

UNAIDS/WHO Working Group on
Global HIV/AIDS and STI Surveillance

Guidelines for Conducting HIV Sentinel Serosurveys among Pregnant Women and Other Groups



World Health
Organization



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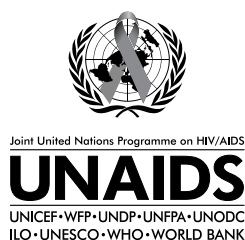
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World Health Organization (WHO)
Joint United Nations Programme on HIV/AIDS (UNAIDS)
US Centers for Disease Control and Prevention (CDC)



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1. INTRODUCTION

1.1 HIV serosurveillance in the context of second-generation surveillance

Every country needs information about its human immunodeficiency virus (HIV) epidemic in order to combat its spread. Information about trends in HIV prevalence—whether and by how much the prevalence is increasing or decreasing and which populations are affected—can help a country monitor its epidemic and provide information on the effectiveness of prevention and control measures. The monitoring of trends in HIV prevalence is conducted through surveillance activities. Surveillance is not a one-time activity, nor should it be a stand-alone programme. National AIDS programmes (NAPs) should build HIV surveillance systems that will perform activities in a routine, standard manner. The cornerstone of surveillance systems is consistency of methods, populations and tools. Consistency is essential in order to provide reliable information, which, over time, will enable a country to monitor trends.

Second-generation surveillance promotes a comprehensive approach towards developing a solid HIV surveillance system. In *Guidelines for Second Generation HIV Surveillance* (UNAIDS/World Health Organization [WHO], 2000), UNAIDS and WHO summarize “core” (essential) surveillance activities and “additional” (useful) surveillance activities for determining HIV prevalence, by a country’s epidemic state (Table 1). Regardless of the state of the epidemic, countries should collect prevalence information from populations that are more or less representative of the general population (such as pregnant women), as well as populations considered to be at high risk of infection and transmission. Conducting serosurveys among pregnant women is a core surveillance activity in concentrated and generalized epidemics and an additional surveillance activity in low-level epidemics. The frequency of serosurveys and the geographic coverage or type of exposure area surveyed may vary, depending on the state of a given epidemic.

Not only are pregnant women considered a good proxy for the general population, they are also fairly easy to access: most use antenatal clinic (ANC) services during their pregnancies and, in that context, blood may be drawn for routine testing. A portion of this blood can be used for HIV testing using several approaches. The selection of HIV testing approaches for serosurveillance depends on contextual factors, such as country policies and settings for HIV testing. Unlinked anonymous HIV testing (without informed consent) is only conducted in clinic settings where blood is collected regularly for other purposes (usually syphilis testing). If feasible, such testing should be conducted in settings where referrals to voluntary counselling and testing are provided. Linked testing (confidential or anonymous) with informed consent is the preferred approach when the specimens are collected solely for HIV testing. In such cases, unlinked anonymous testing with informed consent may also be used, depending on the country’s relevant policies and guidelines. See box overleaf for a summary of the different HIV testing approaches used for HIV serosurveillance.

Linked and unlinked HIV testing.**Unlinked anonymous testing (without informed consent)**

- Testing of unlinked specimens collected for other purposes
- No personal identifiers obtained, no informed consent, no counselling required
- Coded specimen

Unlinked anonymous testing (with informed consent)

- Testing of unlinked specimens collected solely for surveillance purposes
- Informed consent required
- No personal identifiers or names obtained, no counselling required.
- Coded specimen

Linked confidential testing (with informed consent)

- Testing of samples linked to the person by name, collected primarily for surveillance
- Informed consent and pre-test and post-test counselling required
- Personal identifiers or names obtained
- Coded specimen; code linked to personal identifying information

Linked anonymous testing (with informed consent)

- Testing of samples linked to the person by code, collected primarily for surveillance
- Informed consent and pre-test and post-test counselling required
- No personal identifiers or names obtained
- Coded specimen; code given to patient so that only he or she may obtain results

The age of pregnant women affects how representative they are of the general population. HIV prevalence among younger pregnant women may overestimate the prevalence among other women of the same age in the general population: these pregnant women (who may represent a small percentage of all younger women) have, by definition, been sexually active and thus may have been exposed to infection. Other women of the same age may not yet be sexually active and thus not exposed. HIV prevalence among older pregnant women may underestimate that of other women of the same age in the general population: HIV-infected women are less likely to become pregnant and thus do not attend ANCs and will not be captured in a serosurvey. Nevertheless, in countries with generalized epidemics, data from ANC serosurveys continue to be a valuable, convenient source of information and currently present the best source of information on the current epidemiological situation in the general population. As such, results of these serosurveys are frequently used for the estimation of national prevalence.

