Measles and Beyond: A Seroprevalence Study in Belgium, 2025-2026

Study protocol

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General information

This study will be coordinated by the service Epidemiology of Infectious Diseases (contacts: Laura Cornelissen, Amber Litzroth, Giulietta Stefani and Clara Mazagatos Ateca) of Sciensano. Partners of the study are volunteering laboratories from Epilabo (contact) for the collection of blood samples, UHasselt (contact: Niel Hens) for the sample size calculation, the Immune response service (contact: Isabelle Desombere) and the National Reference Center (NRC) of the concerned pathogens (NRC contact) for the coordination of laboratory testing and analyses. The study is financed by the Federal Public Services for Health.

Objective of the study

The overall aim of this study is to establish a national serum bank, which will be used for a period of five years, to estimate the age-specific prevalence of biomarkers for infectious diseases in Belgium, based on priorities set by the scientific steering committee.

As a first priority, the focus will be on estimating age-specific seroprevalence of IgG antibodies against measles in Belgium, in order to inform public health interventions and possible catch-up vaccination programs.

Additional pathogens to be analyzed over the following four years will be chosen based on the prevailing public health situation. The steering committee will prioritize the potential pathogens accordingly.

Background - justification

Serological studies

A seroprevalence study offers valuable insights into the extent of immunity or past infection within a population, providing a clearer picture of the spread of infectious diseases. By measuring the presence of pathogen-specific biomarkers, such a study can identify individuals who have been exposed to a pathogen, even if they were asymptomatic or did not seek medical attention, and hence, assess a population's immunological profile. This helps to assess the burden of disease, measure the proportion of undiagnosed infections, inform outbreak response measures and inform public health interventions. Seroprevalence data can also guide vaccine distribution strategies, estimate herd immunity levels, and monitor trends over time, making it an essential tool for both immediate disease control and long-term epidemic preparedness (1). Moreover, serological data are paramount to inform mathematical models for infectious diseases (2). Therefore, by offering a

more comprehensive understanding of disease dynamics, seroprevalence studies ultimately help shape more effective public health strategies, both for diseases with existing vaccines and those for which preventive options are still under development.

Serological data are of great value in the monitoring of vaccine-preventable diseases. Indeed, vaccination programs can be evaluated using data on vaccination coverage and disease incidence, but those data might be incomplete. Seroprevalence data provide useful additional information for the identification of under-vaccinated groups, that would benefit from additional vaccination efforts, thereby guiding public health policies (3–6).

For diseases such as hepatitis B, serological testing algorithms can distinguish susceptible persons, people with an acute infection, or those chronically infected. Serology can distinguish infection-induced from vaccine-induced immunity, thus allowing not only to monitor immunity but also infection status in the population.

Moreover, serological data are crucial for understanding the prevalence and transmission dynamics of non-vaccine-preventable diseases. Indeed, they can help to better understand the occurrence of specific diseases, such as for Lyme borreliosis, the course of their evolution, such as for hepatitis E virus (HEV), as well as the associated risk factors , such as for hantavirus (7–9).

In Belgium, previous national sero-surveys conducted in 2002, 2006 and 2013 have provided valuable insights in the disease and immune status of the Belgian population; key results were, amongst others, the identification of specific age groups remaining at risk for infection with measles, mumps, rubella, diphtheria and tetanus, despite decades-long free universal vaccination (10) and the estimation of a low prevalence of HCV seropositivity and chronic infection, providing valuable information for decision makers regarding treatment reimbursement (11).

Measles

Measles is a severe, highly contagious viral infection. The virus is transmitted by infected persons through respiratory droplets that can linger in the air for hours. Mathematical models place the basic reproductive number between 12 and 18, meaning that one infected person can lead to 12 to 18 secondary cases in a fully susceptible population (12). The rate of complications differs by setting and access to healthcare, but is about 25% in the EU. Worldwide, measles remains one of the leading causes of childhood mortality, with an estimated 140,000 children dying each year due to complications from the disease (12).

Vaccination against measles began in the 1960s and has significantly reduced its incidence in Europe. However, because of its extremely high contagiousness, WHO puts forward a vaccination coverage of ≥95% (2 documented doses of the measles-containing vaccine) to obtain herd immunity and eliminate measles (13). Despite high vaccination coverage, measles still leads to frequent outbreaks in Belgium and in the EU.

Based on seroprevalence studies, adults born in Belgium before 1970 are considered as naturally immune as the virus was omnipresent in their childhood (10,14). The MMR vaccine has been included in the childhood vaccination schedule since 1985, but coverage is thought to have been rather low initially. Therefore, to avoid outbreaks, catch-up vaccination programs are needed (15). In the Flemish Community, catch-up vaccination for all non-immune adults born after 1970 is free of charge. In contrast, in Wallonia, catch-up vaccination for adults is only partially reimbursed by the National Institute for Health and Disability Insurance (RIZIV/INAMI), which may hinder efforts to achieve universal coverage (16). While vaccination coverage for MMR1 has been ≥95% for more than 10 years, coverage for the second dose remains below the target even for the most recent birth cohorts. Current coverage stands at 82.6% nationally, with regional differences: 89% in Flanders, but only 75% in Wallonia and Brussels (17). These estimates are based on vaccination coverage surveys from 2019 and 2020, more recent information is lacking. In 2019, to increase coverage and reduce the number of susceptible children, the Belgian Superior Health Council recommended lowering the age for the second MMR dose. This was implemented in the French-speaking Community in 2020 for children aged 7-8 (instead of the previous age of 11-12) (18) and

in the Flemish Community in 2023 for children aged 9-10 (instead of the previous age of 10-11). Although there is so far no indication that coverage has declined, high-quality data on the impact of these changes on vaccination coverage are lacking. Due to GDPR issues, recruitment issues and increasing costs of door-to-door surveys, the next estimates for vaccination coverage (expected to be available in 2026) will be calculated using administrative data only. This change in methodology is expected to cause a change in estimates, and hence interpretation of longitudinal trends will be more challenging. Moreover, because of fragmented political mandates in the bilingual Brussels-Capital Region, information on a large part of the Brussels population will continue to be lacking (i.e. of pupils enrolled in Dutch-speaking schools, in international schools or living in Brussels but going to school outside Brussels, and of all persons moving to Belgium after childhood). All these challenges highlight the importance of a seroprevalence study to correctly estimate population immunity.

Belgium is committed to eliminating measles, in line with WHO's goal, which requires maintaining an annual incidence of less than 1 case per million inhabitants (equivalent to no more than 11 cases nationwide) and swiftly interrupting of chains of transmission after imported cases. After a significant reduction in reported measles cases for the period 2020-2022, partly due to COVID-19 containment measures and potential underreporting, there has been a clear and alarming increase in the number of cases in 2023 (16) and especially 2024.

Indeed, there were 67 cases in 2023, giving Belgium an incidence of 4.4 cases per million inhabitants, which is almost 10 times higher than in 2021-2022 and above the proposed elimination threshold (16). Still, WHO Regional Verification Committee has classified Belgium as 'measles eliminated' since 2020, because transmission chains were quickly interrupted, with relatively small outbreaks, typically linked to repeated importations (19). In 2024, 526 cases were reported, the highest number in over 10 years. Countries like Morocco and Romania, that are closely linked to Belgium through immigrant workers, are reporting large outbreaks which could affect Belgium through import (20). In light of these evolutions, it is crucial to continue efforts to improve vaccination coverage.

Rationale

By assessing immunity across different demographics (age and geographical area), this study aims to highlight specific populations at risk of measles outbreaks. This information will enable targeted public health interventions, such as tailored vaccination campaigns in areas or age groups with lower seroprevalence, ultimately improving overall vaccination coverage and preventing future outbreaks. Additionally, it will enable us to estimate the population's overall susceptibility to measles and monitor progress toward measles elimination goals (21).

In addition to assessing seroprevalence for measles, this study will also explore the seroprevalence of other biomarkers of infectious diseases that are of public health concern in Belgium. These may include diseases such as mumps, rubella, diphtheria, tetanus, pertussis and hepatitis, amongst others. A preliminary list of potentially included pathogens, along with the rationale for their inclusion, is provided in Appendix 1. This list is intended to support further discussions; final decisions on which pathogens will be included will be made in consultation with the steering committee. These data aid in identifying at-risk populations, guiding public health interventions, and providing a more accurate measure of disease burden, including underreported infections.

Proposed methods

Objectives

1. Establish a national serum bank to estimate the age-specific prevalence rates of biomarkers for infectious diseases in Belgium:
    a. Estimate the measles seroprevalence in the Belgian population
        i. across different age groups and geographical areas to identify potential gaps in immunity,
        ii. identify risk factors for susceptibility (age, region),
        iii. assess the relationship between immunity estimates and vaccination coverage,
        iv. compare the findings with results from previous studies to interpret trends over time.
    b. Estimate the seroprevalence of other pathogen-specific biomarkers in the Belgian population, based on the priority list determined by the steering committee, to guide public health interventions.

Study population

The target population of this study is the entire Belgian population. The study population consists of individuals living in Belgium, of all ages, who have had a blood sample analyzed at one of the laboratories participating to the sample collection during the sample collection time period.

To prevent overrepresentation of immunosuppressed individuals, specific selection criteria will be implemented if the information about them is readily available. Samples should ideally be obtained from emergency, surgical/orthopedic, and otorhinolaryngology wards, as well as from first-line settings such as general practitioner (GP) consultations and private practices. Samples from oncology or intensive care units, as well as those with available information on immunosuppressed conditions or multiple transfusions, will be excluded. Samples should be obtained from individuals living in Belgium, with a registered Belgian postal code. These criteria have also been applied in previous Belgian sero-surveys (10,22,23).

