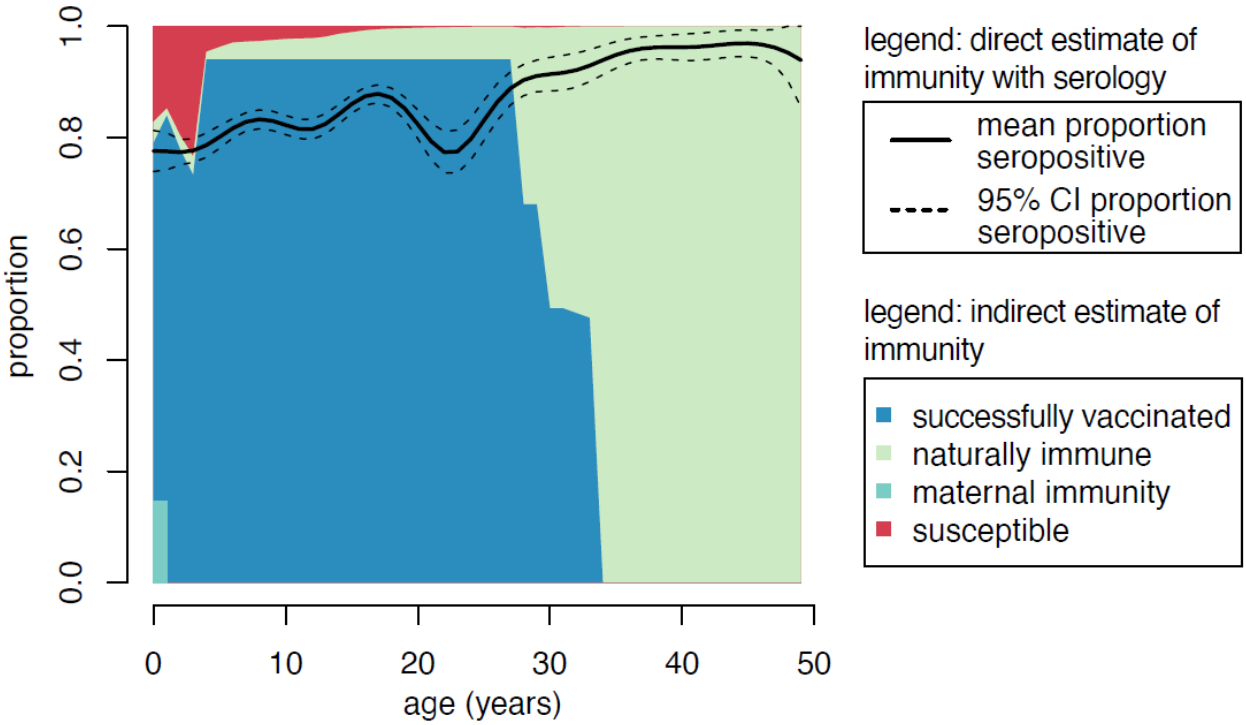
Figure 1. Hypothetical population showing how vaccination coverage can misclassify immune and susceptible individuals



Examples from SISS

We leveraged a nationally representative biorepository from the 2016 Zambian Population HIV Impact Assessment (ZAMPHIA) study and tested nearly 10,000 blood specimens for measles and rubella IgG antibodies (Carcelen AC et al, 2022). As shown in Figure 2, the age-specific measles seroprevalence (shown in the dark line) gives a very different immunity profile compared to an indirect estimate of measles immunity using vaccine coverage and measles case reports. The immunity gap among adults in their early 20s would be impossible to identify using administrative coverage and case surveillance data.

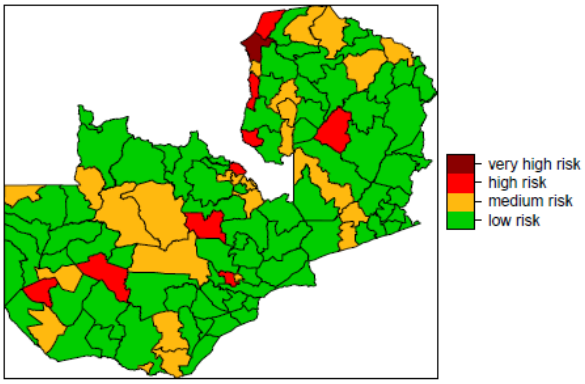
Figure 2. Comparison of age-specific measles seroprevalence in Zambia in 2016 with reconstruction of an immunity profile using vaccine coverage and case surveillance data



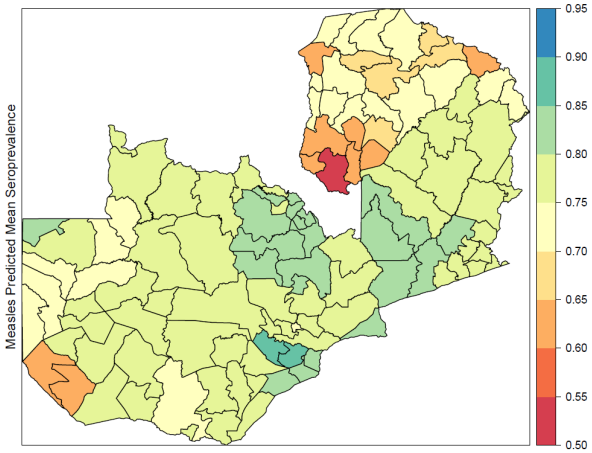
Measles seroprevalence estimates can also differ in the identification of high-risk districts compared to other estimates and tools, such as the commonly used Measles Programmatic Risk Assessment Tool (MPRAT) as shown in Figure 3. Although not unexpected as these are different measures, these differences highlight the need for more accurate risk assessment tools, both informed by and validated with seroprevalence estimates.

Figure 3. Serological data (right) identify areas of concern that differ from the Measles Programmatic Risk Assessment Tool (left), Zambia 2016

Measles Programmatic Risk Assessment Tool (MPRAT)



Mean measles seroprevalence



Seropositivity by number of measles-containing vaccines

Given the potential for some vaccinated children to fail to seroconvert, and some children to be misclassified as having received vaccine, we explored the potential for serological data to better understand susceptibility and evaluate vaccine program performance. To do this, we compared the number of vaccine doses reported to have been administered to the seropositivity. This analysis was restricted to children who had routine vaccination cards available, as verbal recall can introduce additional bias. We found that seropositivity by vaccine dose administered ranged across our studies. Among children who had at least 2 doses of measles containing vaccine administered, seroprevalence was sometime the expected 95% (Table 2). This could have been in part due to misclassification of doses; whereby these vaccines were not actually received, poor immunogenicity, poor sensitivity of the enzyme immunoassay test.

Table 2. Seropositivity by number of documented measles-containing vaccines among children with routine vaccination cards

	0 doses	1 dose	2+ doses
Zambia 2020 Nested SIA	39.0 (31.5, 46.9)	88.2 (85.6, 90.3)	91.4 (89.8, 92.8)
India post-campaign[a]			
Palghar, Maharashtra	–[b]	87.3 (65.0, 96.2)	96.3 (91.3, 98.5)
Kanpur Nagar, Uttar Pradesh	–[b]	88.5 (47.4, 98.5)	84.2 (77.2, 89.4)
Hoshiarpur, Punjab	–[b]	95.9 (62.4, 99.7)	93.5 (88.8, 96.3)
Dibrugarh, Assam	–[b]	86.4 (52.8, 97.3)	98.7 (96.7, 99.5)
Thiruvananthapuram, Kerala	–[b]	97.9 (80.2, 99.8)	95.0 (91.0, 97.3)
Zambia FIA HH survey[a]			
Choma	64.4 (31.0, 87.9)	81.7 (71.8, 88.6)	97.8 (94.1, 99.2)
Ndola	–[b]	78.3 (61.1, 89.2)	87.1 (76.9, 93.2)

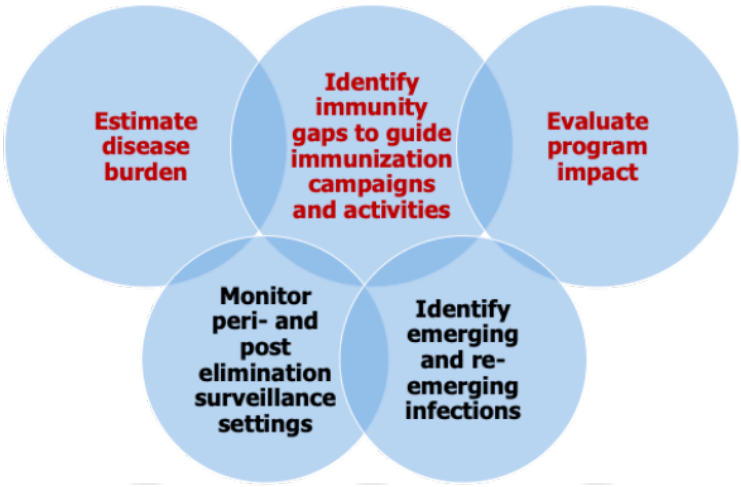
a. Survey weighted estimates
b. Not presented due to small sample size (< 10 children).

Lesson #2: Serology can guide immunization activities and evaluate program impact

Serology has multiple use cases (Figure 4), including:

1. Estimate disease burden
2. Identify immunity gaps to guide targeted immunization campaigns and activities
3. Evaluate program impact
4. Monitor peri- and post elimination surveillance settings
5. Identify emerging and re-emerging infections

Figure 4. Use cases for serosurveillance, with those addressed in the SISS project in red



In the SISS project, we explored the first three use cases and described these in more detail below and in a publication from SISS team members related to this work (Winter AK et al 2018). The SISS project also influenced the development of use cases discussed during the 2023 Serosurveillance Summit (Carcelen AC et al, 2024).