Table 1. HIV surveillance by epidemic state^{1,2}

	Epidemic state		
	Low-level	Concentrated	Generalized
Core surveillance	<p>(HIV prevalence has not consistently exceeded 5% in any defined subpopulation)</p> <ul style="list-style-type: none"> • <i>HIV serosurveillance in identified groups at high risk³</i> • Analysis of available blood donor screening data 	<p>(HIV prevalence is consistently >5% in at least one defined subpopulation and is <1% in pregnant women in urban areas)</p> <ul style="list-style-type: none"> • <i>HIV serosurveillance in identified groups at high risk³</i> • Annual HIV serosurveillance in pregnant women in a limited number of urban areas <i>and in bridging populations⁴</i> • Analysis of available blood donor screening data 	<p>(HIV prevalence is consistently >1% in pregnant women)</p> <ul style="list-style-type: none"> • Annual HIV serosurveillance in pregnant women in urban and rural areas (increase sample size in high-volume sites to allow for analysis by age groups)
Additional surveillance/studies	<ul style="list-style-type: none"> • Larger coverage and increased frequency of HIV serosurveillance in identified groups at high risk³ • HIV sentinel surveillance in pregnant women and <i>bridging populations⁴</i> in urban areas • Serosurveillance of other groups (e.g., military, occupational workers, tuberculosis [TB] patients, hospital patients) every 2 years • HIV surveillance among patients (TB patients, hospital patients) 	<ul style="list-style-type: none"> • Wider geographical coverage and increased frequency of HIV serosurveillance in identified groups at high risk³ • Wider coverage of HIV serosurveillance in pregnant women and <i>bridging populations⁴</i> • Serosurveillance of other groups (e.g., military, occupational workers, TB patients, hospital patients) every 2 years • HIV surveillance among patients (TB patients, hospital patients) 	<ul style="list-style-type: none"> • <i>HIV sentinel serosurveillance in groups considered to be at high risk³</i> • HIV sentinel serosurveillance in a larger number of sentinel sites of pregnant women and <i>bridging populations⁴</i> • <i>Population-based HIV prevalence studies to calibrate and validate surveillance data</i> • HIV surveillance among patients (TB patients, hospital patients)

¹ Updated from: *Guidelines for Second Generation HIV Surveillance* (UNAIDS/World Health Organization [WHO], 2000)

² Italicized text indicates serosurveillance/data analysis **not** addressed in these guidelines.

³ For example, patients at sexually transmitted infection clinics, sex workers, men who have sex with men, and injecting drug users (IDUs).

⁴ Groups linking subpopulations at high HIV risk with the general population, e.g., clients of sex workers.

1.2 Purpose of the guidelines

These guidelines are written for NAP managers and epidemiologists responsible for monitoring trends in HIV prevalence in resource-constrained countries. They provide technical guidance on conducting HIV serosurveys among pregnant women attending ANCs. They also describe how to collect and/or use serosurveillance data from the military, occupational groups, and blood donors, which can help characterize an epidemic, as well as assist in defining prevention activities, clinical services, and resources. The document also briefly discusses the use of HIV seroprevalence data among patients with tuberculosis (TB) and hospital inpatients, who are important sources of information for determining the impact of HIV on health services and for programme planning and service delivery.

These guidelines propose specific approaches for conducting HIV serosurveillance among pregnant women, using the principles of second-generation surveillance. They describe how to select sites, design and implement a serosurvey, analyse and interpret the data gathered, and disseminate the findings. Also included is a glossary of terms used in the document. These guidelines are part of a series of technical guidelines for developing and implementing second generation surveillance systems. Conducting serosurveys of groups with high risk behaviour—for example, injecting drug users, men who have sex with men, and sex workers—is addressed elsewhere (Kaldor et al., 2001). As part of the second generation guideline series and to complement topics addressed in these guidelines, additional documents are being developed to more comprehensively address sampling of populations, effective use of data, and ethical issues surrounding the conduct of HIV surveillance.