Study design and period

This will be a cross-sectional study, providing a snapshot of the seroprevalence of biomarkers of pathogens under investigation at a single point in time, for the period from mid-2025 to mid-2026.

Operational definitions

- Seroprevalence: The proportion of individuals who have disease-specific biomarkers in their blood above a certain threshold as defined in literature, the specifications of the laboratory test, or, in absence thereof, in collaboration with the NRC of the related pathogen.
- Seroprevalence for measles: The proportion of individuals who have measles-specific IgG antibodies in their serum above the threshold of >120 IU/ml (considered to offer protection as defined in literature (24,25).

Sampling method

The sampling method used will be convenience sampling. We will collect leftover samples from participating laboratories of the Belgian sentinel laboratory network as they are easy to obtain and cost-effective to collect. Research has demonstrated that convenience samples of sera can yield immunity estimates for vaccine-preventable diseases that are comparable to those obtained from randomized cluster sampling methods (26,27).

Sampling procedure

Since 1983, Sciensano has managed a network of microbiological laboratories, called the Belgian sentinel laboratory network or *Epilabo*. This network relies on the voluntary participation of clinical laboratories spread throughout the country that send weekly data to Sciensano from routine clinical tests on a number of different pathogens (28).

All laboratories (n=52) from Epilabo will be invited to participate to the collection of samples for this seroprevalence study. Depending on the number of laboratories agreeing to participate and to ensure geographical representativeness, participating laboratories will be allocated a fixed number of age-specific leftover samples to collect proportional to the population density in that area. To ensure equal proportion of males and females within the same age group, the number of samples to collect by the laboratories will equally be stratified by sex.

Sample size

The distribution of age-stratified samples will follow the simulation-based framework developed by Blaizot et al (2019), which aims to optimize the precision of seroprevalence estimates for a fixed total sample size (29). Using statistical and mathematical models, we will compare several age-based sampling structures: (i) a survey-based distribution corresponding to the age patterns observed in previous serological surveys, (ii) a population-based structure proportional to the current demographic distribution, and (iii) a uniform structure with equal sampling between age groups. These strategies will be evaluated to determine which provides the most accurate and precise seroprevalence estimates. In addition, we will compute the optimal number of samples to allocate within each age group in order to minimize uncertainty in the final estimates. We apply this method to two pathogens — measles virus and hepatitis B virus — selected for their distinct sero-epidemiological profiles and the availability of historical serological data. These pathogens provide representative models for the wider range of infections covered by the proposed seroprevalence study.

For measles, the most recent national serological survey was conducted in 2013. To evaluate the sample size needed to estimate the current seroprevalence by age, we use a cohort-based modelling approach to project immunity levels to 2025. This model incorporates historical vaccination coverage, age of vaccination and waning immunity, following methods described by Hens et al. (2015) and Abrams et al. (2014) (30,31). The aim was to obtain both overall and agegroup-specific seroprevalence estimates with a precision of +-2% (width of confidence interval max. 4%). To ensure sufficient sample size of the serumbank for pathogens other than measles, these calculations were repeated for 2 additional pathogens. The additional pathogens (CMV and Hepatitis B) were selected based on their seroprevalence patterns in the population who differ importantly from the measles seroprevalence patterns.

Appendix 5 contains the full methodology and results. With a total sample size of 3,900 samples, precision of overall national seroprevalence estimates for all 3 pathogens would be <2% (and even <1% for measles and Hepatitis B). Not all agegroup-specific estimates would have <2% precision, but all are within acceptable ranges (all <3% precision for measles). To obtain equal precision in the region-specific estimates, similar absolute number of samples would need to be collected from Flanders, Walllonia and Brussels. However, as both the population size as well as

the number of labs in Brussels is much smaller than in Flanders, this is deemed unfeasible. We therefore plan a pragmatic approach and adjust the regional allocation to reflect practical constraints and the actual number of participating laboratories, accepting wider confidence intervals in Brussels. The final distribution of samples by agegroup is then presented in Table 1.

Table 1. Samples distribution - National

Age groups	Number of samples - National
1-4yo	450
5-9yo	450
10–14yo	300
15-19yo	450
19-31yo	450
31-40yo	750
40-65yo	1050
Total	**3900**

Sample collection and analysis

Sample collection and transport

Each participating laboratory will be allocated with a specific number of samples to be collected, stratified by age (in years) and sex. Tubes and labels with a code indicating the laboratory number, age and sex of the patient's sample, will be provided by Sciensano. For each participant, we will collect (if possible) 2ml leftover serum, with a minimum of 0.5ml. For pediatric samples (children <5y of age), minimal required sample volume is 0.2ml. Each sample will be divided in two tubes by the laboratory to ensure minimal freeze-thaw cycles. Depending on the number of samples to collect, every laboratory will be provided with the corresponding number of tubes and labels as well as some additional tubes and labels, in case of errors.

Participating laboratories will be instructed to keep the samples in a refrigerator (4-8°C) for no more than 4 days following centrifugation, and to then store them at -20°C until collection by Sciensano.

Sciensano will organize two transports of samples at -20°C (on dry ice) per participating laboratory, one 6 months after the start of the collection period, and one at the end. Additional transports might be organized in consultation with Sciensano if deemed necessary by the laboratory based on its storage capacity. At Sciensano, samples will be stored at -80°C.

The serum bank will be stored and managed by Sciensano. Samples will be registered within the biobank *"Sciensano Biobank module WD 11 prospectief"* (FAMHP reference number: BB190137), according to the Sciensano standardized operational procedure for biobanks management. Samples will be stored at Sciensano for a period of 20 years for potential use in future research studies. After 20 years they will be destroyed, with the approval of the biobank professional manager.

Sample analysis

Sciensano will organize the transport to the laboratory in charge of the analyses, with samples kept at -20°C (on dry ice) during transit. Once at Sciensano, all samples will be kept at -80°C. Laboratory analysis will be performed using the appropriate testing methodology as defined in agreement with the respective National Reference Center.

Samples will be tested either at the respective National Reference Center, or, in case of availability of multiplex testing, at the Service of immune response at Sciensano.

Residual human body material samples (as defined by the "HBM law" of 19.12.2008 and for which the presumed donor consent principle applies) that were already used in the *Seroprevalentiestudie van infectieziekten in België 2013–2015* (i.e. a past study with ethical committee registration number: BE300201316922) and stored in the *Sciensano Biobank module WD 11 prospectief* (FAMHP reference: BB190137), were collected using a similar methodology. These samples will be re-tested in the current study to validate the current testing approach and enable cautious longitudinal data comparison.

Data collection

Data transfer

1. Information transferred from participating laboratories to Sciensano

Laboratories will share, on a monthly basis, anonymized information about the samples they have collected so far (unique sample code, age, sex and arrondissement) through a password-protected Excel document. Based on lab preference, data will be sent through a Secured Filed Transfer Protocol (sFTP) or a dedicated mailbox with restricted access, specifically set up for this project.

2. Information transferred from laboratory in charge of testing to Sciensano

Serology test results will be reported through spreadsheets using the unique sample codes. Data will be shared through e-mail or, if laboratory testing is done at Sciensano, by storage in a dedicated folder on the Sciensano file server with access restricted to the study team and laboratory responsible.

Data collection procedure

Collection of data at labs participating in sample collection

Participating laboratories will collect the samples requested and label them with a unique sample code. The study sample code cannot be linked back to the patient, thereby ensuring anonymity in the further process.

Participating laboratories will fill in a password-protected Excel document containing an overview of the pre-filled sample codes (Appendix 2). In the first tab of the document, the laboratory fills for every included sample date of birth, gender and postal code of the patient whose blood/serum sample was labelled with the corresponding sample code. Using this information, the built-in validation rules of the spreadsheet will flag potential duplicates and ask the lab collaborator to verify and exclude samples from the same patient. The date of birth and postal code variables will then be automatically converted into age and arrondissement on a second tab of the Excel-sheet. Only the second tab, containing sample code, age (in years), gender and arrondissement, will be transferred to Sciensano. This procedure allows to avoid multiple samples from the same patient being included (since the sample collection will run over several months) whilst at the same time ensuring only anonymous data are shared with Sciensano. Laboratories will be asked to destroy the original Excel files after the collection period has ended.

A verification will be performed by an epidemiologist of Sciensano to ensure that information on age and gender collected through the Excel document matches the sample labels.

The final serum bank and the data files will be the responsibility of Sciensano.

Laboratories will be invited to an online meeting beforehand, outlining the study methodology and sampling procedure. A recording of the meeting as well as additional written documentation will be available afterwards. A project web-page on the Sciensano website will be developed to enable participating laboratories to access useful information as well as contact details in case of further questions.

Collection of data at laboratory in charge of testing

Laboratory testing results will be communicated to the study team through an Excel file only containing unique sample codes and test results, to ensure a maximum protection of data. The file will be stored at Sciensano on a secured and confidential file server. Since the data are anonymized, it will not be possible to link serological results to specific individuals, and therefore, no individual feedback will be provided.

Data analysis

General

For each pathogen studied, we will calculate crude prevalence of seronegative, equivocal (if applicable) and seropositive results for Belgium. Since all collected samples will be tested for a certain pathogen by a single laboratory, we avoid any potential inter-laboratory variability in testing methodology. We assume that differences observed between laboratories reflect geographical variation rather than laboratory artifacts. However, since laboratories serve patients residing outside their province, the actual geographical distribution of samples may deviate from the intended stratification. To address this discrepancy, post-stratification weights will be applied to adjust for the spatial disparity between the data and the Belgian population. Moreover, seroprevalences will be adjusted for test sensitivity and specificity for the pathogen under investigation.