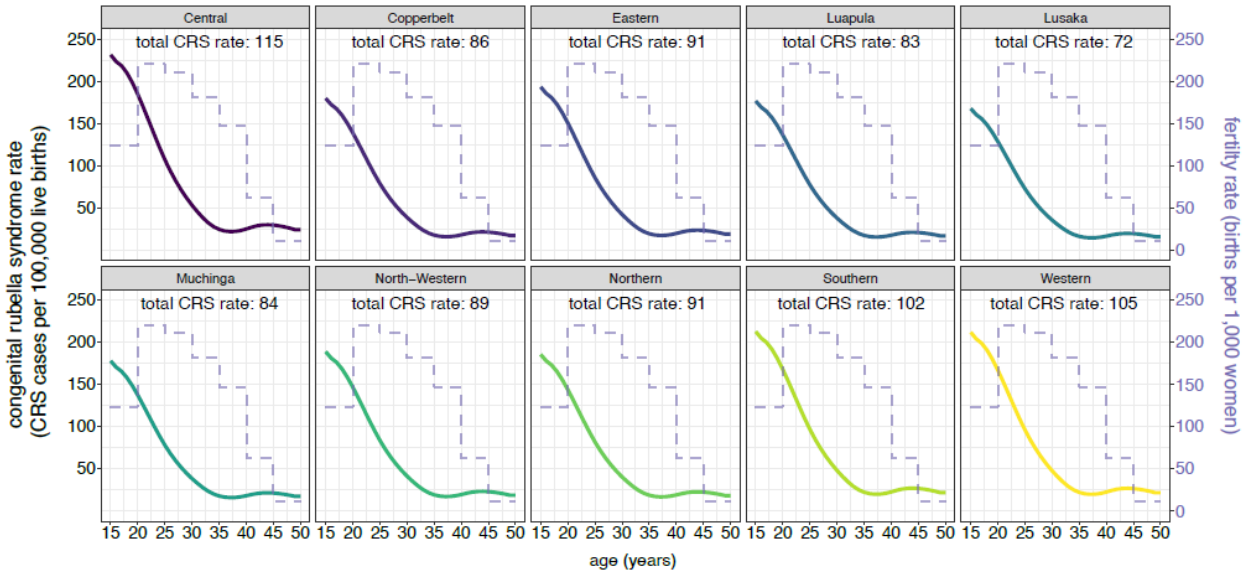
Estimate disease burden

Measles and rubella serosurveys provide estimates of exposure to wild-type and vaccine viruses. Current methods do not allow the differentiation of these exposures but this may be possible in the near future. For some vaccine-preventable diseases such distinction is possible. An example is the measurement of IgG antibodies to the nucleocapsid protein of SARS-CoV-2 that is not a component of some vaccines. One of the best use cases has been to use age-specific rubella seroprevalence to estimate the burden of congenital rubella syndrome, a condition for which routine surveillance is often poor.

Example from SISS

We derived the burden of congenital rubella syndrome from age-specific seroprevalence estimates obtained from the measles and rubella IgG antibody testing of nearly 10,000 blood samples from the nationally representative biorepository from the 2016 Zambian Population HIV Impact Assessment (ZAMPHIA) (Carcelen AC et al, 2022). Age-specific rates of congenital rubella syndrome per 100,000 live births were calculated across the 12 provinces in Zambia that, when combined, resulted in a national annual rate of 96 CRS cases per 100,000 live births (Figure 5).

Figure 5. Age-specific rates of congenital rubella syndrome per 100,000 live births across 12 provinces in Zambia.



We have explored additional strategies to distinguish exposure to wild-type and vaccine viruses. One approach leverages quantitative data, on the assumption that natural infection results in higher antibody levels than vaccination and uses a latent class model to assign the probability of natural infection and vaccination. A second approach has been to use a combination of measles and rubella seroprevalence estimates. For example, a child who should have received a measles-rubella vaccine but was measles seropositive and rubella seronegative most likely had wild-type measles virus infection given the greater immunogenicity of rubella vaccine. Work on these strategies is on-going.

Identify immunity gaps to guide targeted immunization campaigns and activities

The most important use case for measles and rubella serosurveys is to identify immunity gaps to guide targeted immunization campaigns and activities, although more work is needed to demonstrate this in practice. Given current resource constraints, more countries will need to do more with less, including geographic targeting of immunization campaigns. The recently published "WHO Interim Guidance: Targeted and Selective Strategies in Measles and Rubella Vaccination Campaigns", to which we contributed, provides a framework for such targeting. Measles and rubella seroprevalence estimates can be used in conjunction with vaccine coverage and case surveillance data to identify immunity gaps by age, space, and special populations for targeted campaigns.

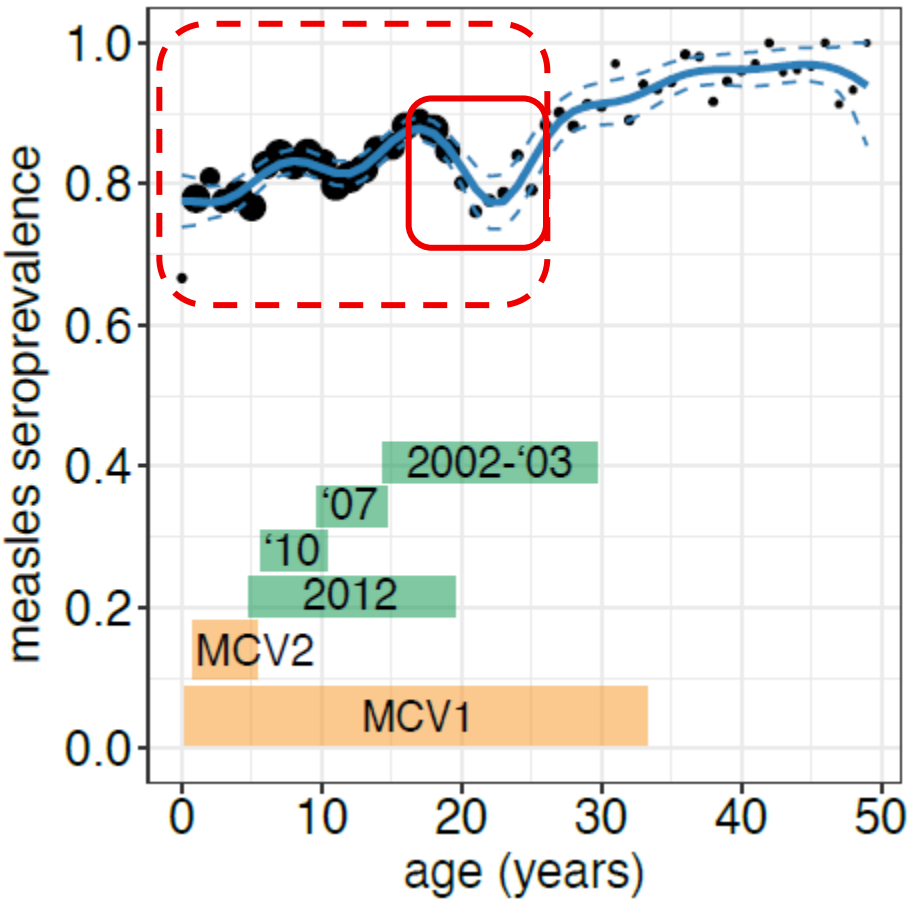
Immunity gaps by age, space, and special populations

Examples from SISS

We have many examples from the SISS project that demonstrate immunity gaps by age, space, and special populations and highlight several here.

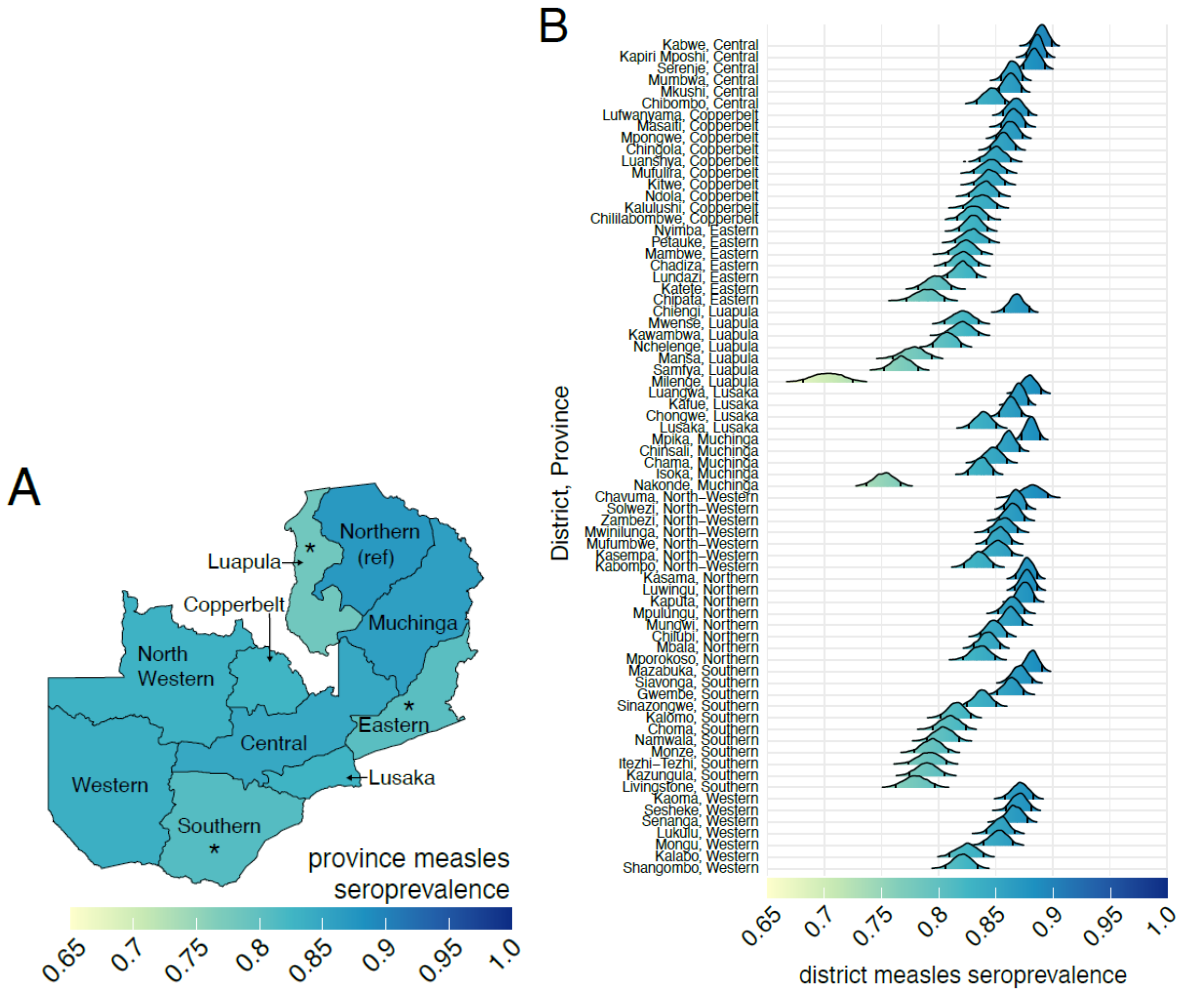
In the PIRMZ project in Zambia that leveraged the ZamPHIA biorepository, we identified a measles immunity gap not only in children younger than 5 years of age (measles seroprevalence 71%) but also in adults 20-25 years of age that would not otherwise have been identified (Figure 6) (Carcelen AC et al, 2022).

Figure 6. National age-specific measles seroprevalence in Zambia, 2016 showing immunity gaps. The points represent the data grouped by age in years. The size of the point is proportional to the number of observations in each age group. The blue lines represent fitted generalized additive model mean (solid line) and 95% confidence intervals (dashed lines). The age cohorts eligible for vaccination campaigns by campaign year (green boxes) and routine doses of measles-containing vaccine dose 1 (MCV1) and dose 2 (MCV2) (orange boxes) are shown.