The recommendations in these guidelines are based on lessons learned from the field. Following these recommendations in conducting serosurveys will help ensure the quality and sustainability of the serosurveys. National AIDS Programmes should use these recommendations as appropriate, given their own country situation and epidemic state.

1.3 Conducting HIV serosurveys

HIV serosurveys are conducted by a country's NAP or the national institution responsible for communicable disease surveillance. Conducting a serosurvey requires several essential steps, from deciding on the survey design to disseminating data and evaluating the surveillance system. Serosurvey operations should be evaluated regularly, and evaluation results should be used to revise and improve how the programme conducts its serosurveys.

A successful serosurvey relies on effective training of health-care workers; supervision; and quality assurance in the laboratory, serosurvey operations, and data management. A well-conducted serosurvey will provide reliable and valid information and also will have the commitment of the Ministry of Health and donors.

1.4 Ethical considerations in conducting HIV serosurveys

The basic principles of ethical medical research in human subjects should be applied to HIV serosurveys: persons should be respected, the endeavour should provide benefits—if not to the individual, to the population—and no harm should be done (Declaration of Helsinki, revised October 2000). However, not all medical concerns are public health concerns. The field of public health focuses on populations, rather than individuals, and on preventive efforts, rather than curative ones. NAPs should review and incorporate the basic principles of both medical and public health ethics in developing protocols for HIV serosurveys. A national ethics committee should review the protocol to ensure that it respects and meets the country's national ethics code.

In 1990, WHO/GPA issued the first guidelines for developing HIV sentinel surveillance systems. In 2000, UNAIDS and WHO produced *Guidelines for Second Generation HIV Surveillance* (UNAIDS/00.03, and WHO/CDS/CSR/EDC/2000.5). The guidelines proposed the use of unlinked anonymous testing (UAT). Since that time, most sub-Saharan African countries conducting serosurveillance have used this approach to collect data. In recent years, there have been increasing concerns in some countries about the use of this strategy. The principal concern is that UAT can lead to missed opportunities for referring patients/clients to available prevention services such as voluntary counselling and testing and the prevention of mother-to-child HIV transmission. However, surveillance and prevention activities have different objectives: surveillance aims to measure the level of an HIV epidemic and to monitor trends, while prevention activities aim to provide services to prevent HIV infections. Inadequate prevention, treatment and support services in many countries are often a result of limited resources (human and financial) and inadequate infrastructure. These services are important and should be offered, but generally not through surveillance activities, given that the objectives of the two activities (i.e. voluntary counselling and testing, and surveillance) are different. Surveillance data may be helpful in monitoring and evaluating prevention and support services that do exist. Yet, due to limited service coverage and selection biases that result in differences between those who use services and those who do not, using data from prevention and support services as an alternative to surveillance data is not advisable at this time.

As coverage of prevention and support services (e.g., programmes to prevent mother-to-child HIV transmission) increases, data originating from such services should be increasingly used for programme planning and evaluation. Comparison of data originating from programmes with data from surveillance activities should be made to identify potential biases and limitations of either data source on a continual basis, wherever both data collection systems exist.

If UAT is selected as the method for use in HIV serosurveys, the following steps should be taken:

- Protocols should be reviewed and approved by the national ethics committee.
- No additional blood should be collected specifically for HIV testing.
- No additional information should be requested from the participant; only the socio-demographic or clinical information required on the clinic form should be collected.
- All identifiers (e.g., names, patient codes) should be removed before testing.
- Information on referrals to local voluntary counselling and testing services should be made available.

In settings where UAT without informed consent is not permitted or not recommended, UAT with informed consent (see Box 1) may be considered. If UAT with informed consent is conducted, voluntary counselling and testing services should be provided for participants. When these services are provided, UAT with informed consent addresses some of the ethical concerns about UAT, without compromising the objectives of surveillance. Only if participation rates are affected, and if those who refuse to participate have a different HIV prevalence from those who consent, does it become a problem. Whichever method is selected, confidentiality should be ensured for all patients included in a serosurvey.

Guidelines that further address the ethical issues of HIV surveillance are being prepared in a forthcoming publication.