Statistical analysis will be done with R. Dummy tables are presented in Appendix 3. The specific analysis performed for each of the pathogens will be stored in an R file, containing explanation of the analysis. For each pathogen, these files will be stores in a secured folder at Sciensano.

Measles

For measles, measles vaccine target groups will additionally be defined as those targeted by measles vaccination (at least 1 year old and born in or after 1985) versus those not targeted (born before 1985, or not yet 1 year old at time of sampling). Within the measles vaccine-targeted group, two subgroups will be defined, with the 2-10 year olds as the ones targeted by minimal 1 dose and the 11-40 year olds as the ones targeted by 2 doses. The association between seronegativity and age group, sex, and province will be estimated using log binomial regression per measles vaccine target group. Adjusted prevalence ratios (aPR) and 95% confidence intervals (CI) will be calculated. P-values <0.05 will be considered significant.

The geometric mean titers (GMTs) for anti-measles IgG will be calculated and adjusted for clustered sampling and standardized for age, sex and population per province according to the Belgian population structure in 2025. The 95% confidence intervals (95%CI) will be estimated. Differences in GMTs between sex, region and age group will be assessed with the adjusted Wald chi-square test.

Quality assurance

To ensure the quality of the research study, a scientific steering committee was established to validate the study protocol, monitor the progress of the study, review and discuss results, decide on additional diseases to include, and address requests from external institutions for the use of the study results. The committee members are listed in Appendix 3.

All participating laboratories are BELAC-certified. The NRCs have been selected by an independent committee based on their proven expertise in the corresponding pathogen and must be accredited ISO15189 within two years from their official selection (Royal Decree of February 9th, 2011).

To ensure data quality and to support participating laboratories, an online meeting will be held in advance to explain the study methodology and sampling process. A recording, written documentation, and a dedicated project webpage on the Sciensano website will be made available for ongoing reference. The webpage will also include contact details, including a project-specific email address, for any further inquiries.

Bias and limitations

This study includes potential bias and limitations.

First, selection bias might have been introduced by the use of convenience sampling. Indeed, if certain population groups (e.g., lower socioeconomic classes, populations with limited/restricted access to health care) are underrepresented, this may skew results. Furthermore, no data on individual vaccination status or specific risk factors will be available, limiting the scope of possible analyses. In addition, information on socioeconomic status (SES) is not collected, and, due to the anonymous nature of the sampling, no detailed geographical data (e.g., at the statistical sector level) can be obtained that could otherwise serve as a proxy for SES. However, research has demonstrated that convenience samples of sera can yield immunity estimates for vaccine-preventable diseases that are comparable to those obtained from randomized cluster sampling methods (26,27). This limitation will nonetheless be cautiously taken into consideration when interpreting the data.

Second, serological testing has some intrinsic limitations. False positives or false negatives can occur due to the nature of serological tests. We will use validated assays to minimize error and perform confirmatory tests where necessary. Moreover, distinction between natural and vaccine-induced immunity (e.g. for measles) might not always be possible. Finally, interpretation of serological results and their clinical significance is not always clear. However, even if a cut-off or correlate of protection has not been established in the published literature, comparison of levels of biomarkers across different groups can yield valuable information.

Protection of human subjects

Vulnerable populations

The targeted population of this study is the entire Belgian population. The study population consists of individuals from Belgium, of all ages, who have had blood samples analyzed at one of the participating laboratories within the Belgian sentinel laboratory network. While vulnerable populations are included within the Belgian population and therefore might be included in the study population, vulnerable populations are not specifically targeted in this study.

Data collected are anonymous as only unique sample code, age, sex and arrondissement will be transferred. Hence, no identification will be possible.

Risks

As only leftover samples of blood/serum will be used, there will not be any direct risk/consequences on the health of the population under study.

The risk of sharing private or sensitive information about the study participants will be minimal. All data collected will be anonymous. All data will be stored at Sciensano on a secured and confidential file server. Access will be restricted to the study team, only through Sciensano-laptops.

Identifying information is not collected, therefore participating individuals cannot be informed about the results of the serological test (unlinked anonymous).

Benefits

This seroprevalence study, through its results and conclusion, will guide public health interventions and vaccination programs in Belgium. Hence, it will be beneficial for the Belgian community.

Confidentiality

See "Data collection – *Data collection procedures*".

Biological specimen

See "Samples collection and analysis".

Informed consent

This seroprevalence study uses leftover samples (blood samples are taken for diagnostic purposes without any additional amount of blood being taken). As by the Law of 19 December 2008 relating to *the obtaining and use of human body material intended for human medical applications or for scientific research purposes* (20§2)[1] and that data are provided unlinked anonymous, no personal informed consent is needed.

Ethical committee clearance

The study protocol will be submitted for approval to the Ethical committee of the University of Antwerp.

Practical considerations

Field work

Data collection will be done by participating laboratories. Training and information will be given as explained in the "*data collection procedure*" section. All logistical aspects will be taken care of by Sciensano. Data analysis and reporting will be done by the Service of *Epidemiology of Infectious Diseases* at Sciensano.

Timeline

The timetable of activities and milestones is presented below.

- Milestone 1: study protocol validated by the scientific steering committee.
- Milestone 2: List of participating laboratories available.
- Milestone 3: Approval of the Ethical Committee and the Commission to Protect the Personal Privacy.
- Milestone 4: All samples collected.
- Milestone 5: All samples tested for measles.
- Milestone 6: Data analyzed
- Milestone 7: Report on the measles results validated
- Milestone 8: Decision taken on following pathogen to be studied on the serum bank (annually).

[1] Loi du 19/12/2008 relative a l'obtention et a l'utilisation de materiel corporel humain destine a des applications medicales humaines ou a des fins de recherche scientifique - Wet van 19/12/2008 inzake het verkrijgen en het gebruik van menselijk lichaamsmateriaal met het oog op de geneeskundige toepassing op de mens of het wetenschappelijk onderzoek

	2025												2026											
	Jan	Feb	March	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	March	April	May	June	July	Aug	Sep	Oct	Nov	Dec
Protocol				M1																				
Laboratory recruitment				M2																				
Ethical procedures							M3																	
Samples collection																		M4						
Testing measles																					M5			
Data cleaning & analysis measles																						M6		
Report measles																								M7
Decision following pathogen													M8											

Expected benefits

Output

The primary outcome of this seroprevalence study is the creation of a national serum bank. Over the following years (2026–2030), this resource will support the development of a series of manuscripts estimating the seroprevalence of pathogen-specific biomarkers in the Belgian population. The selection of pathogens will be guided by the priority list set by the steering committee, aiming to inform public health interventions. The first manuscript will focus on measles.

Outcome

The primary benefit of this study will be a clearer understanding of the current measles immunity levels in Belgium, helping public health officials to:

- Identify populations at high risk of measles outbreaks and target vaccination campaigns more effectively;
- Inform policy decisions regarding vaccination strategies;
- Improve public health messaging on the importance of vaccination and the risks of measles;
- The results will also contribute to global knowledge of measles seroprevalence and vaccination effectiveness, potentially informing similar studies in other countries;

In a second phase, this seroprevalence study will enable us to acquire similar knowledge about immunity levels for other pathogens, which have yet to be determined by the steering committee.

Appendices

Appendix 1

Pathogen	Biomarker	Age group	Rationale	Similar studies
Hep B	• anti-HBs • anti-HBc • HBsAg	• Adults • Children <5y	• To assess burden of hepatitis B in Belgium. Chronic HBV has important health implications and, despite existence of effective preventive measures, it incidence seems NOT to be decreasing (cfr Hep report). • To monitor our progress towards the WHO elimination goals of 2030 • To evaluate the impact of HBV vaccination since introduction in routine immunization • To compare results with other studies.	2020, Belgium
Hep C	• Anti-HCV • HCV RNA	• Adults	• To assess burden of hepatitis C in Belgium, and monitor our progress towards the WHO elimination goals of 2030. • To assess the impact of HCV treatment on prevalence of active HCV infection in Belgium, by comparing the results with the previous seroprevalence study you did (before extension of treatment criteria) • To estimate the proportion of people ever in contact with HCV	2013-2015, Belgium
Hep E	• Anti-HEV	• All ages	• Assess real burden and transmission dynamics of HEV which is the most common cause of acute viral hepatitis world-wide and can lead to severe outcomes. • There is no solid surveillance in place for HEV (very few samples sent to NRC), but it seems that there is a rising trend both in Belgium and in Europe. It is currently unclear to what extend this is due to increased detection or a true increase. • Compare results with previous study • Better understanding of the epidemiology of HEV in Belgium may allow for planning of more actions in the food industry.	2006-2014, Belgium
Influenza A	• anti-H5 (HI) • anti-H7 (HI) • anti-H9 (HI)	• All ages	• Assess the prevalence of asymptomatic cases (undetected infections) resulting from prior zoonotic exposure to animal influenza viruses in Belgium to provide complementary evidence for risk assessments and public health preparedness measures. • Evaluate the zoonotic potential of specific virus subtypes across different age groups.	