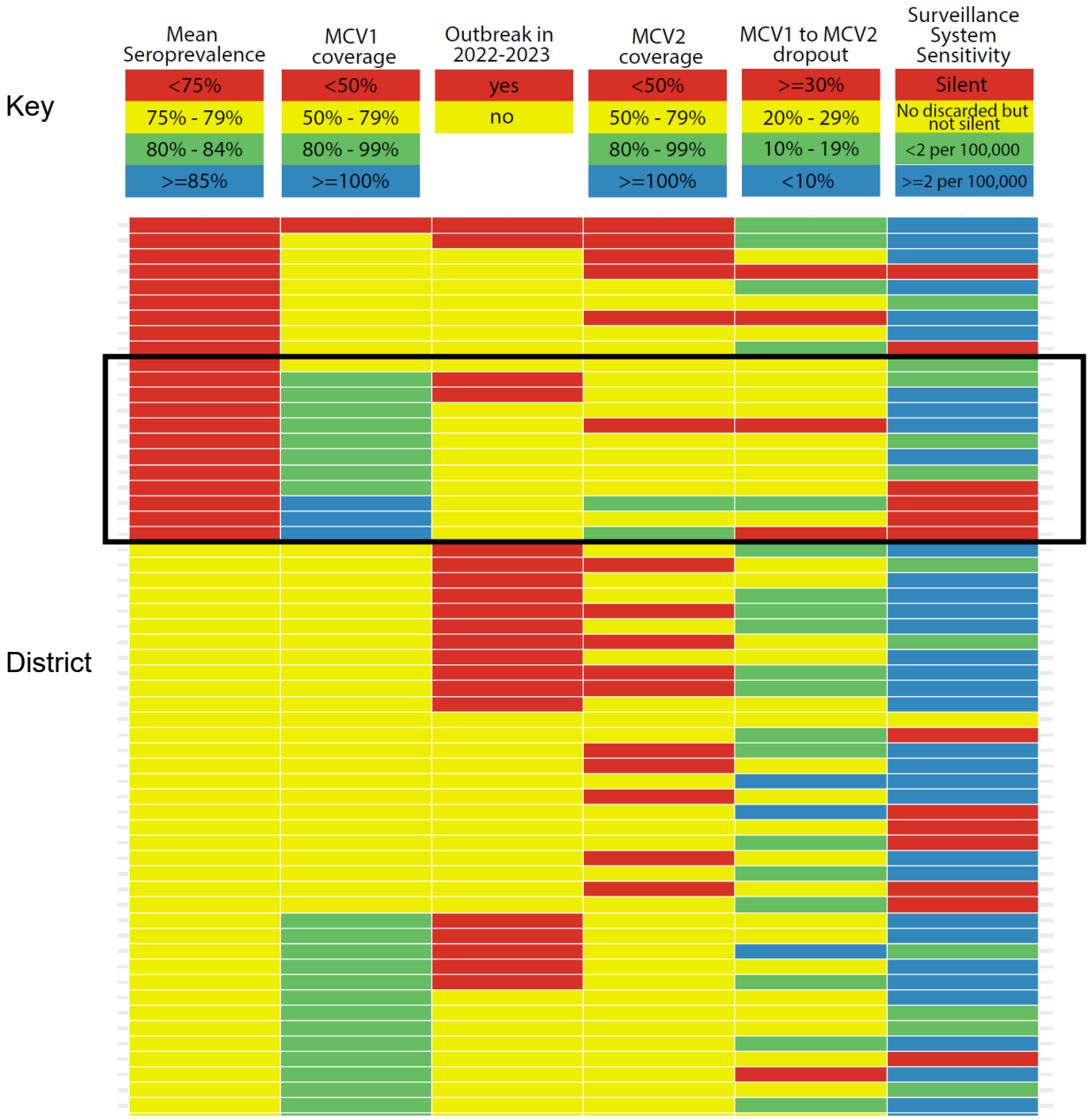
The same measles seroprevalence survey allowed us to identify immunity gaps across Zambia at both the provincial and district levels (Figure 7). Such estimates could be used to guide targeted SIAs.

Figure 7. Measles seroprevalence estimates by (A) province and (B) district in Zambia, 2016.



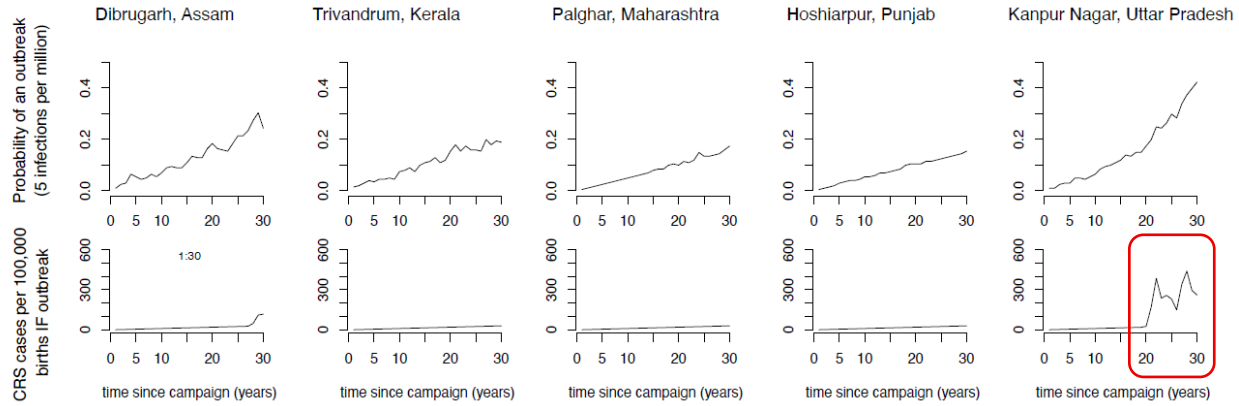
Seroprevalence estimates are of particular value where there is a high level of uncertainty in the vaccine coverage estimates or discrepancies between the coverage and case surveillance data (i.e., high coverage estimates but also high number of cases). Figure 8 is an example of how we compared preliminary district-specific seroprevalence data from the Zambian Demographic and Health Survey to different immunization and surveillance metrics for policymakers, using color coding to highlight areas where seroprevalence was low but vaccination coverage high.

Figure 8. Comparison of measles seroprevalence with immunization metrics by district in Zambia, highlight areas with discrepancies.



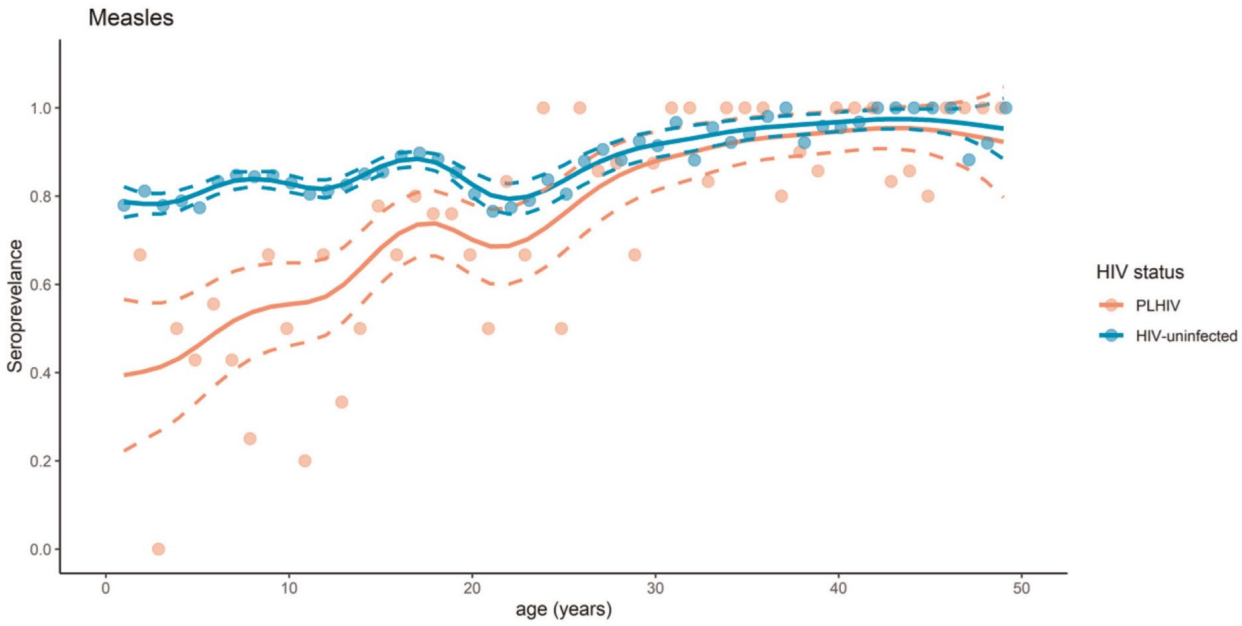
Measurement of immunity gaps by age and space can be used to inform models of outbreak risk, as we did for rubella outbreaks in different states in India (Figure 9).

Figure 9. Probability of a rubella outbreak and the number of expected CRS cases per 100,000 births if an outbreak were to occur, highlighting the risk in Kanpur Nagar based on age-specific rubella seroprevalence estimates.



Lastly, the PIRMZ project also allowed age-specific estimates of measles seroprevalence among persons living with HIV because it was nested within the ZamPHIA study through which HIV infection status was determined (Mutembo S et al, 2023). We identified measles immunity gaps in children, adolescents, and adults living with HIV up to the age of 30 years (Figure 10).