2. SEROSURVEYS AMONG PREGNANT WOMEN

Summary box: Serosurveys among pregnant women

Sampling

- Design
- Sample size
- Timeframe
- 'Oversampling for youth'

Operational procedures

- Staffing and training
- Data collection and management
- Specimen collection and testing
- Data collection and management

Quality assurance

- Measures to protect anonymity

Serosurveys conducted among pregnant women in resource-constrained countries have traditionally been conducted in public ANCs in urban settings. These clinics provide ready access to a cross-section of sexually active women from the general population who are not using contraceptive methods. In addition, blood collected for routine antenatal diagnostic tests (e.g., syphilis) can be made available for UAT. In most resource-constrained countries, pregnant women are likely to visit an ANC at least once during their pregnancy, especially in more urban settings. For example, in selected sub-Saharan African countries, an estimated 80–94% of all pregnant women access antenatal care services. Myanmar and Thailand have rates of approximately 80% (WHO, 1997; WHO, 2000).

Conducting serosurveys among pregnant women requires that operational procedures be documented in a protocol where they are described in detail.

The following are important operational procedures that should be included in such a protocol:

- Selection criteria for sites (including the number of sites and location)
- Patient eligibility criteria
- Use of data collection forms
- Data and specimen collection (local and national levels)
- HIV testing
- Supervision of operations (local and national levels)
- Data analysis and dissemination
- Budgeting

Surveillance staff at the national level should tailor the protocol in accordance with the country's situation, and a national ethics committee should review the protocol before it is implemented. The local-level serosurvey personnel who will conduct the serosurvey should receive the protocol and be trained to use the procedures before the serosurvey begins. (See Appendix 1 for an example of an outline of a serosurvey protocol.)

2.1 Sampling for serosurveys

Sampling is the process of selecting and surveying a small, part of a larger population to determine characteristics (e.g., HIV prevalence) of the larger population. Different sampling methods can be used in serosurveys. The success of the serosurvey will depend on the sampling method used, good organization and records, and good judgement. It is important to

find the appropriate sampling method, while considering the limitations—such as human and financial resources and time.

Before HIV serosurveys are conducted, the sentinel sites and populations (e.g., pregnant women attending ANCs) for inclusion in the serosurvey must be selected. The sampling method used will affect the design of the serosurvey and the analysis of data from the serosurvey—specifically, what analyses can be performed, whether data must be weighted, and how data should be interpreted.

Sampling is usually performed in two phases:

- Phase I: Selection of the sample of ANCs
- Phase II: Selection of the sample of pregnant women attending the selected ANCs

2.1.1 Sample design

Either probability or nonprobability sampling can be used to select a sample in Phases I and II. In probability sampling, random sampling methods are used to determine which population members are chosen. Each population member has a known and nonzero probability of being selected in the sample. Examples of probability sampling include simple random sampling, stratified sampling, systematic random sampling, and cluster sampling with probability proportional to size. In nonprobability sampling, subjective judgement, convenience, or quota are used to determine which population members are included in the sample.

Sampling terms

Population: Group that is being sampled and targeted (e.g., ANCs, pregnant women)

Sample design: How a sample is selected from the population (e.g., convenience sample, simple random sample, cluster sampling with probability proportional to size)

Sample unit: Unit for listing and selecting in a serosurvey (e.g., an ANC, a pregnant woman)

Sample frame: List of all sample units from which a sample is drawn (e.g., list of all ANCs, villages, or households in a province or country)

2.1.1.1 Phase I: Sampling antenatal clinics

In HIV serosurveys, ANCs are usually selected based on convenience sampling—a nonprobability sampling design.

Considerations when selecting antenatal clinics

The selected ANCs should not only be able to obtain the sample size needed for the serosurvey, but they should also be able to provide a national picture of the epidemic, which entails obtaining information from:

- different geographical locations, including those with a high risk of HIV infection (e.g., borders, transport corridors);
- areas with different population densities and sizes;
- both urban clinics and rural clinics;
- women with different socioeconomic status. Women from poor socioeconomic backgrounds are often overrepresented, and so some clinics in the private sector (e.g., mission hospitals) could be considered for selection, if they cater for a significantly different population of pregnant women (if feasible); and geographic areas with other ongoing HIV surveillance and prevention activities (e.g., behavioural surveillance, population-based HIV serosurveys, VCT programmes) in order to obtain comprehensive information about the area's epidemic and evaluate any ANC serosurvey biases.