			• Assess the severity and fatality rates, acknowledging potential overestimation due to undetected cases. • Determine immunity levels in the general population based on seroprevalence data.	
Measles	• Anti-measles IgG	• All ages	• Increasing incidence of measles, important public health problem • WHO elimination goals • Unclear population immunity in children as last survey in infants from 2019, important methodological changes in planned vaccine coverage studies • Unclear population immunity in adolescents in Brussels, as not all are captured by current vaccine coverage studies • Unclear population immunity in adults due to lack of historical records and potential of waning immunity	Belgium 2002, 2006, 2013
Mumps	• Anti-mumps IgG	• All ages	• Waning immunity and outbreaks in adolescents, unclear optimal timing of MMR vaccine in schedule • Compare with previous studies • Integrate with findings on measles and rubella as part of 1 combination vaccine	
Rubella	• Anti-rubella IgG	• All ages • women of reproductive age	• Congenital rubella = WHO target for elimination • Unclear optimal timing of MMR vaccine in vaccination schedule	
Pertussis	• anti-PT IgG	• All ages	• Recent important increases in incidence; unclear which proportion reflects an increased reporting versus a true rise in cases • Waning immunity over time, unclear optimal timing of booster vaccinations • Compare findings with previous studies to understand evolution over time	
Tetanus	• anti-TT IgG	• All ages, incl. 65+	• Classic marker of vaccination coverage • Unclear population immunity in elderly	
Diphtheria	• Anti-DT	•	• 2022-2023 outbreak in Belgium including 1 fatal case (16y) • Emergence of macrolide-resistant C. Diphtheria • C. Ulcerans infections in elderly	Risk groups Belgium 2014-2016 Belgium, 2012
CMV	• anti-CMV IgG	• all ages • women reproductive age	• Ph3 clinical trials vaccine underway • cCMV big public health impact	Belgium 2002,2006

Appendix 2

Data collection sheet for laboratories (only seen by laboratories)

Laboratory Nb 1

Unique code	Sample date	Date of birth	Gender	Postcode
Laboratory/gender/age				
….				

Data collection sheet delivered from laboratories to Sciensano

Laboratory Nb 1

Unique code	Sample date	Age	Gender	Arrondissement
Laboratory/gender/age				
….				

Appendix 3

Dummy tables

1. Study population characteristics

Variable	Category	N (%) – Study population	N (%) – Belgian population
Age	1-4 yo		
	5-9 yo		
	10-14 yo		
	15-19 yo		
	20-29 yo		
	30-39 yo		
	40-49 yo		
	50-59 yo		
	60-69 yo		
	≥ 70 yo		
Sex	Female		
	Male		
Provinces	Antwerp		
	West Flanders		
	East Flanders		
	Limburg		
	Flemish Brabant		
	Walloon Brabant		
	Hainaut		
	Namur		
	Liege		
	Luxembourg		
	Brussels		

2. Seroprevalence by Demographic Characteristics – one table per pathogen

Characteristic	n tested	n seropositive	% Seropositive (95% CI)
Age group (years)			
Sex			
Region/provinces			

3. Comparison of Seroprevalence over time (with previous seroprevalence study) – one table per pathogen

Birth cohort	Time point	n tested	n seropositive	% Seropositive (95% CI)
Targeted by measles vaccination	Study 2013/2015			
	Study 2025/2026			
Not targeted by measles vaccination	Study 2013/2015			
	Study 2025/2026			

4. Log binomial regression analysis (Prevalence Ratios) – one per pathogen

Variable	Adjusted Prevalence Ratios (95% CI)	p-value
Age		
Sex		
Region/provinces		

Appendix 4

Scientific steering committee – Composition

Function/entity	Expertise	Number of representatives
FOD Volksgezondheid (federal authorities)	Public Health	1
DepZorg (regional authorities)	Public Health	1
AViQ (regional authorities)	Public Health	1
Vivalis (regional authorities)	Public Health	1
ONE (regional entity)	Public Health (vaccination)	1
University of Hasselt – University of Antwerpen	Biostatistics	2
University of Gent (UGent) – University Hospital of Gent (UZ Gent)	Infectious diseases (adults)	1
University of Brussels (ULB) - University Hospital of St Pierre	Infectious diseases (adults)	1
Catholic University of Louvain-la-Neuve (UCL) – University Hospital of Dinant Godinne UCL Namur	Infectious diseases (children)	1
University Hospital of Brussel (UZ Brussel)	Infectious diseases (children)	1
University of Brussels (ULB) – Plotkin institute	Immunology	1
Sciensano	Public Health Biostatistics Microbiology	3
Representatives of the Epilabo network	Clinical microbiology	7

Scientific steering committee – Role

Provide input and feedback on main aspects as study, such as

- Choice of pathogens and priority research questions
- Methodology aspects
- Any technical issues that might arise
- Interpretation of results and preparation of communication and publication
- If applicable, evaluate external demands on the use of the serumbank

To be able to fulfill this role, it is expected that the selected committee members will participate to ca. 1 meeting / year in addition to provide ad-hoc written input (through e-mail) if required.

Appendix 5

Allocation of serological samples

The distribution of age-stratified samples follows the simulation-based framework developed by Blaizot et al. (2019) [1]. More specifically, the procedure aims to optimize the precision with respect to seroprevalence estimators (based on single cross-sectional serological survey data) for a fixed total sample size (i.e., number of blood serum samples collected in a random fashion). Using statistical and mathematical models, we compare several age-based sampling structures: (i) a survey-based age distribution corresponding to the observed age patterns in previous serological surveys, (ii) a population-based structure proportional to the current demographic age distribution, and (iii) a uniform age structure with equal sampling probabilities across different age groups. These strategies will be evaluated to determine which approach provides the most accurate and precise seroprevalence estimates. In addition, we compute the optimal number of samples to allocate within each age group in order to minimise uncertainty in the final estimates.We apply this method to three pathogens—measles virus, hepatitis B virus and cytomegalovirus—selected for their distinct sero-epidemiological profiles and the availability of historical serological data. These pathogens provide representative models for the wider range of infections covered by the multiplex test.

For measles, the most recent national serological survey was conducted in 2013. To estimate the current seroprevalence by age, we used a cohort-based modelling approach to project immunity levels to 2025. This model incorporates historical vaccination coverage information, age at which vaccination occurred and waning of humoral immunity, following methods described by Hens et al. (2015) and Abrams et al. (2016) (see [2], [3], respectively).

1. Materials and methods

1.1. Data

1.1.1. Serological data

Serological testing for the presence of measles antibodies was conducted on large representative national serum banks in Belgium [4]. Serum samples were collected in 2013, from residual blood samples used for routine laboratory testing.

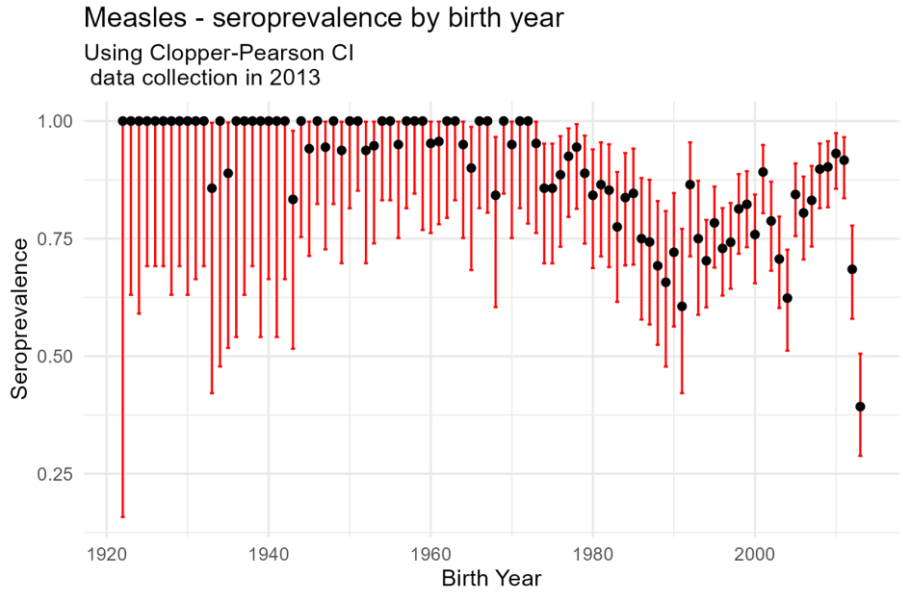

Figure 1. Seroprevalence of measles by birth year in Belgium (2013). The black points represent the empirical seroprevalence estimates for each birth year, while the red vertical bars indicate the 95% Clopper-Pearson confidence intervals.

1.1.2. Vaccination coverage

Vaccination coverage estimates for Belgium for both recommended measles-mumps-rubella (MMR) vaccine doses were taken from [5], [6], [7], [8], [9], [10], [11], [12], [13]. Detailed coverage information can be found in Hens et al. (2016) [2]. Most recent vaccination coverage estimates are updated annually by Sciensano using population-weighted estimates, but new survey data are not collected every year (Table 1, [14]). For years without reported coverages between 2001 and 2025, we applied a linear interpolation approach based on the available data in order to generate a continuous perspective on annual coverage estimates for MMR1 and MMR2 (Figure S1). When regional data were missing, national estimates were used as a proxy.

Year	Region	MMR1	MMR2
2019	Wallonia	96,50%	
2019	Brussel	94,80%	
2019	National	96%	82%
2020	Flanders	96,10%	89,20%
2020	National	96%	83%
2021	Wallonia		73,00%
2021	National	96%	83%
2022	National	96%	83%
2023	National	96%	82%

Table 1. Vaccination coverage estimates for the period 2019-2023 [14].

1.2. Cohort model to project seroprevalence to 2025

The model builds on the multicohort framework introduced by Abrams et al. (2014) in the context of mumps and further exemplified for measles by Hens et al. (2015) [2], [3], which uses historical serological data and vaccination coverage information to estimate age-specific immunity profiles in later years. In our case, we adapted the aforementioned approach to project measles seroprevalence from the 2013 national survey to 2025. This was done in two steps: (i) modelling the age-specific seroprevalence data from 2013 using a generalised additive model (GAM), and (ii) projecting the immunity profile to 2025 using a dynamic cohort model that accounts for birth year, vaccination coverage (MMR1 and MMR2), and waning immunity over time.