Figure 10. Age-specific measles seroprevalence by HIV infection status in Zambia, 2016



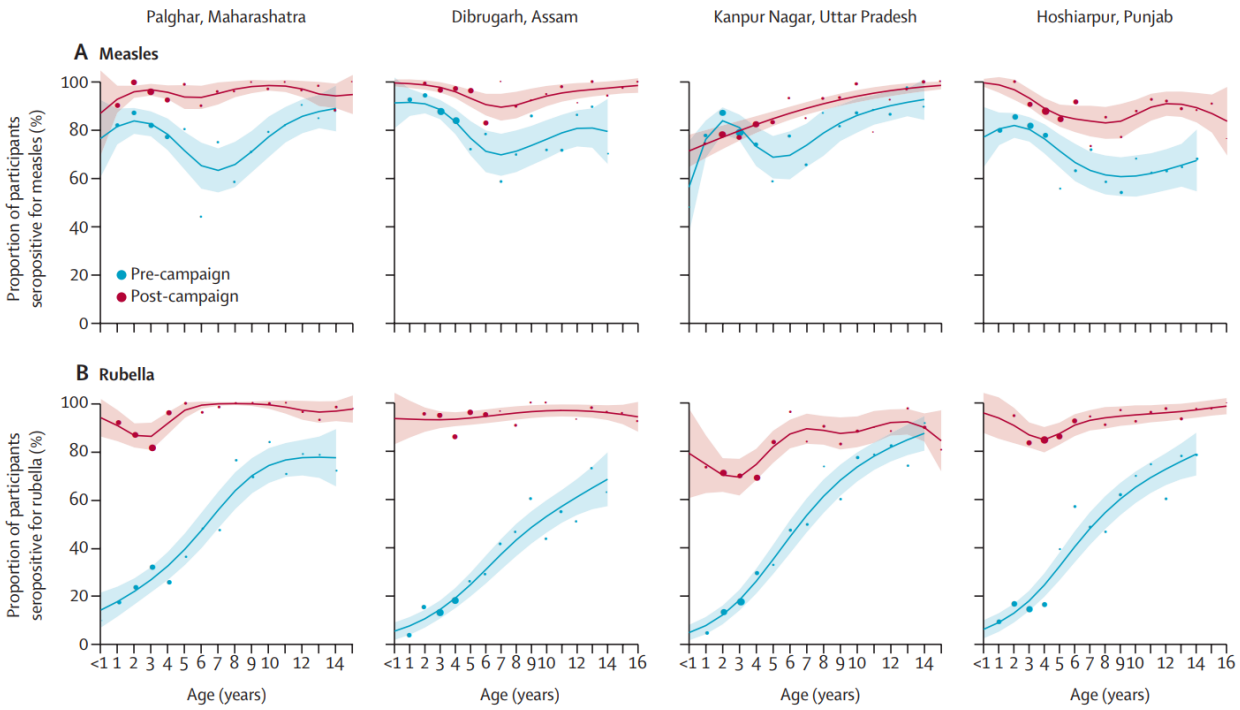
Evaluate program impact

By measuring immunity gaps across age, space, and special populations, measles and rubella serosurveys can evaluate program impact. This is most clearly demonstrated by measuring the impact of measles and rubella SIAs.

Examples from SISS

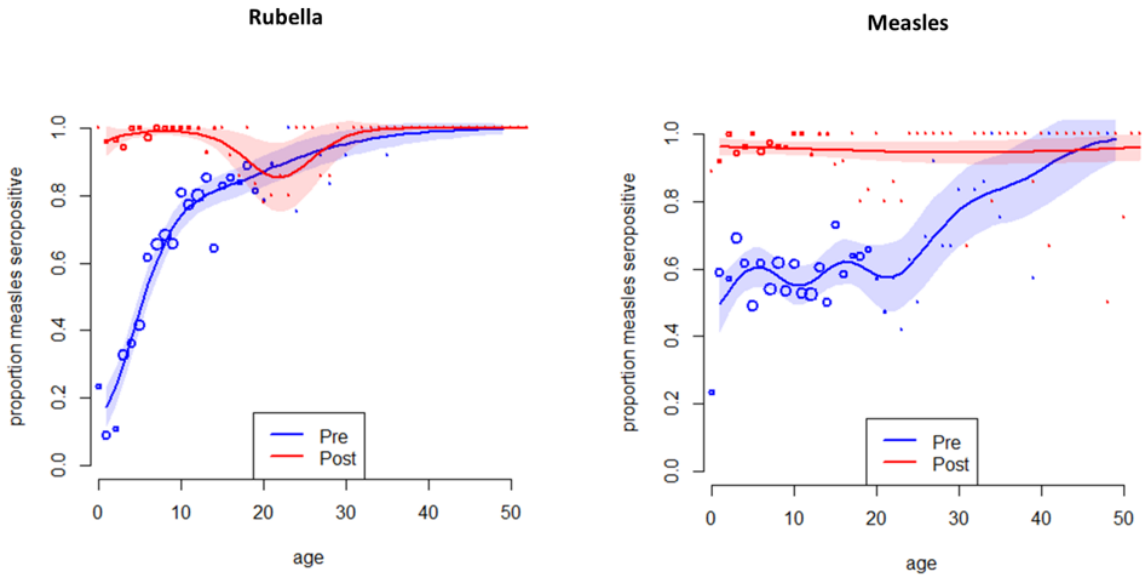
In India, we measured age-specific measles and rubella seroprevalence in four districts before and after mass vaccination campaigns that included the introduction of the rubella vaccine in the Universal Immunization Programme (Figure 11) (Murhekar MV et al, 2022). Immunity gaps were filled in most districts except for Kanpur Nagar in Uttar Pradesh, where measles and rubella immunity gaps persisted among young children.

Figure 11: Age-specific measles and rubella seroprevalence among serosurvey participants, by district



In Southern Province, Zambia we also measured the impact of a measles and rubella SIA that first introduced rubella vaccine into the Essential Immunization Program, leveraging different sampling strategies (Carcelen AC et al, 2021). The measles immunity gaps were effectively filled but a rubella immunity gap persisted among adolescent and young adults, a critical age to prevent congenital rubella syndrome (Figure 12). This immunity gap could not be identified through a post-campaign coverage survey.

Figure 12. Age-specific measles and rubella seroprevalence in Southern Province, Zambia before and after a measles and rubella SIA in 2016.

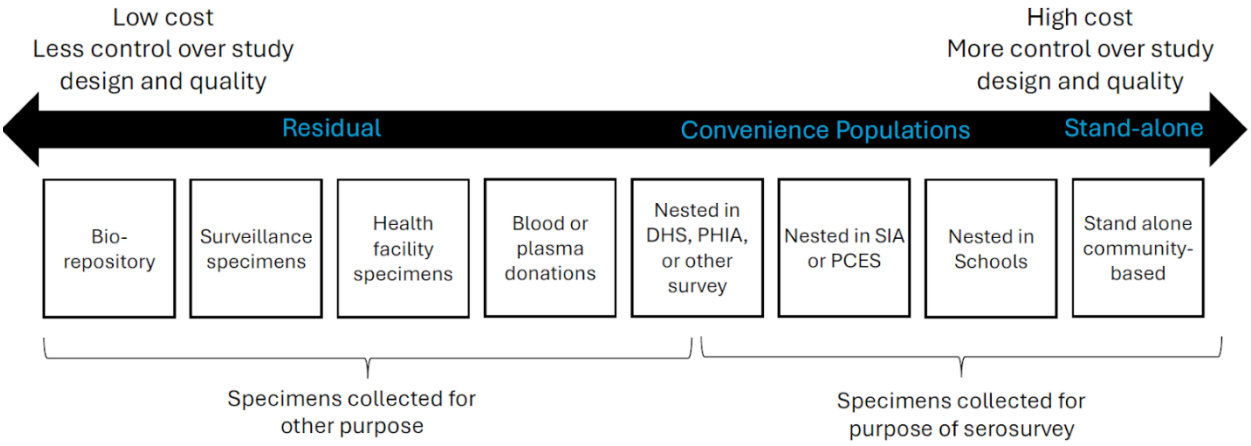


Lesson #3: Serosurveillance is feasible and sustainable

In addition to assessing the utility of measles and rubella serosurveys, we also explored multiple sampling strategies to show that serosurveillance is feasible and potentially sustainable. We largely used measles and rubella IgG enzyme immunoassays, although we also performed tetanus and SARS-CoV-2 serology. Through other work supported by the Gates Foundation, we have conducted and are conducting multi-pathogen serosurveillance in Mozambique and Zambia using multiplexed bead assays (Carcelen AC, 2025 COMSA). In addition, through the Seroanalytics Hub supported by the Gates Foundation, we are expanding and improving analytical methods and developing analytical tools for multiplexed serological data.

In conducting serosurveys, several questions need to be addressed in collaboration with policy makers and key stakeholders: 1) What antigens should be included; 2) What specimens should be collected; 3) What assays should be performed; 4) What study designs and sampling strategies should be used; and 5) What analyses can and should be conducted to answer relevant policy questions. Although we explored each of these questions in the SISS project, here we highlight work related to different study designs and sampling strategies, the use of residual specimens, considerations for laboratory assays, and the cost of serosurveys - noting how these can facilitate the feasibility and sustainability of serosurveillance. We recognize that stand-alone community-based household serosurveys are expensive and labor intensive. To make serosurveys feasible and sustainable, different sampling strategies need to be used as shown in Figure 13.

Figure 13: Range of study designs and sampling strategies for serosurveys



Study designs and sampling strategies

In Zambia, we conducted measles and rubella serosurveys using diverse study designs and sampling strategies.

1. Post-campaign coverage surveys

We demonstrated the feasibility and utility of nesting a measles and rubella serosurvey within a post-campaign coverage survey in Southern Province, Zambia in 2016 (Hayford et al, 2019; Mutembo et al, 2018). Among households that participated in the post-campaign coverage survey, 80% also participated in the serosurvey and 86% of individuals available in the household provided a blood sample for the serosurvey. Seroprevalence to measles and rubella viruses in children younger than 16 years of age was significantly higher than expected from vaccination coverage estimates, likely reflecting exposure to wild-type viruses and underreporting of vaccination. The serosurvey revealed rubella immunity gaps among women 16–30 years of age, precisely the age group for which protection from rubella is most important to prevent congenital rubella syndrome.

2. Biorepositories

We have described key findings from our measles and rubella serosurvey using specimens from the 2016 ZamPHIA biorepository, a provincially representative HIV incidence and prevalence survey (Carcelen et al, 2022). A subsample of residual specimens was selected from the ZAMPHIA biorepository to generate age-specific seroprevalence estimates for measles and rubella in each province. A total of 11,500 participants were subsampled from the 25,383 ZAMPHIA participants younger than 50 years of age who had blood collected. Specimens were selected based on HIV infection status, geographic cluster, and age to maintain provincial representativeness. The final sample size was 9854 residual specimens that were tested for IgG antibodies to measles and rubella viruses. This study demonstrated the value of leveraging a large, nationally representative biorepository, significantly reducing the cost of the measles and rubella serosurvey.