The number of sites selected will depend largely on the local surveillance programme's human and financial resources and ability to coordinate the sites. Ideally, one to two sites (one urban and one rural) should be selected per province or district, depending on surveillance needs and geographical coverage. If too many sites are selected and adequate monitoring cannot be ensured, the quality of serosurvey operations, and thus the data obtained, may be compromised. Selecting too few sites will result in limited information about the epidemiologic situation. It is preferable to start with a small number of urban sites accessed by a large number of pregnant women. As both financial and human resources permit, additional sites can be added, leading to enhanced coverage of different geographical areas and both urban and rural areas. No matter how clinics are selected, if at all possible, they should be included in the sample each year the serosurvey is conducted so that reliable trend data may be obtained over time.

In selecting clinics for inclusion in the surveillance system, it is important to consider the goals of collecting surveillance data. If one wishes to only look at trends in a few key areas (e.g., urban areas, border crossing), a few clinics just from these areas suffice. This is often the case in countries with low-level or concentrated epidemics. If one wishes to measure prevalence in rural areas, then clinics must be chosen from those areas. There is no simple set of rules for selecting sites; rather, one must consider what questions need to be answered and then design the system that can best provide that information, given the financial and logistical limitations that the programme faces.

Once sites have been selected, information concerning specific characteristics of each site should be gathered before commencing the serosurvey. Information on the number of pregnant women who access the clinic per month, a description of the site's geographic catchment area, general characteristics of the clinic population, such as age distribution, and residence (rural, semi-urban, urban) will be helpful during interpretation of survey findings.

The following information on site-specific characteristics should be gathered for each of the selected sites:

- Number of patients per month as well as proportion who are repeat visitors
- Age distribution of clients
- Geographic locality of clinic (urban, semi-urban, rural)
- Residence of clinic population (urban, semi-urban, rural)
- Description of services provided at clinic (family planning, voluntary counselling and testing, prevention of mother-to-child transmission).

2.1.1.2 Phase II: Sampling pregnant women attending the selected antenatal clinics

After the sample of ANCs has been selected in Phase I, a sample of pregnant women at each of the sampled ANCs should be selected. Then the inclusion and exclusion criteria can be used to select the pregnant women. Pregnant women can be chosen from a clinic intake registry at each clinic, using probability sampling, such as simple random sampling or systematic random sampling, or using nonprobability sampling, such as convenience sampling. Traditionally, consecutive sampling (a type of convenience sampling) is most frequently used to select the women whose blood specimens will be tested for HIV. With consecutive sampling, the first eligible pregnant woman and each subsequent eligible pregnant woman attending the clinic during the serosurvey period thereafter is included in the serosurvey until the desired sample

size (see 2.1.2) is achieved or until the serosurvey period ends. Site personnel should consult the intake registry to ensure that pregnant women who meet the inclusion criteria are consecutively selected, and test specimens are drawn from these women for HIV.

Consecutive sampling has several advantages:

- It facilitates the process of obtaining a sufficient sample size in a given time frame.
- It is convenient, feasible, and easy to employ.
- It reduces the likelihood of selection bias introduced by on-site personnel.

Considerations affecting the sample size

- Initial or baseline HIV prevalence in a geographic area.
- Magnitude of change in HIV prevalence one wants to be able to detect.
- Accuracy needed for the seroprevalence estimate (width of the confidence interval).
- Degree of certainty that the change in HIV prevalence is statistically significant (level of significance or alpha), usually 0.05.
- Degree of certainty that a statistically significant change, if it occurred, can be detected (power or beta), usually 0.80.
- Percentage of pregnant women eligible to be included in the serosurvey. This will involve being able to estimate or determine the patient volume in the ANC during the serosurvey period.
- Resources available for conducting the serosurvey (e.g., costs, logistics).

2.1.2 Sample size determinations

The total sample size of pregnant women for the serosurvey must be determined, as must the number of women per selected ANC. If the sample size is too small, the results are imprecise and of little use, and if the sample size is too large, resources are wasted. It is important to document the sample size per serosurvey site (ANC) in the protocol and in any reports that are generated from the surveillance data, especially if data are aggregated for the analysis.

Sample sizes must be sufficiently large to provide a reasonably accurate estimate of prevalence at each sentinel site selected. The HIV prevalence calculated during a serosurvey for a specific clinic is usually only an *estimate* of the true prevalence for that clinic. If a 95% confidence interval (CI) is calculated for this estimate, a range is created within which one can be 95% confident that the true prevalence falls (see 3.1.1 regarding how to calculate confidence intervals). The narrower the confidence interval, the more precise and reliable the estimated prevalence will be in describing the true prevalence in the selected sentinel sites. Seroprevalence estimates based on small samples will have wide confidence intervals.