1.2.1. A model for the serological data in 2013

We used a GAM for binary outcome data (with binary response variable indicated seropositivity status) to estimate age-specific measles seroprevalence in Belgium based on the 2013 serological survey data. A complementary log-log link function was used to link the seroprevalence to age. The model included a smooth function of age to capture a potential non-linear trend in humoral immunity. Therefore, the GAM [15] can be formulated as follows:

$$Y|a \sim B(\pi(a))$$
$$\text{cloglog}(\pi(a)) = f(a),$$

where f(.) represents a smooth function for age which is constructed based on cubic spline basis functions.

The resulting age-specific seroprevalence profile served as the baseline immunity profile (distribution) (i.e., allowing us to describe the age-specific susceptibility to measles in the population in 2013) for the dynamic cohort model projecting to 2025.

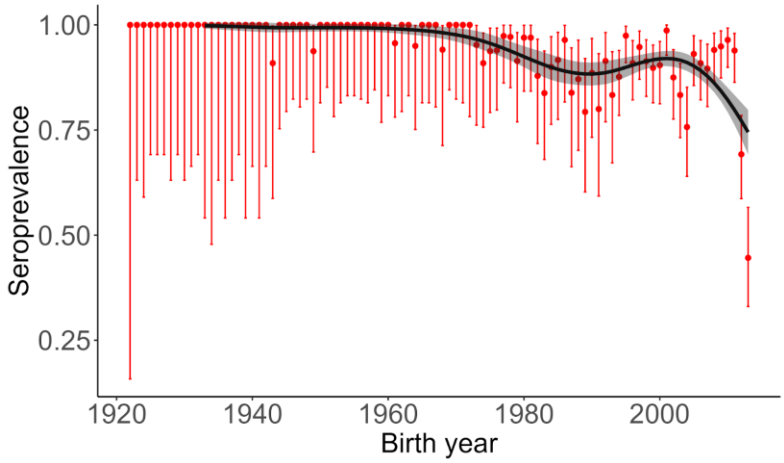

Figure 2. Observed seroprevalence and smoothed estimates by region in 2013.
Age-specific seroprevalence (red dots with binomial confidence intervals) and fitted GAM estimates (black line) based on 2013 serological data in Belgium. The generalised additive model was fitted using a complementary log-log link with a smooth term for age.

1.2.2. Age-dependent susceptibility profiles in 2025

We denote sb(a)as the proportion of susceptible individuals of age *a* and birth year *b*, such that calendar time is t=b+a. The multicohort model, adapted from Hens et al. (2015) and Abrams et al. (2014) [2], [3], was used to project age-specific seroprevalence to the year 2025.

We excluded those under one year of age, as maternal antibodies generally disappear in the first few months of life (3-9 months), so immunity of children beyond this age is determined by vaccination coverage. The age-specific immunity profile at baseline (2013) was estimated using a GAM (see Section 1.2.1).

For individuals born before 1984, immunity was assumed to result from natural infection; hence, we used the predicted seroprevalence from the GAM model (based on 2013 seroprevalence data) without applying waning of humoral immunity. For those born between 1984 and 2000 (who were vaccinated during the early years of MMR implementation), observed seroprevalence in 2013 was used as the baseline, and waning of immunity was applied from 2013 to 2025. For cohorts born after 2000, immunity was modeled entirely based on (extrapolated) vaccination coverage information and accommodating waning of humoral immunity, without using serological data directly. We assumed no natural infection occurred between 2013 and 2025, consistent with the absence of major measles outbreaks in Belgium during this period.

To project immunity forward to 2025, we applied a cohort simulation model using the following age-specific rules for the probability of being seropositive:

$$1\text{-sb}(a)=e\text{-}1(a\text{-}1)1, \quad \text{if } 1a12$$

$$1\text{-sb}(a)=e\text{-}2(a\text{-}12)2, \quad \text{if } 12a$$

Here, ρ is the seroconversion probability following vaccination, 1 and 2 are the waning rates after MMR1 and MMR2, respectively. Values of these parameters were extracted from Schenk et al. (2020) [16] (=0.96; 1=0.008; 2=0.009). The quantities 1 and 2 represent the age- and cohort-specific coverage of the first and second MMR doses.

1.3. Age-based sampling structures

To compare the impact of different sampling strategies on the precision of seroprevalence estimates, we considered three age-based sampling structures, as described in Blaizot et al. (2019) [1]. First, a population-based structure, proportional to the demographic distribution from the 2021 national census (Figure S2). Second, a survey-based structure, reflecting the age distribution observed in the 2013 Belgian serological survey, in which children and adolescents were oversampled (Figure S3). Third, a uniform structure, assuming equal sampling across all ages. These three structures were used to simulate synthetic datasets under varying total sample sizes, allowing comparison of the resulting precision across strategies.

1.4. Simulation framework for sample allocation and precision

To assess the precision obtained under different age-based sampling structures, we simulated synthetic datasets by drawing binomial outcomes from the projected age-specific seroprevalence profile (Section 1.2). For each individual in the simulated sample, seropositivity status was assigned using a Bernoulli distribution with probability equal to the predicted seroprevalence at their age. This process was repeated independently 500 times for each sampling scenario. The resulting datasets were used to estimate the seroprevalence in each of the eight predefined age groups. Following the approach described by Blaizot et al. (2019) [1], precision was defined as half of the width of the 95% simulation-based confidence interval across the 500 replicates (simulation runs), calculated for each age group and overall. This framework enabled direct comparison of the expected estimation precision under each sampling design.

1.5. Optimisation of age-specific sample allocation

To identify the best way to distribute 3,000 samples across age groups, we tested a wide range of candidate allocations. Each allocation defined the proportion of samples assigned to seven age groups (1–2, 2–6, 6–12, 12–19, 19–31, 31–40, and 40–65 years), using proportions that varied by steps of 5% and always summed to 100%.

For each candidate allocation, we simulated 500 synthetic datasets, assigned serostatus based on the predicted seroprevalence by age, and fitted a generalized additive model (GAM) to estimate the overall seroprevalence. We then computed the precision of each scenario as half the width of the 95% confidence interval across simulations. The allocation that achieved the best precision was selected for each pathogen and region.

2. Results

2.1. Allocation of samples for measles

We conducted the analysis at the national level. As described in the previous section, we estimated the seroprevalence of individuals in 2025 thanks to a multicohort model based on 2013 serological data (Figure 3).

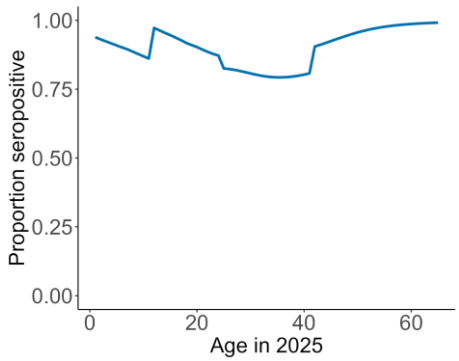

Figure 3. Estimated seroprevalence profiles in 2025 in Belgium (measles). Estimates were derived from the multicohort model that incorporates vaccination coverage, waning of vaccine-induced immunity, and baseline seroprevalence from 2013.

Figure 4 presents the expected overall seroprevalence and associated uncertainty (95% simulation interval) for each region across increasing sample sizes (1000, 2000, 3000, 4500 individuals), under three age-based sampling structures: survey-based (sero), population-based (pop), and uniform (unif) sampling structures. The differences between sampling structures were generally modest for overall seroprevalence, although the population-based structure often yielded slightly better precision due to the higher number of samples among young people.

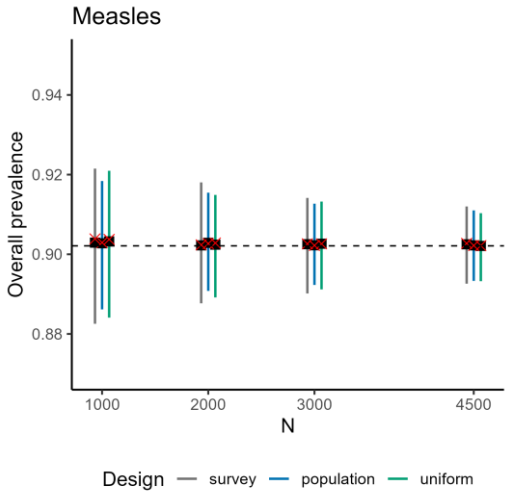

Figure 4. Measles serological data: mean, median and 95% confidence interval for the overall seroprevalence over 500 simulations as a function of the total number of sampled individuals (N).

2.2. Allocation of samples for Hepatitis B

We estimated age-specific hepatitis B seroprevalence using data from the 1993 Belgian serological survey. Figure S4 shows the observed seroprevalence along with smoothed estimates obtained from a generalized additive model (GAM) using a complementary log-log link and a smooth function of age. These estimates were used as input to simulate the precision of overall seroprevalence under varying sample sizes and sampling structures.

Figure 6 presents the resulting estimates of overall seroprevalence (mean, median, and 95% confidence intervals) based on 500 simulations across four total sample sizes (1,000 to 9,000) and three age-based sampling strategies: survey-based, population-based, and uniform. Only marginal differences are observed between the three sampling strategies. For a sample size of 1,000, the population-based design yielded the best precision, whereas for 3,000 samples, the survey-based design performed slightly better. A summary of the precision values obtained for each scenario is available in Supplementary Table S2.