3. Measles and rubella supplementary immunization activities

We demonstrated the feasibility and utility of nesting a measles and rubella serosurvey within an SIA by conducting a serosurvey nested within the November 2020 measles-rubella SIA integrated with the Child Health Week activities in Zambia (Carcelen et al, 2024; Prosperi et al, 2025). The serosurvey successfully enrolled 90% of children from Child Health Week due to close coordination between the vaccination and serosurvey teams. Eighty-six percent and 90% of children were measles and rubella seropositive, respectively, before vaccination during the SIA. Thirty-six percent of children with no

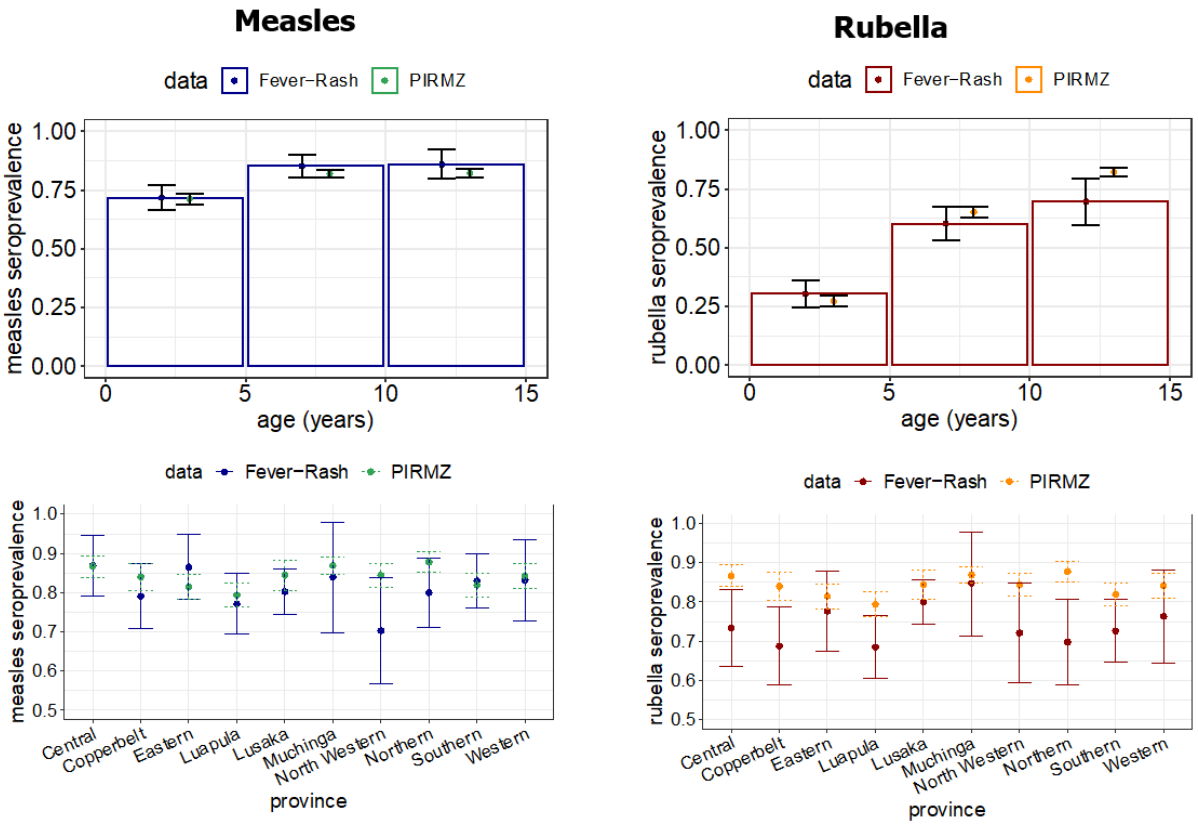
prior routine MR dose also were measles seropositive, while nine percent of children with two prior routine MR doses were measles seronegative.

4. **Fever-rash specimens collected for IgM antibodies to measles and rubella viruses**

We demonstrated the feasibility and utility of conducting a measles and rubella IgG serosurvey using specimens collected and sent to the Virology Laboratory in Lusaka, Zambia for measles and rubella IgM antibody testing as part of their fever-rash surveillance system. The advantage to using these specimens for IgG antibody testing is that they are readily available in a laboratory capable of performing enzyme immunoassays. We tested 2,052 residual samples collected between 2015-2020 for measles and rubella IgG antibodies and compared the results to age-specific seroprevalence estimates from a nationally representative serosurvey (the PIRMZ study described above). In general, we found reasonable correlation between the two estimates although there were some differences (Figure 14). We subsequently tested an additional 1,500 specimens collected from January 2021 through May 2024, providing ten years of data from the fever-rash surveillance system.

Figure 14: Age-specific and province-specific measles and rubella seroprevalence:

Population-based (PIRMZ) serosurvey vs. 2015-2016 Fever-Rash



5. **Demographic and Health Surveys**

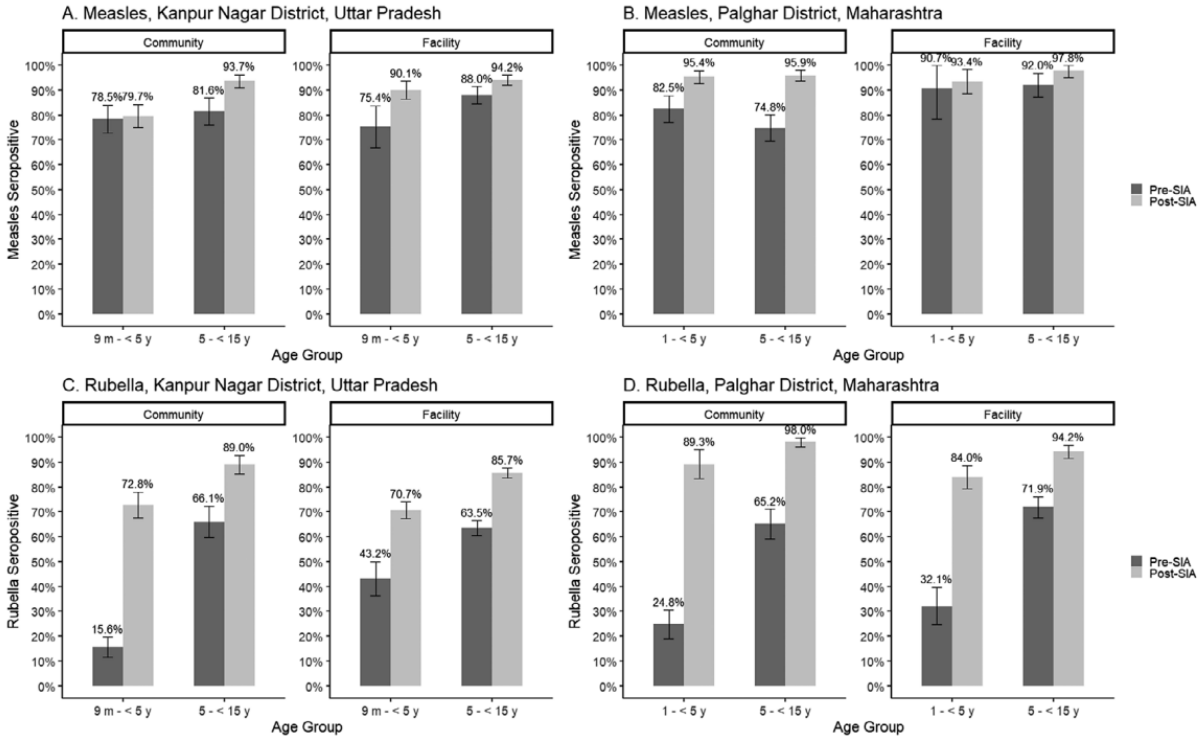
We demonstrated the feasibility and utility of nesting a measles serosurvey within the 2024 Zambia Demographic and Health Survey, leveraging this large, nationally representative survey. Specimens from 6,126 children six months to five years of age were tested for IgG antibodies to measles virus. Overall measles seroprevalence was 82% prior to assay adjustments. Analyses are in progress, but we will be able to estimate age-specific measles seroprevalence and prevalence by province and by district using a hierarchical spatial model.

Use of Residual Specimens

Stand-alone, community-based serosurveys are expensive and labor intensive and likely not sustainable. One alternative is nesting measles and rubella serosurveys in other surveys or biorepositories as described above. A second alternative is to use residual specimens collected for other purposes and stored at health facilities or laboratories. We conducted a scoping review of the use of residual blood specimens in seroprevalence studies for vaccine-preventable diseases and found that residual blood specimens are widely used, particularly during emerging disease outbreaks when rapid estimates are critical (Pilewskie, 2025). However, there were inconsistencies in how researchers analyzed and reported the use of residual specimens; we thus proposed a set of recommendations to improve the analysis, reporting, and ethical considerations of serological surveys using residual specimens.

We also conducted measles and rubella serosurveys in India and Zambia using residual specimens, with the goal of assessing potential biases in the seroprevalence estimates by comparing the results with those of parallel or concurrent community-based household serosurveys. In two districts in India, significant increases in seroprevalence were observed among children following the measles and rubella SIA using facility-based specimens but there were discrepancies between the two estimates (Prosperi et al, 2024) (Figure 15). However, we concluded that, despite challenges with representativeness and limited metadata, residual specimens can be useful in estimating seroprevalence and assessing trends through facility-based sentinel surveillance.

Figure 15: Measles and rubella seroprevalence before and after the measles-rubella supplemental immunization activity among children 9 months to < 15 years, by specimen source, India 2018-2019



Among adults in Palghar District, Maharashtra, India, age-specific measles and rubella seroprevalence estimates were similar between residual and community sources (Prosperi et al, 2024). Although measles seropositivity was slightly higher among adults attending the facilities, both facility and community measles seroprevalence estimates were 95% or higher. The similarity in measles and rubella seroprevalence estimates between the community-based and facility serosurveys for adults highlights the potential value of residual specimens to approximate community seroprevalence.

To further explore potential use cases for residual specimens, we conducted community-based surveys alongside residual specimen collection at hospitals in two districts in Zambia to see if they provided similar conclusions to programmatic questions. For measles, the goal was to determine whether seroprevalence exceeded 85% among children 1-4 years of age and 95% among children 5-15 years of age. For SARS-CoV-2, the goal was to assess whether changes to SARS-CoV-2 seroprevalence over time could be detected, since specimens were collected at the beginning of the pandemic. After accounting for sampling bias by space, gender, and inpatient samples in the facility-based serosurvey, only minor differences in measles seroprevalence remained between the two specimen sources. Regardless of specimen source, measles seroprevalence was statistically significantly less than the desired 95% in the 5-to-14-

year age groups, signaling the presence of measles immunity gaps (Figure 16). Using the facility-based specimens, we observed increasing SARS-CoV-2 seroprevalence over time consistent with increases in natural exposure or immunization (Figure 17).