A number of considerations affect the minimally acceptable sample size (see box). In general, the most influential are the expected HIV prevalence in the population and the desire to monitor trends in HIV prevalence over time. The importance of resources cannot be underestimated, as the sample size needed may be larger than a country's resources (human and financial) or available time permit.

In countries where HIV-2 may be prevalent and surveillance programmes wish to also monitor HIV-2 infection levels, sample sizes for serosurveys should be calculated separately to provide periodic estimates of seroprevalence for HIV-2 and for HIV-1.

Sample sizes can be calculated by using published tables based on setting the confidence interval for a given level of prevalence (from a previous survey) (Table 2), or by using computer programs (e.g., Epi Info's STATCALC for simple random sample) (see 2.2.5 and Appendix 2).

Table 2. Ninety-five per cent confidence intervals for observed prevalence by sample size*

Observed prevalence (%)	Sample size				
	50	100	250	500	1000
0	0-7	0-4	0-2	0-1	0-0
2	0-11	0-7	1-5	1-4	1-3
10	3-22	5-18	7-14	8-13	8-12
20	10-34	13-29	15-26	16-24	18-23
30	18-44	21-40	24-36	26-34	27-33
40	27-55	30-50	34-46	36-44	37-43
50	36-64	40-60	44-56	46-54	47-53

*Based on a binomial distribution.
Sources: Fleiss, 1981; Snedecor and Cochran, 1967.

Table 2, which is based on 95% confidence intervals, shows that, for a given level of observed HIV prevalence, 95% confidence intervals become smaller as the sample sizes become larger. For a given sample size, the 95% confidence intervals grow wider as the observed prevalence increases. However, the precision in the observed HIV prevalence increases because the variation is proportionally lower. For example, for a sample of 250 women, if the observed prevalence is 2%, the 95% CI is 1–5%; and, if the observed prevalence is 30%, the 95% confidence interval (CI) is 24–36%. Proportionally, the 95% CI for the 2% prevalence is more than twice the prevalence, while it is only $\pm 20\%$ for the 30% observed prevalence, and thus provides more precise information. Therefore, in low-prevalence areas, a large sample size is needed, even if the 95% CI is small.

To use Table 2 to calculate sample size, find the observed prevalence closest to the one in the geographic area of interest¹, set the width of the 95% confidence interval, and then select the sample size. This table may be used to determine the minimum sample sizes needed for serosurveys at each sentinel site. For example, if the observed prevalence is 20% and the 95% confidence interval is set at 15–26%, the sample size needed would be 250.

In some cases, it may be important that each sentinel site monitor its own level of infection and trends of HIV seroprevalence; in other cases, one wants to detect provincial or regional trends. If the goal is to track prevalence in each site, then each site will need to have a sufficiently large sample for prevalence and trends to be reliably measured. If, on the other hand, the goal is to obtain district or regional trends and/or information about prevalence in more remote areas, then the approach of combining a number of smaller clinics may be more appropriate. If clinics are aggregated to reach the required sample size, there will be limited statistical reliability around the estimate for the individual sites. However, one will have a measure of prevalence for those more remote areas and, if the same sites are used and aggregated in each round of surveillance, trends can be analysed. National programmes must decide how to balance their needs for information with their financial and logistical capacity in selecting sites to be included in their surveillance system.

¹ If an estimate of the HIV prevalence for the geographic area of interest is unknown, one can use an estimate from a similar geographic area or from studies or surveys believed to be similar for that area.

2.1.2.1 Sample size determination to monitor trends in HIV seroprevalence

Monitoring trends in HIV seroprevalence over time requires a larger sample size than does determining one estimate at one point in time. To monitor seroprevalence trends, NAP staff should select a sample size large enough for changes to be detected over several years. The smaller the increase or decrease in the estimates of seroprevalence over time, the larger the sample size required to detect a statistically significant change. Therefore, NAP staff must decide on the magnitude of change they wish to be able to detect and at what level of granularity (individual clinics, district or provincial level), and determine if resources allow for the increased sample size that may be needed. In concentrated and low-level epidemics, it may be very difficult and expensive to obtain a large enough sample size to detect changes over time in HIV prevalence at the level of a single ANC site.