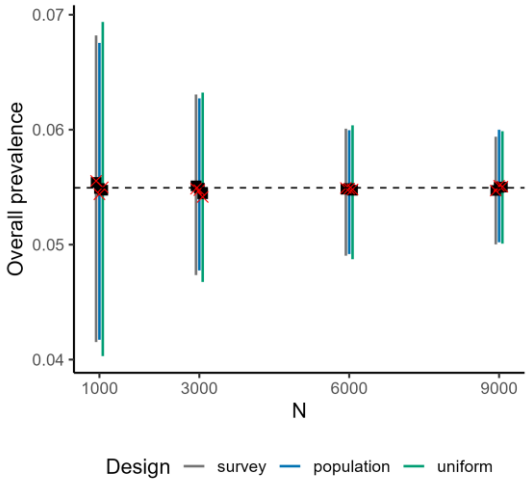

Figure 6. Hepatitis B serological data: mean, median and 95% confidence interval for the overall seroprevalence over 500 simulations as a function of the total number of sampled individuals.

2.3. Allocation of samples for CMV

We estimated age-specific cytomegalovirus (CMV) seroprevalence using data from the 2011 Belgian serological survey. Figure S5 displays the observed seroprevalence and the fitted estimates obtained from a GAM. These model-based estimates were used to simulate the precision of overall CMV seroprevalence under various sample sizes and sampling strategies.

Figure 9 summarizes the results from 500 simulations, showing the mean, median, and 95% confidence intervals of overall seroprevalence across four total sample sizes (1,000 to 9,000) and three sampling structures (survey-based, population-based, and uniform). The three sampling designs yielded very similar results. The population-based structure showed slightly better precision at 1,000 samples, whereas the survey-based design performed best at 3,000 samples.

Based on these results, we recommend allocating at least 1,000 samples per region for CMV, corresponding to 3,000 samples nationally. With this total sample size, the estimated precision for the overall CMV seroprevalence at the national level ranges between ±2.8% and ±3.4%, depending on the sampling strategy. Although this is sufficient for generating national estimates, it does not provide acceptable precision for regional-level estimates. To ensure adequate precision at the regional scale (e.g., ±2%), a higher number of samples per region would be required. Detailed precision values for each sample size and sampling design are provided in Supplementary Table S3.

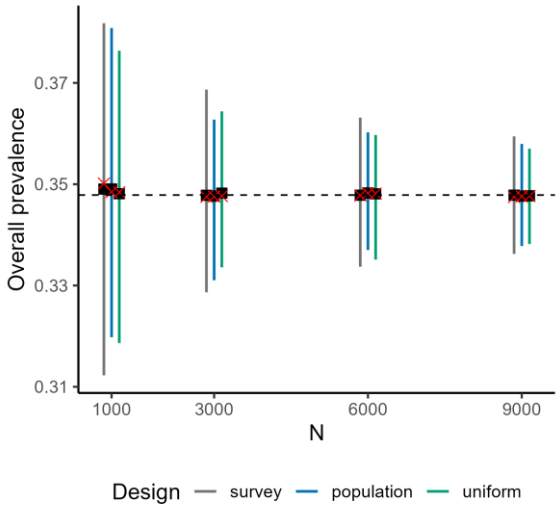

Figure 9. CMV serological data: mean, median and 95% confidence interval for the overall seroprevalence over 500 simulations as a function of the total number of sampled individuals.

3. Conclusions

We recommend allocating 3,900 samples nationally, corresponding to approximately 1,300 samples per region (Flanders, Brussels, and Wallonia), to ensure acceptable precision (below ±2%) for estimating the overall seroprevalence of measles, hepatitis B, and CMV at the regional level.

An optimisation algorithm was used to test multiple age-based sampling distributions and identify the most efficient allocation for each pathogen. Table 2 summarises the optimal proportions of samples by age group, which differ slightly across pathogens. These proportions were then translated into absolute sample counts (Table 3), assuming 3,000 samples per pathogen at the national level.

To ensure sufficient precision for all pathogens, we selected the maximum number of samples per age group across the three pathogens as the final recommendation. This results in a total of 3,900 samples nationally, which we divide equally across the three regions, yielding 1,300 samples per region.

Table 4 reports the expected precision of the overall seroprevalence estimates under this allocation. At the national level (3,900 samples per pathogen), the expected precision is 0.82% for measles, 0.85% for hepatitis B, and 1.69% for CMV. At the regional level (1,300 samples per region), expected precision decreases slightly to 1.69% for measles, 1.45% for hepatitis B, and 2.64% for CMV, still within acceptable bounds.

Age group	Measles	HepB	CMV
1-4yo	5%	5%	15%
5-9yo	15%	15%	5%
10–14yo	5%	10%	10%
15-19yo	15%	5%	10%
19-31yo	15%	10%	10%
31-40yo	20%	25%	15%
40-65yo	25%	30%	35%
Precision	0,88%	0,74%	1,52%

Table 2. Optimal proportion of samples per age group for measles, hepatitis B, and CMV based on national-level simulations.

	Measles	HepB	CMV	National	Regional
1-4yo	150	150	**450**	450	150
5-9yo	**450**	**450**	150	450	150
10–14yo	150	**300**	**300**	300	100
15-19yo	**450**	150	300	450	150
19-31yo	**450**	300	300	450	150
31-40yo	600	**750**	450	750	250
40-65yo	750	900	**1050**	1050	350
Total	3000	3000	3000	3900	1300

Table 3. Absolute number of samples per age group, assuming 3,000 samples per pathogen. The final allocation (bold values) corresponds to the highest required count across pathogens, resulting in a total of 3,900 samples nationally (or approximately 1,300 per region).

	Measles	HepB	CMV
National	0,82%	0,85%	1,69%
Regional	1,69%	1,45%	2,64%

Table 4. Expected precision (half-width of the 95% simulation-based confidence interval) for overall seroprevalence estimates under the final allocation. National precision is based on 3,900 samples; regional precision is based on 1,300 samples per region.

		Precision by age - national allocation		
Age group	Allocation	Measles	HepB	CMV
1-4yo	450	2,25%	0,50%	4,23%
5-9yo	450	2,61%	0,53%	3,25%
10–14yo	300	1,76%	0,63%	3,50%
15-19yo	450	1,95%	0,73%	3,14%
19-31yo	450	2,88%	1,20%	2,94%
31-40yo	750	2,40%	1,51%	2,78%
40-65yo	1050	1,25%	1,42%	3,02%

Table 5. Expected precision (half-width of the 95% simulation-based confidence interval) for prevalence estimates of each age group under the final allocation.

4. References of Appendix 5

[1] S. Blaizot, S. A. Herzog, S. Abrams, H. Theeten, A. Litzroth, et N. Hens, « Sample size calculation for estimating key epidemiological parameters using serological data and mathematical modelling », *BMC Med. Res. Methodol.*, vol. 19, nº 1, p. 51, déc. 2019, doi: 10.1186/s12874-019-0692-1.

[2] N. Hens *et al.*, « Assessing the risk of measles resurgence in a highly vaccinated population: Belgium anno 2013 », *Eurosurveillance*, vol. 20, nº 1, janv. 2015, doi: 10.2807/1560-7917.ES2015.20.1.20998.

[3] S. Abrams, P. Beutels, et N. Hens, « Assessing Mumps Outbreak Risk in Highly Vaccinated Populations Using Spatial Seroprevalence Data », *Am. J. Epidemiol.*, vol. 179, nº 8, p. 1006-1017, avr. 2014, doi: 10.1093/aje/kwu014.

[4] J. Schenk *et al.*, « Identifying immunity gaps for measles using Belgian serial serology data », *Vaccine*, vol. 40, nº 26, p. 3676-3683, juin 2022, doi: 10.1016/j.vaccine.2022.05.009.

[5] H. Theeten, C. Vandermeulen, M. Roelants, K. Hoppenbrouwers, A. Depoorter, et P. Van Damme, « Coverage of recommended vaccines in children at 7–8 years of age in Flanders, Belgium », *Acta Paediatr.*, vol. 98, nº 8, p. 1307-1312, août 2009, doi: 10.1111/j.1651-2227.2009.01331.x.

[6] H. Theeten *et al.*, « Infant vaccination coverage in 2005 and predictive factors for complete or valid vaccination in Flanders, Belgium: an EPI-survey », *Vaccine*, vol. 25, nº 26, p. 4940-4948, juin 2007, doi: 10.1016/j.vaccine.2007.03.032.