Figure 16: Measles seroprevalence by age group and source of samples after adjustment, Zambia 2022

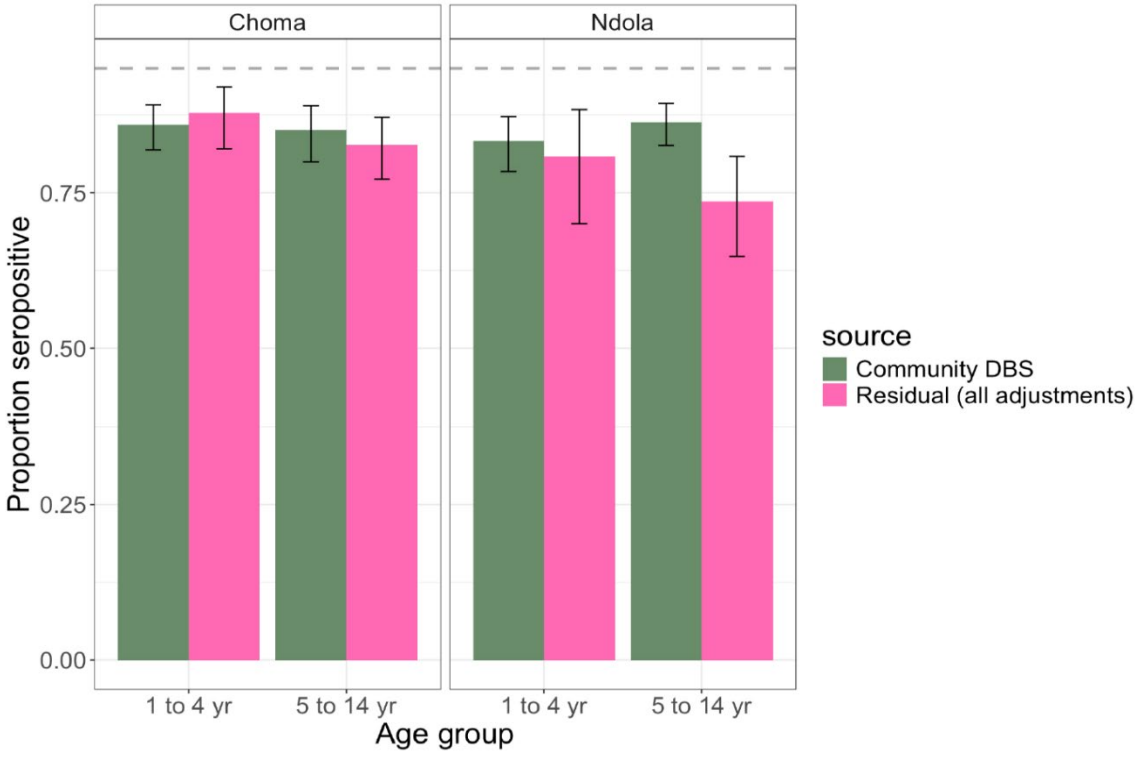
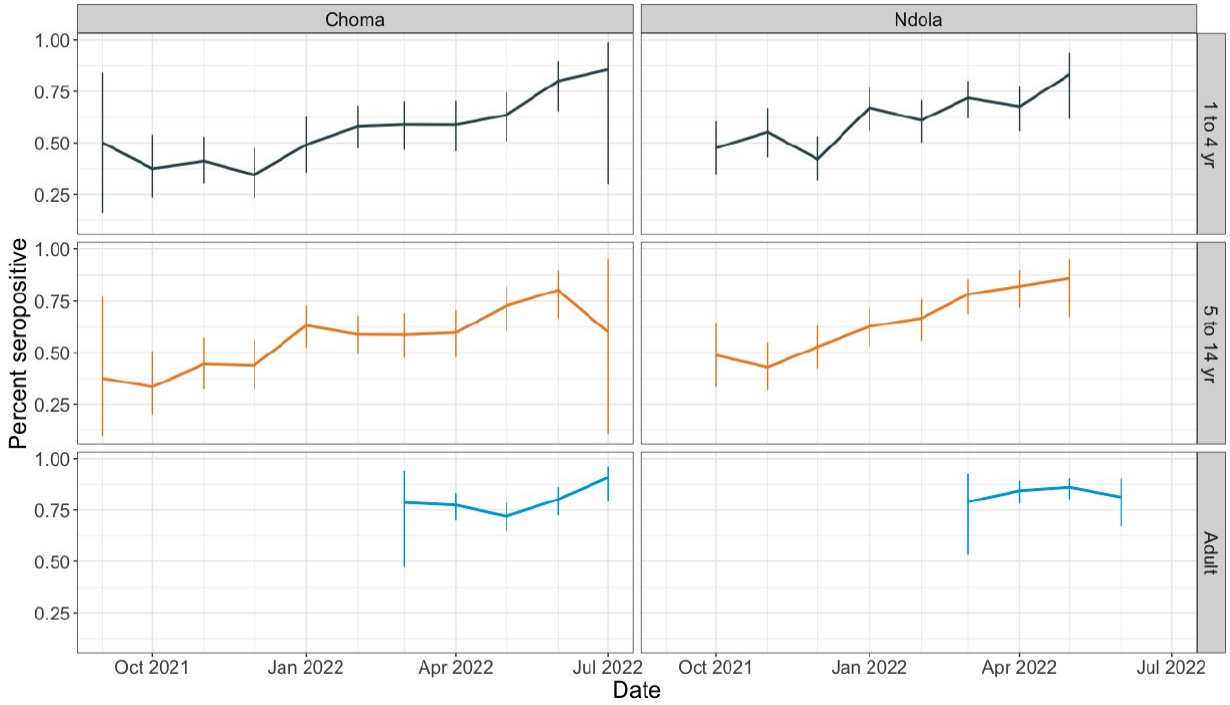


Figure 17: SARS-CoV-2 residual seroprevalence over time using residual specimens collected from district hospitals, Zambia 2022



Laboratory Assays and Threshold Adjustments

Early in the SISS project we worked with the Division of Viral Products, Office of Vaccines Research and Review, Center for Biologics Evaluation Research at the Food and Drug Administration to develop and validate a high-throughput microneutralization assay based on an RT-qPCR detection platform, resulting in a rapid, sensitive, specific, and robust assay for detecting measles neutralizing antibodies (Alvarado-Facundo et al, 2019). Current bead-based assays correlate well with neutralization assays and can be multiplexed and thus are likely to be the best assay for measles and rubella serosurveillance.

Enzyme Immunoassays (EIAs) are commonly used to estimate IgG antibody concentrations and compared to the gold-standard PRNT are quick, cost-effective, and high-throughput tests suitable for most laboratories. Measles EIA commercial kits are designed to estimate individual level seropositivity with a focus on optimizing specificity, whereas epidemiological studies are interested in estimating population level seroprevalence with a focus on optimizing sensitivity. The Dade-Behring Enzygnost measles IgG ELISA was the preferred kit until it became unavailable in 2017. Alternative kits, such as the Euroimmun measles IgG EIA, have demonstrated lower sensitivity and issues with reliability (Lutz et al, 2023). For example, we observed measles seroprevalence estimates lower than expected among children after a vaccination

campaign in India and among children with two documented MR doses in Zambia using the Euroimmun measles IgG EIA kit. To address this, we collaborated with the CDC's Viral Preventive Diseases Branch to retest a random subsample of 300 dried blood spot specimens collected during the serosurvey nested in the 2020 MR SIA using their measles multiplex bead assay. An adjustment to the measles quantitative results was calculated by comparing the MBA and EIA results and then applied to the measles quantitative results (Prosperi et al, 2025). A similar calibration study was performed to generate an adjustment for the serosurvey nested in the 2024 Zambia Demographic Health Survey. A manuscript describing the use of calibration studies and analytic techniques to adjust EIA serosurvey output is in development.

Cost of Serosurveys

We estimated the cost of measles and rubella serosurveys in different contexts and using different methods. Nesting a serosurvey within a post-campaign coverage survey in Southern Province, Zambia increased the cost by approximately one-third but was conducted as a parallel research study (Carcelen et al, 2020). The total nested serosurvey cost was US $68,558 to collect dried blood spots from 658 participants, with personnel costs the largest contributing input to overall serosurvey costs (51%), followed by transportation costs (23%) and field consumables (9%). Programmatically integrating the serosurvey within the post-campaign coverage survey should reduce these costs.

A modeling study compared the cost of vaccinating one zero-dose child under three vaccination scenarios: standard nationwide SIA, targeted subnational SIA informed by MRAT, and targeted subnational SIA informed by both MPRAT and measles seroprevalence data (Mak et al, 2024). The standard nationwide SIA was found to be the least cost-efficient strategy at 13.75 USD per zero-dose child vaccinated. Targeted SIA informed by MPRAT was the most cost-efficient at 7.63 USD per zero-dose child, assuming that routine immunization is just as effective as subnational SIAs in reaching zero-dose children. Under similar conditions, a targeted subnational SIA informed by both MPRAT and seroprevalence data resulted in 8.17 to 8.35 USD per zero-dose child vaccinated, suggesting that use of seroprevalence to inform SIA planning may not be cost prohibitive.

Ongoing work includes exploring the potential cost savings of using residual specimens rather than conducting household-based serosurveys. Preliminary analyses indicate that the cost to collect and prepare a single dried blood spot specimen from a community-based serosurvey for testing was nearly eight-times higher than that of a residual specimen ($106.72 and $13.50, respectively). Personnel costs were the largest cost category for community-based and residual specimen serosurveys (44.6% and 68.6%, respectively), followed by transportation (20.9%) and consumables (21.0%) for

the community serosurvey and training for the residual specimen serosurvey (21.7%). Our probabilistic sensitivity analysis revealed an average cost savings of $91.44 (95% confidence interval: $90.67-$92.21) when using residual specimens.

How Serosurveys Can Go Wrong

Although we argue for the feasibility and sustainability of serosurveillance, we recognize that conducting serosurveys is not simple nor straightforward and there are multiple potential pitfalls in the design, implementation, analysis, and interpretation of serosurveys. A real danger is a poorly designed or executed serosurvey that provides misleading or inaccurate information to immunization policy makers, leading to misguided policy decisions and a loss of trust in serological data. Some of the pitfalls include:

- Poor study design and biased sampling strategy
- Problems with sample collection, transport, and storage
- Problems with laboratory processing of samples
- Problems with assay quality and controls
- Assay validity (even if performed correctly)
- Improper thresholds for seropositivity
- Improper analytical methods
- Invalid interpretation of seroprevalence estimates and uncertainty bounds

Because of the many potential pitfalls, it is critical that persons with knowledge and expertise in the conduct of serosurveys be engaged. The biggest danger to this field is that poorly designed and executed serosurveys undermine confidence in their feasibility and utility.

We explicitly addressed several of these potential pitfalls through the SISS project..

<u>Who is missed in a community-based survey?</u>
In attempting to understand sampling biases in the use of residual specimens for serosurveys, we also wanted to understand biases in community-based surveys, often considered the gold standard sampling strategy, knowing that not everyone participates in such surveys. We carried out a follow-up study among individuals missed from the sampling frame of a measles and rubella serosurvey in Zambia in 2022, enrolling 672 individuals (Kostandova 2024). We assessed the potential for and impact of biases in the community-based serosurvey by i) estimating differences in characteristics of households and individuals included and excluded from the sampling frame of the serosurvey and ii) evaluating the magnitude these differences make on healthcare-

seeking behavior, vaccination coverage, and measles seroprevalence. We found that missed households were 20% smaller and 25% less likely to have children. Missed individuals resided in less wealthy households, had different distributions of sex and occupation, and were more likely to seek care at health facilities. Despite these differences, simulating a survey in which missed households were included in the sampling frame resulted in less than a 5% estimated bias in these outcomes.