Sample sizes required to detect a change (decrease or increase) in seroprevalence rates at a specific clinic between two serosurvey periods are shown in Table 3. For example, if the baseline prevalence is 20%, a sample size of 197 is required to detect a decline of 50% in seroprevalence between two time periods (from 20% to 10%).

Table 3. Sample size required for determining a significant change between two proportions¹

Baseline prevalence (%)	Sample size, given % proportional change								
	10%	20%	30%	40%	50%	60%	70%	80%	90%
1	145,800	34,000	14,000	7290	4280	3000	2070	1459	1060
5	28,000	6550	2800	1500	903	585	400	282	204
10	13,300	3200	1350	718	432	280	190	135	97
15	8500	2030	850	457	275	178	122	86	62
20	6000	1425	612	326	197	128	87	61	44
25	4500	1090	463	247	149	97	66	46	33

¹ With a power of 80% (beta = 0.80) and a significance level of $P < 0.05$.

2.1.3 Eligibility criteria for ANCs and pregnant women

Regardless of the sampling design used to select ANCs or pregnant women, surveillance programmes should specify eligibility criteria for inclusion in the serosurvey. Eligibility criteria for ANCs are defined below.

Criteria for selecting ANCs

- Blood is routinely collected from clients.
- A reliable laboratory for processing of specimens and transport to the laboratory that will be conducting HIV testing.
- The site is accessible to surveillance staff.
- On-site staff members are willing to cooperate and are trained to conduct the serosurveys.
- The site provides services to a sufficiently large number of clients.
- On-site HIV counselling and testing services **or** referral to such services should be available to clients included in the serosurvey.

Eligibility criteria (inclusion and exclusion) should be specified so that only women meeting the inclusion criteria will be part of the serosurvey. Ideally, all women attending an ANC should be eligible to allow for a relatively unbiased assessment of the women at the clinic. However, in reality, eligibility should be restricted to the following recommended criteria, with NAP surveillance coordinators determining the final eligibility criteria.

Criteria for selecting pregnant women

Inclusion criteria

- Pregnant women attending the clinic for the first time during their current pregnancy during the serosurvey period
- Pregnant women aged 15–49 years
- Candidate for routine blood draw

Exclusion criteria

- Pregnant women who previously visited the clinic during the serosurvey period (to avoid duplicate sampling)
- Pregnant women aged ≤ 14 years or ≥ 50 years

2.1.4 Sampling period

2.1.4.1 Duration

The serosurvey's duration in each ANC will be determined by the time required to reach the desired sample size. Therefore, the length of the survey will depend on the following: number of first time visits by eligible pregnant women to the sentinel sites per week, or month; and, if testing is not UAT, proportion of eligible patients who agree to participate.

Although it is not possible to calculate HIV prevalence among ANC attendees at a single point in time, the closer the sample collection period represents a single point in time, the more comparable the data are over time. Therefore, it is recommended that the serosurvey be conducted during a period of 4–12 weeks to reach the desired sample size. In some instances, it may be necessary to extend the serosurvey period up to an agreed-upon time for a maximum period of 4–6 months. For example, if an NAP has decided to use a larger sample size among younger age groups (15–24), (see 2.1.6), or if rural clinics cannot reach the desired sample size in the given period, the serosurvey period should be extended. In that case, strict

rules on sampling duration (such as data only being collected for 8 or 12 weeks) are less appropriate, as HIV prevalence will not change drastically over the course of a few months.

2.1.4.2 Frequency

Data from sentinel serosurveys among pregnant women provide information for advocacy and planning/evaluating prevention programmes. Therefore, serosurveys should be conducted annually (whenever possible) in order to monitor trends, using the same methods and same sentinel sites, if feasible. In countries or areas where human or financial resources are constrained, serosurveys can be conducted every two years using the same methods and sentinel sites. In the case of low-level epidemics, it may be appropriate to conduct serosurveys every two years in the selected urban ANCs. It is critical to maintain a list of the clinics in which surveys were conducted and in which years they were conducted so that the appropriate adjustments may be made to the HIV prevalence estimates (see 3.1.5).

2.1.5 Options to achieve the required sample size

As a rule, serosurveys should not be conducted in settings where the minimum sample size cannot be achieved. NAPs that have not been able to achieve the desired sample size at the selected sites—most commonly in rural sites—within the suggested sampling period, can use one of the three methods below to address this situation.