[7] A. Vellinga, A. Depoorter, et P. Van Damme, « Vaccination coverage estimates by EPI cluster sampling survey of children (18–24 months) in Flanders, Belgium », *Acta Paediatr.*, vol. 91, nº 5, p. 599-603, mai 2002, doi: 10.1111/j.1651-2227.2002.tb03283.x.
[8] E. Robert et B. Swennen, « Enquête de couverture vaccinale des enfants de 18 à 24 mois en communauté française (Bruxelles excepté), 2009. [Vaccination coverage survey in infants at 18-24 months of age in the French Community except Brussels, 2009] ». Brussels: Provac, Université Libre de Bruxelles, novembre 2009. [En ligne]. Disponible sur: http://ddata.over-blog.com/xxxyyy/3/27/09/71/Enquete-Provac-2009.pdf
[9] E. Robert et B. Swennen, « Enquête de couverture vaccinale des enfants de 18 à 24 mois en Région de Bruxelles-Capitale, 2006. [Vaccination coverage survey in infants at 18-24 months of age in the Brussels Capital Region, 2006]. » Université Libre de Bruxelles, Ecole de santé publique., décembre 2006. [En ligne]. Disponible sur: http://www. observatbru.be/documents/graphics/rapports-externes/ enquete-de-couverture-vaccinale-des-enfants-de-18-a-24-mois-en-region-de-bruxelles-capitale.pdf
[10] C. Vandermeulen, M. Roelants, H. Theeten, A.-M. Depoorter, P. Van Damme, et K. Hoppenbrouwers, « Vaccination Coverage in 14-Year-Old Adolescents: Documentation, Timeliness, and Sociodemographic Determinants », *Pediatrics*, vol. 121, nº 3, p. e428-e434, mars 2008, doi: 10.1542/peds.2007-1415.
[11] P. Van Damme, H. Theeten, T. Braeckman, T. Lernout, N. Hens, et K. Hoppenbrouwers, « Studie van de vaccinatiegraad bij jonge kinderen en adolescenten in Vlaanderen in 2012. [Vaccination coverage in infants and adolescents in Flanders in 2012]. » Brussels: Vlaamse overheid, Vlaams Agentschap Zorg en Gezondheid, Team Infectieziekten, 2013.
[12] E. Robert et B. Swennen, « Enquête de couverture vaccinale des enfants de 18 à 24 mois en Région de Bruxelles-Capitale, 2012. [Infant vaccination coverage at 18-24 months of age in the Brussels Capital Region, 2012]. » Brussels: Université Libre de Bruxelles, Ecole de santé publique;, décembre 2012. [En ligne]. Disponible sur: http://www.observatbru.be/documents/graphics/ rapports-externes/enquete-vaccination-2012-fr.pdf
[13] E. Robert et B. Swennen, « Enquête de couverture vaccinale des enfants de 18 à 24 mois en Fédération Wallonie-Bruxelles (excepté Bruxelles). [Vaccination coverage survey in infants at 18-24 months of age in the French Community (except Brussels)] ». Brussels: Provac, Université Libre de Bruxelles, décembre 2012.
[14] Grammens, « Couverture vaccinale des vaccinations de base », sciensano.be. Consulté le: 27 mai 2025. [En ligne]. Disponible sur: https://www.sciensano.be/nl/biblio/couverture-vaccinale-des-vaccinations-de-base
[15] S. N. Wood, *Generalized Additive Models: an introduction with R.*, Boca Raton, Florida: Chapman and Hall/CRC Press. 2006.
[16] J. Schenk, S. Abrams, H. Theeten, P. Van Damme, P. Beutels, et N. Hens, « Immunogenicity and persistence of trivalent measles, mumps, and rubella vaccines: a systematic review and meta-analysis », *Lancet Infect. Dis.*, vol. 21, nº 2, p. 286-295, févr. 2021, doi: 10.1016/S1473-3099(20)30442-4.

5. Supplementary Materials of Appendix 5

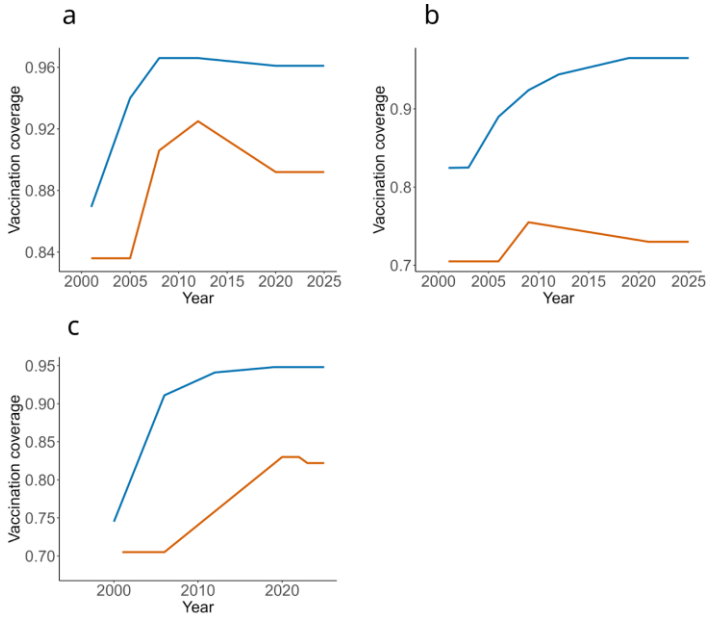

Figure S1. Vaccination coverages (MMR1 in blue and MMR2 in orange) for (a) Flanders, (b) Wallonia, (c) Brussels.

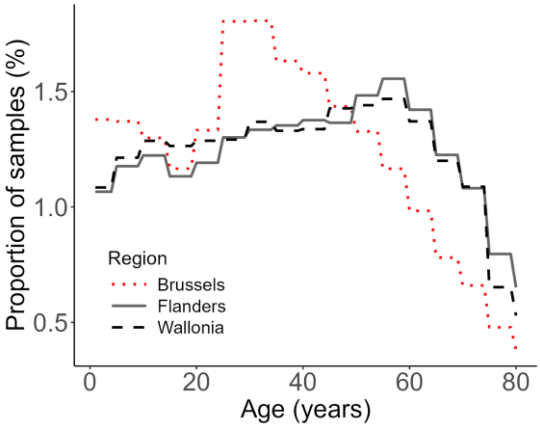

Figure S2. Age structure of samples under the population-based allocation, based on 2021 demographic data.

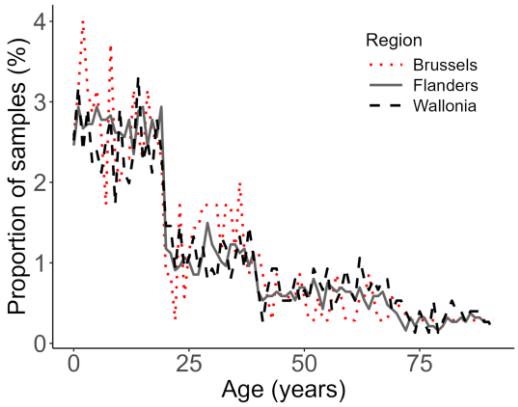

Figure S3. Age distribution of samples under the survey-based allocation, based on the empirical age structure observed in the 2013 serological survey (measles).

sample size	structure	precision	overall prevalence
1000	sero	0,0195	0,903
1000	pop	0,0161	0,903
1000	unif	0,0184	0,903
2000	sero	0,0152	0,902
2000	pop	0,0123	0,903
2000	unif	0,0129	0,902
3000	sero	0,0120	0,903
3000	pop	0,0102	0,902
3000	unif	0,0110	0,903
4500	sero	0,0097	0,903
4500	pop	0,0089	0,902
4500	unif	0,0085	0,902

Table S1. Precision of the overall prevalence for measles.

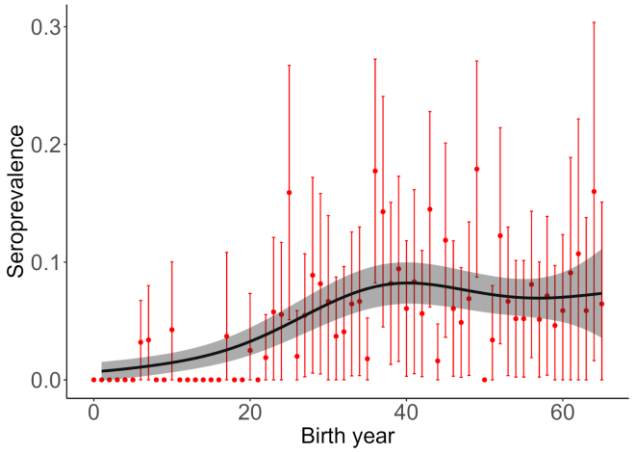

Figure S4. Observed seroprevalence and smoothed estimates in 1993 for HBV.
Age-specific seroprevalence (red dots with binomial confidence intervals) and fitted GAM estimates (black line) based on 1993 serological data. The generalised additive model was fitted using a complementary log-log link with a smooth term for age.

sample size	structure	precision	overall prevalence
1000	sero	0,01334	0,05541
1000	pop	0,01291	0,05489
1000	unif	0,01453	0,05473
3000	sero	0,00785	0,05511
3000	pop	0,00748	0,05491
3000	unif	0,00824	0,05443
6000	sero	0,00552	0,05482
6000	pop	0,00539	0,05488
6000	unif	0,00582	0,05473
9000	sero	0,00468	0,05471
9000	pop	0,00489	0,05498
9000	unif	0,00489	0,05501

Table S2. Precision of the overall prevalence for Hepatitis B.

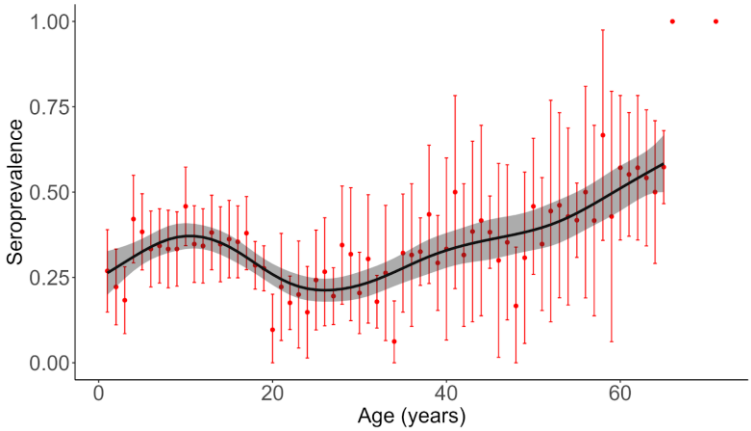

Figure S5. Observed seroprevalence and smoothed estimates in 2011 for CMV.