Acceptability of serosurveys

Suboptimal participation in community-based serosurveys can impact the validity of the seroprevalence estimates. We used a multiple methods approach to characterize reasons for serosurvey participation in communities in Southern Province, Zambia where a measles serosurvey was conducted in 2016 (Carcelen 2023). The first phase consisted of focus group discussions and in-depth interviews with 24 data collectors who participated in a measles-rubella serosurvey in 2016. The second phase surveyed 34 caregivers at health facilities to identify barriers and facilitators to serosurvey participation. At the individual level, providing incentives was a facilitator, and some religious beliefs were described as a barrier to serosurvey participation. At the interpersonal level, family dynamics and community peer influences could help or hinder serosurvey participation. At the structural level, concerns about specimen collection, who was selected for serosurveys, and not receiving test results arose as potential barriers. The most frequently reported facilitator was provision of information about the purpose of the serosurvey (85% of respondents). The most frequently reported barrier was lack of clarity regarding use of their blood specimen (53% of respondents). For specimen collection type, caregivers consistently preferred finger prick blood collection over both venous blood draw and oral swabs.

Designing and implementing community serosurveys

Serosurveys can be logistically challenging, expensive, and have higher refusal rates than vaccine coverage surveys. We described the lessons learned through implementing nine measles and rubella household serosurveys in five districts in India —the challenges faced, the potential impact on results, and recommendations to facilitate the conduct of serosurveys (Hasan 2021). Specific lessons learned arose from challenges related to community mobilization owing to lack of cooperation in certain settings and populations, limitations of outdated census information, non-response due to refusal or unavailability during survey enumeration and enrollment, data collection issues, and specimen collection and handling issues. Although some experiences are specific to serosurveys in India, these lessons are generalizable to other household surveys, particularly vaccination coverage and serosurveys conducted in low- and middle-income settings.

Use of dried blood spots for serosurveys

Dried blood spots are often the preferred specimen type for serosurveys because of their ease of collection, transport, and storage. We optimized a protocol to elute IgG antibodies against measles and rubella viruses in four dried blood spot devices, demonstrating high concordance with paired venous sera for most devices, and conducted stability studies with various temperature and storage conditions in the laboratory and in the field using HemaSpot HF DBS devices (Kaduskar 2021). We also assessed the diagnostic accuracy of dried blood spots collected using HemaSpot HF devices against venous sera in measuring measles- and rubella-specific IgG antibodies in a household serosurvey conducted in two districts in India (Prosperi 2021). Finally, we conducted a systematic review of the diagnostic accuracy of dried blood spots for serology of vaccine-preventable diseases, recommending practical considerations to improve standardized reporting for DBS validation studies (Holroyd 2021).

Sharing results of a community-based serosurvey with participants

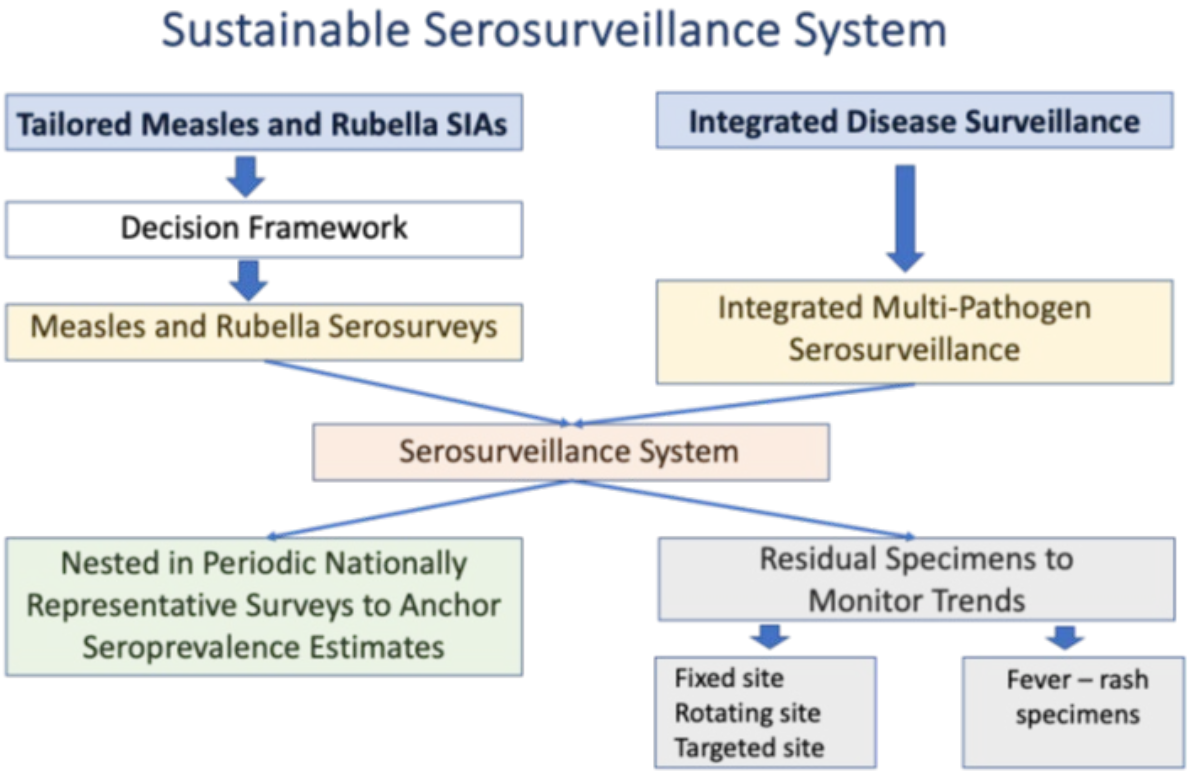
Although the results of serosurveys are not typically shared with study participants, we described the procedures, experiences, and considerations in returning results to participants in a community-based measles and rubella serosurvey in Palghar District of Maharashtra, India. Overall, 140 individuals enrolled in the survey tested seronegative for IgG antibodies to measles and/or rubella viruses; were provided the reports and informed to seek medical advice. Upon follow up by phone, 10% (14) of the 140 participants reported to have been vaccinated (Salvi 2022).

Lesson #4: A serosurveillance system should consist of a combination of sampling strategies

Although we did not have the opportunity to build a sustainable serosurveillance system in Zambia, we developed a conceptual framework for what such a system could look like, driven by a need for tailored and targeted measles and rubella immunization activities and a broader integrated disease surveillance system (Figure 18). This framework leverages our exploration of tailored measles and rubella SIAs funded by Gavi, the Vaccine Alliance and our work on multipathogen serosurveillance funded by the Gates Foundation. We envision a serosurveillance system comprised of different study designs and sampling strategies, including the use of residual specimens and sentinel sites, as well as sample registration systems and other nationally representative surveys.

We conducted a pilot project in two districts in Zambia to demonstrate the feasibility and utility of residual specimen collection embedded within tertiary-level facilities to estimate measles seroprevalence. Consultative planning meetings with hospital staff were held to understand the characteristics and operational details of the hospitals and laboratories to co-create the residual specimen collection that aligns with routine laboratory procedures and existing data systems. Although the generic flow of residual specimens was similar across facilities, the specific procedures for identifying specimens were influenced by how specimens were stored after testing, the daily number of specimens received in the laboratory, age groups of interest, and the format of specimen metadata in the laboratory. For sustainability at the hospital level, residual specimen collection should be integrated with routine lab activities rather than in parallel as a research study. A manuscript summarizing our experience designing and implementing residual specimen collection, including the lessons learned and recommendations for collecting residual specimens at health care facilities is in development.

Figure 18. Conceptual framework for a sustainable multipathogen serosurveillance system linked to tailored measles and rubella SIAs



Additional SISS studies

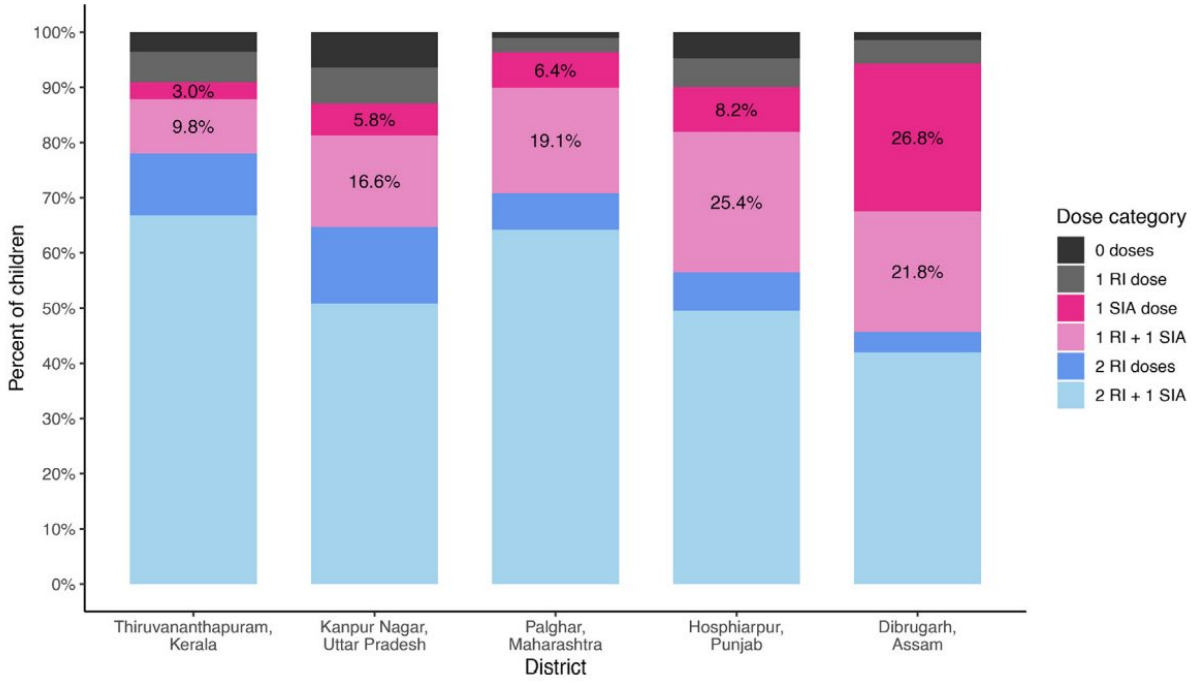
Supplemental Immunization Activities

By nesting measles and rubella serosurveys within other survey platforms, particularly post-campaign coverage surveys and supplementary immunization activities, we were able to measure the impact of measles and rubella campaigns on vaccinating zero-dose and unvaccinated children and develop new metrics for vaccine activity effectiveness and efficiency.

India

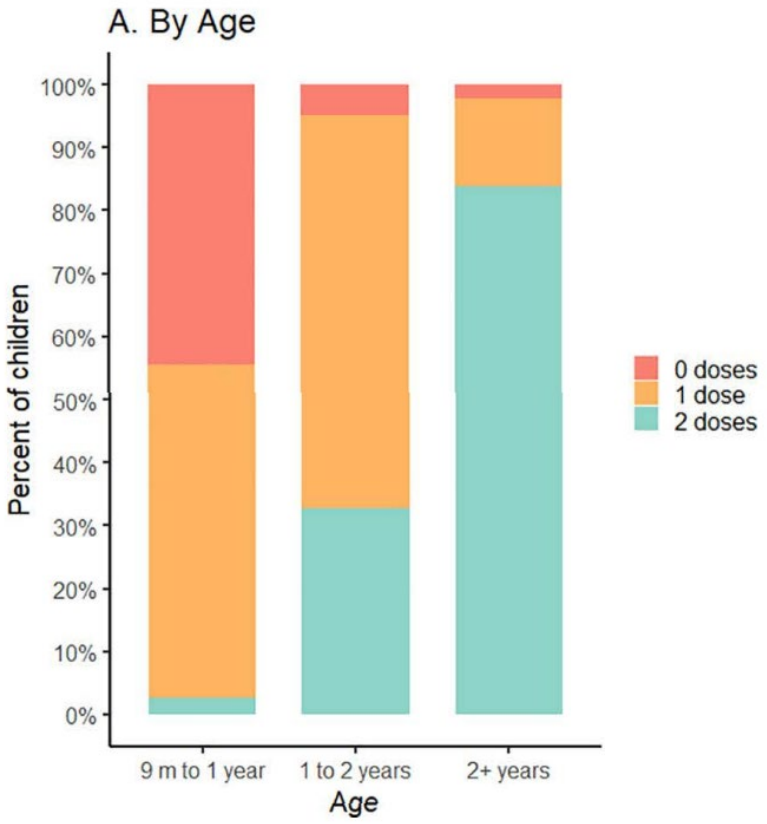
Among children aged 9 months to younger than 5 years enrolled in the post-SIA surveys in five districts in India following the 2017-2019 measles and rubella SIA (N=1,675), the percentage of children receiving a 1st or 2nd measles vaccine dose through the SIA ranged from 12.8% in Thiruvananthapuram District to 48.6% in Dibrugarh District (Prosperi et al, 2023). Although the number of zero-dose children prior to the SIA was small in most sites, the proportion reached by the SIA ranged from 45.8% in Thiruvananthapuram District to 94.9% in Dibrugarh District. We demonstrated that the measles and rubella SIA provided considerable added value in terms of measles vaccination coverage, although there was variability across districts due to differences in routine and SIA coverage (Figure 19). Campaign coverage among children aged 9 months to 5 years documented or by recall ranged from 74.2% in Kanpur Nagar District to 90.4% in Dibrugarh District, Assam, with similar coverage observed for children 5 to 15 years (Thangaraj JWV et al, 2024). Caregiver awareness of the campaign varied from 88.3% in Hoshiarpur District, Punjab to 97.6% in Dibrugarh District, Assam, although 8% of children whose caregivers were aware of the campaign were not vaccinated during the campaign. Failure to receive the campaign dose was associated with urban settings, low maternal education, and lack of school attendance although the associations varied by district.

Figure 19: Receipt of measles-containing vaccine among children 9 months to younger than 5 years of age post-SIA by site and strategy, including documented or recall evidence of vaccination, India 2018-2020



We found high measles and rubella vaccine coverage among Zambian children participating in a 2020 measles and rubella supplementary immunization activity (Prosperi et al, 2025). Among children enrolled with measles and rubella vaccination data (N=2,364), 2,214 (94%) reported at least one MR dose before the campaign (Figure 20). One hundred and fifty children (6%) received their first dose through the SIA. We estimate 4.9% (117/2364) of children would not have otherwise received MCV1 without the SIA and 20.3% (481/2364) would not have otherwise received MCV2. Thus, 1 in 4 doses were given to a child who may not have received that dose in the absence of an SIA. Monitoring SIA effectiveness, efficiency, and equity is important to understand the benefits of vaccine delivery strategies in reaching zero-dose and under-vaccinated children and may guide alternative strategies. We developed two new metrics to evaluate success of an SIA in the context of having representative data on children seen by the activity: vaccination activity effectiveness and vaccination activity efficiency. Through these metrics the 2020 MR SIA in Zambia had mixed effectiveness and efficiency.

Figure 20: Number of measles-containing vaccine doses received prior to the SIA, Choma and Ndola, Zambia, November 2020



We conducted a secondary analysis of the post-campaign coverage survey following the November 2020 MR SIA to measure the proportion of measles zero-dose and under-immunized children who were reached by the 2020 MR-SIA and identified reasons associated with persistent inequalities following the MR-SIA (Yang et al, 2023). Among the 4,640 children enrolled in the nationwide coverage survey, only 68.6% received MR during the SIA. The MR-SIA provided MR1 to 4.2% and MR2 to 6.3% of enrolled children, but 58.1% of children receiving the MR-SIA dose had received at least two prior MR doses. Twenty-eight percent of measles zero-dose children were vaccinated through the MR-SIA.

Using data from the community-based serosurveys conducted in Zambia in 2022 in parallel with the residual specimen collection, we estimated measles and rubella vaccination coverage and assessed vaccine timeliness and factors linked to delayed vaccination. While MCV1 coverage was estimated to be high and timeliness generally good, MCV2 coverage remains sub-optimal, with one in five children missing the second dose. We observed that delays in MCV1 were associated with living in rural areas and being in poorer wealth groups, highlighting persistent access barriers.

Development of new metrics for evaluating supplementary immunization activities

Traditionally, 95% coverage is the target for measuring SIA success; however, this does not take into account whether a campaign is reaching the most important populations of un- and under-vaccinated children. To better account for the ability of SIAs to reach the most vulnerable, we developed two novel metrics: *vaccine activity efficiency* (VAEC) and *vaccine activity effectiveness* (VAET) (Table 3) (Prosperi et al, 2025). V*AEC*, represents how many doses it takes the SIA to capture a single un- or under-vaccinated child who would not have otherwise received routine MR doses, adjusting for age and routine vaccination in that population. V*AET* represents the ability of the SIA to proportionately reach MR un- and under-vaccinated children in the population, as compared to a random selection of individuals in that population, in other words, does the activity do better, the same, or worse than random sampling. These metrics also allow comparisons between different vaccination activities to assess how well they reach un and under-vaccinated children.

Table 3: Vaccine activity efficiency (VAEC) and vaccine activity effectiveness (VAET)

	Vaccine Activity Efficiency	**Vaccine Activity Effectiveness**
Definition	Number of doses given to capture a single un- or under-vaccinated child	Ability of the SIA to proportionately reach un- and under-vaccinated children in the population as compared to a random sample
Calculation	The total number of administered SIA doses that went to children who otherwise would never have received each routine MR dose, divided by the total number of administered SIA doses Requires calculation of age-specific probabilities receiving each dose using hazard of children receiving routine MR dose by age from DHS data	Ratio of the odds of a child included in the vaccination activity having received a routine dose to the odds of a child in the general population having received the same routine dose
Value range	Proportion, ranging from 0 to 1 Can the be interpreted as 1 child reached per N doses given	Odds ratio (OR), ranging from 0 to infinity
Interpretation	*Lower efficiency* *VAEC* = 0.05 indicates 20 doses given to reach 1 unvaccinated child *Higher efficiency* VAEC=0.25 indicates 4 doses given to reach 1 unvaccinated child	<1 SIA is less effective at reaching un/undervaccinated children =1 SIA is reaching children at same rate as random sample >1 SIA is more effective at reaching un/undervaccinated children than a random sample

Tetanus seroprevalence estimates using residual blood specimens

We conducted a tetanus serological survey in rural Choma and urban Ndola Districts in Zambia. The serosurvey sampled residual routine clinical and diagnostic specimens from two district hospitals among children 1 year through 14 years of age. A commercial enzyme immunoassay (VaccZyme) was used to measure tetanus-specific IgG antibody concentrations. Estimated tetanus seroprevalence was 85% and 88% in Choma and Ndola Districts, respectively, with no statistically significant difference. Tetanus seropositivity decreased with increasing age and was lower among children attending outpatient wards compared to inpatient wards. Estimated seroprevalence at age 1 was 93%-97%, declining to 48%-71% by age 14 years. Using residual clinical specimens is a feasible approach for conducting tetanus serosurverys and assessing tetanus vaccination programs. High reported tetanus vaccination coverage in both districts was consistent with serological evidence of high exposure to tetanus toxin. However, declining seropositivity with age suggests vulnerability to tetanus infection among adolescents and the potential need for booster doses.

Covid-19

During the serosurvey nested in Zambia's November 2020 MR SIA, we asked parents and caregivers a set of questions to gauge their knowledge, attitudes, and beliefs about COVID-19 and COVID-19 vaccination. This survey was conducted prior to the significant increase in reported COVID-19 cases and deaths which occurred in early 2021 and before vaccines were available locally. We conducted an analysis to assess the intent to vaccinate for caregivers and children, and how these correlated with knowledge and concerns of COVID-19 disease and vaccines (Carcelen et al, 2021). Among parents bringing their children to receive a measles-rubella vaccine, we found high acceptability of COVID-19 vaccination of their children, but substantial uncertainty and hesitancy about receiving the vaccine themselves. COVID-19 vaccination hesitancy was correlated with beliefs around COVID-19 severity and risk, as well as vaccine safety and effectiveness.

We also evaluated the impact of the COVID-19 pandemic on Zambia's routine childhood immunization program in 2020 using multiple sources of data (Winter AK et al, 2023). Administrative vaccination data and Zambia's 2018 Demographic and Health Survey were used to project national disruptions to district-specific routine childhood vaccination coverage within the pandemic year 2020. Measles serosurvey data from the secondary testing of the nationally representative biorepository from the 2016 ZamPHIA study were used to predict age-specific measles seroprevalence and assess the impact of changes in vaccination coverage on measles outbreak risk in each district. We identified minor disruptions to routine administration of measles-rubella and pentavalent vaccines in 2020. The impact was mitigated in part by Zambia's Child Health Week held

in June of 2020, which helped to reach children missed during the first six months of the year. We estimated that the two-month delay in a measles-rubella vaccination campaign, originally planned for September of 2020 but conducted in November of 2020 as a result of the pandemic, had little impact on modeled district-specific measles outbreak risks.