Age-specific seroprevalence (red dots with binomial confidence intervals) and fitted GAM estimates (black line) based on 2011 serological data. The generalised additive model was fitted using a complementary log-log link with a smooth term for age.

sample size	structure	precision	overall prevalence
1000	sero	0,03476	0,349
1000	pop	0,03053	0,349
1000	unif	0,02886	0,348
3000	sero	0,02002	0,348
3000	pop	0,01585	0,348
3000	unif	0,01540	0,348
6000	sero	0,01472	0,348
6000	pop	0,01162	0,348
6000	unif	0,01230	0,348
9000	sero	0,01160	0,347
9000	pop	0,01009	0,347
9000	unif	0,00942	0,347

Table S3. Precision of the overall prevalence for CMV.

Disease	Sample size for ±2% precision	Sample size for ±1% precision
Mumps	1650	6600
Parvovirus B19	1650	6600
Measles	<1650	1650
Varicella–Zoster Virus (VZV)	<1650	1650
Rubella	<1650	1650

Table S4. Estimation of number of samples for several pathogens obtained by **Blaizot et al (2019).**

References

1. Winter AK, Martinez ME, Cutts FT, Moss WJ, Ferrari MJ, McKee A, et al. Benefits and Challenges in Using Seroprevalence Data to Inform Models for Measles and Rubella Elimination. J Infect Dis. 2 juill 2018;218(3):355-64.

2. Metcalf CJE, Farrar J, Cutts FT, Basta NE, Graham AL, Lessler J, et al. Use of serological surveys to generate key insights into the changing global landscape of infectious disease. Lancet Lond Engl. 13 août 2016;388(10045):728-30.

3. Gilbert GL, Escott RG, Gidding HF, Turnbull FM, Heath TC, McIntyre PB, et al. Impact of the Australian Measles Control Campaign on immunity to measles and rubella. Epidemiol Infect. oct 2001;127(2):297-303.

4. Kim SS, Han HW, Go U, Chung HW. Sero-epidemiology of measles and mumps in Korea: impact of the catch-up campaign on measles immunity. Vaccine. 2 déc 2004;23(3):290-7.

5. Nardone A, Anastassopoulou CG, Theeten H, Kriz B, Davidkin I, Thierfelder W, et al. A comparison of hepatitis B seroepidemiology in ten European countries. Epidemiol Infect. juill 2009;137(7):961-9.

6. Theeten H, Hutse V, Hoppenbrouwers K, Beutels P, VAN Damme P. Universal hepatitis B vaccination in Belgium: impact on serological markers 3 and 7 years after implementation. Epidemiol Infect. févr 2014;142(2):251-61.

7. Burn L, Pilz A, Vyse A, Gutiérrez Rabá AV, Angulo FJ, Tran TMP, et al. Seroprevalence of Lyme Borreliosis in Europe: Results from a Systematic Literature Review (2005-2020). Vector Borne Zoonotic Dis Larchmt N. avr 2023;23(4):195-220.

8. Ho E, Schenk J, Hutse V, Suin V, Litzroth A, Blaizot S, et al. Stable HEV IgG seroprevalence in Belgium between 2006 and 2014. J Viral Hepat. 2020;27(11):1253-60.

9. Krug C, Rigaud E, Siby-Diakite D, Bénézet L, Papadopoulos P, de Valk H, et al. Seroprevalence of Hantavirus in Forestry Workers, Northern France, 2019-2020. Viruses. 25 janv 2023;15(2):338.

10. Theeten H, Hutse V, Hens N, Yavuz Y, Hoppenbrouwers K, Beutels P, et al. Are we hitting immunity targets? The 2006 age-specific seroprevalence of measles, mumps, rubella, diphtheria and tetanus in Belgium. Epidemiol Infect. avr 2011;139(4):494-504.

11. Litzroth A, Suin V, Wyndham-Thomas C, Quoilin S, Muyldermans G, Vanwolleghem T, et al. Low hepatitis C prevalence in Belgium: implications for treatment reimbursement and scale up. BMC Public Health. 8 janv 2019;19(1):39.

12. Measles - Annual Epidemiological Report for 2023 [Internet]. 2024 [cité 4 mars 2025]. Disponible sur: https://www.ecdc.europa.eu/en/publications-data/measles-annual-epidemiological-report-2023

13. World Health Organization (WHO). Measles vaccines: WHO position paper – April 2017 [Internet]. WHO; 2017 [cité 6 mars 2025]. Report No.: 17. Disponible sur: https://www.who.int/publications/i/item/who-wer9217-205-227

14. HGR/CSS. Vaccination de rattrapage [Internet]. 2013 [cité 24 mars 2025]. Disponible sur: https://www.hgr-css.be/file/download/c30f000c-00d9-48e2-b96c-0f3acefb009f/eblup9tl6peqCjJO8cZcYKIhbZZ436l8qzo8AnSYU3d.pdf

15. Cornelissen. sciensano.be. 2022 [cité 4 mars 2025]. Plan d'action 2021-2025 | Comité pour l'élimination de la rougeole et de la rubéole en Belgique. Disponible sur: https://www.sciensano.be/en/biblio/plan-daction-2021-2025-comite-pour-lelimination-de-la-rougeole-et-de-la-rubeole-en-belgique

16. Cornelissen L, Roukaerts I, Abady M. sciensano.be. Sciensano; 2024 [cité 4 mars 2025]. Surveillance épidémiologique de la rougeole (Morbillivirus) - 2023. Disponible sur: https://www.sciensano.be/fr/biblio/surveillance-epidemiologique-de-la-rougeole-morbillivirus-2023

17. Grammens T, Cornelissen L. Vaccine coverage in Belgium, 2020-2021: Report on vaccination coverage and trends [Internet]. Sciensano; 2021 [cité 4 mars 2025]. Disponible sur: https://www.sciensano.be/sites/default/files/vaccine_coverage_2020-21_fr_final.pdf

18. ONE, Federation Wallonie-Bruxelles. Dossier phase transition RRO2 2020-2024 [Internet]. [cité 4 mars 2025]. Disponible sur: https://www.e-vax.be/VaccHelp/help/pdf/dossier_phase_transition_RRO2_2020-2024.pdf

19. World Health Organization (WHO). Twelfth meeting of the European Regional Verification Commission for Measles and Rubella Elimination [Internet]. Copenhagen, Denmark; 2023 sept [cité 6 mars 2025]. Disponible sur: https://iris.who.int/bitstream/handle/10665/376606/WHO-EURO-2024-9722-49494-74055-eng.pdf?sequence=1

20. AViQ, Sciensano. Flash Maladies infectieuses - Janvier 2025 | AVIQ [Internet]. [cité 6 mars 2025]. Disponible sur: https://www.aviq.be/fr/flash-maladies-infectieuses-janvier-2025

21. Schenk J, Abrams S, Litzroth A, Cornelissen L, Grammens T, Theeten H, et al. Identifying immunity gaps for measles using Belgian serial serology data. Vaccine. 9 juin 2022;40(26):3676-83.

22. Kebede M. Sero-epidemiology of vaccine preventable diseases in Belgium. 2005.

23. Van der Wielen M, Van Damme P, Van Herck K, Schlegel-Haueter S, Siegrist CA. Seroprevalence of Bordetella pertussis antibodies in Flanders (Belgium). Vaccine. 2 juin 2003;21(19-20):2412-7.

24. Bolotin S, Hughes SL, Gul N, Khan S, Rota PA, Severini A, et al. What Is the Evidence to Support a Correlate of Protection for Measles? A Systematic Review. J Infect Dis. 27 avr 2020;221(10):1576-83.

25. Arkell P, Tanesi MY, Martins N, Gomes N, Oakley T, Solano V, et al. A population-representative serosurvey estimating vaccine-induced immunity against measles, rubella, hepatitis B and severe acute respiratory syndrome coronavirus 2 in Timor-Leste. Lancet Reg Health Southeast Asia. mars 2025;34:100525.

26. Wright J, Crowcroft N, McLachlan E, Perez-Iratxeta C, Joh E, Osman S, et al. Population immunity to varicella in Canada: A Canadian Immunization Research Network (CIRN) study. PloS One. 2024;19(8):e0309154.

27. Kelly H, Riddell MA, Gidding HF, Nolan T, Gilbert GL. A random cluster survey and a convenience sample give comparable estimates of immunity to vaccine preventable diseases in children of school age in Victoria, Australia. Vaccine. 19 août 2002;20(25-26):3130-6.

28. Muyldermans G, Ducoffre G, Leroy M, Dupont Y, Quolin S, Laboratories PS. Surveillance of Infectious Diseases by the Sentinel Laboratory Network in Belgium: 30 Years of Continuous Improvement. PLOS ONE. 29 août 2016;11(8):e0160429.

29. Blaizot S, Herzog SA, Abrams S, Theeten H, Litzroth A, Hens N. Sample size calculation for estimating key epidemiological parameters using serological data and mathematical modelling. BMC Med Res Methodol. 7 mars 2019;19(1):51.

30. Hens N, Abrams S, Santermans E, Theeten H, Goeyvaerts N, Lernout T, et al. Assessing the risk of measles resurgence in a highly vaccinated population: Belgium anno 2013. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull. 8 janv 2015;20(1):20998.

31. Abrams S, Beutels P, Hens N. Assessing Mumps Outbreak Risk in Highly Vaccinated Populations Using Spatial Seroprevalence Data. Am J Epidemiol. 15 avr 2014;179(8):1006-17.