- *Lengthen the sampling period*

Surveillance coordinators may extend the sampling period beyond 12 weeks. If this is done, the HIV seroprevalence estimate obtained will not be strictly comparable with the estimated seroprevalence found in sentinel sites completing the serosurvey in the designated time frame.

- *Accept the reduced sample size*

Surveillance coordinators may accept the reduced sample sizes achieved at individual sites. If so, the confidence interval should be calculated and reported for the observed seroprevalence (see 2.1.2). With smaller sample sizes, the confidence intervals will be wider. Therefore, statistically significant changes over time will be difficult to determine. A weighted analysis would reduce the influence of this site in any provincial, regional or national estimate.

- *Aggregate data from multiple sites*

Another way to reach the desired sample size is to combine data from various sites. This can be useful in more rural areas, with low population density, where none of the clinics in a district or region may have sufficient women to reach the desired sample size. However, if prevalence data from three or four clinics can be combined, then one will have a reliable measure of the aggregate prevalence for these sites. The advantage in this approach is that smaller and more rural areas are included in the surveillance system. The limitation is that prevalence data for the individual sites (which have been combined) will not be reliable, due to the smaller sample size.

Regardless of the approach taken, it is important to document the methods used at a sentinel site and to report it to the national level so that it can be taken into consideration during analysis.

2.1.6 Methods and rationale for sampling women aged 15–24 years

Second-generation HIV surveillance suggests monitoring new HIV infections in sites and populations where it is feasible to do so. However, because incidence serosurveys require a large number of resources (human and financial), large sample sizes, and long-term follow-up, it is recommended that countries look at trends in HIV seroprevalence in the younger age groups (15–24-year-olds) at a few high-volume sentinel sites as a proxy indicator for incidence. Younger pregnant women (aged 15–24) are more likely to have recently become sexually active and thus are likely to have a higher proportion of new infections than older women.

Data from ANC surveys, particularly among younger women, will also help in measuring the success of HIV/AIDS efforts. To monitor the Declaration of Commitment adopted at the 2001 United Nations General Assembly Special Session on HIV/AIDS, UNAIDS has developed indicators for monitoring the progress of HIV/AIDS efforts in countries most affected by HIV/AIDS (see box), and one of the impact indicators is prevalence among the young.

United Nations General Assembly Special Session on HIV/AIDS: indicators focusing on HIV infection in youth and infants

At the United Nations General Assembly Special Session (UNGASS) on HIV/AIDS in June 2001, governments from 189 countries adopted the Declaration of Commitment on HIV/AIDS. The declaration established goals for achieving specific targets, including reductions in HIV infection among infants and young adults; improvements in HIV/AIDS education, health care and treatment; and improvements in orphan support.

To review and assess the progress made in HIV/AIDS-related efforts, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and its partners developed a set of core indicators. Because the highest rates of new HIV infections typically occur among young adults, among these are impact indicators to measure infection among young adults and infants:

- percentage of people aged 15 to 24 who are HIV-infected
- percentage of HIV-infected infants born to HIV-infected women

Alternative sampling designs may be required to meet the larger sample size required for measuring trends of HIV prevalence among young women. Countries with generalized epidemics have the highest HIV rates among the general population; therefore, in these countries, oversampling can be done in a feasible time frame with adequate resources. In countries with low-level or concentrated epidemics, the financial and human resources required could be prohibitive.

Three sampling designs are described:

1. For ANCs with a large number of patients and a high percentage of women in the 15–24-year-old age group, all age groups can continue to be sampled until the required sample size for women aged 15–24 is reached. The minimum sample size per ANC for women aged 15–24 will differ by HIV prevalence in each geographical area, as when calculating overall sample size (see Table 2).

2. The sample size can be stratified by age. Samples can be collected for women aged 15–24 until the required sample size is met. For example, if a clinic needs to sample 200 women aged 15–24 years and 300 women overall from all age groups have been sampled at the clinic, but only 150 in the age group 15–24 years, sampling would continue until an additional 50 women aged 15–24 years were included in the sample. Stratification requires that the clinic change inclusion criteria during the sampling period. (Initially, all women aged 15–49 who are first-time visitors, to women aged 15–24 who are first-time visitors). This will require

that clinics carefully monitor the number of women who have been tested by age and then alter the inclusion criteria when appropriate. As a cautionary note, when calculating prevalence for this clinic, two different values will need to be calculated. First, overall HIV prevalence for the clinic will be based on the first 300 (size set above) women who were tested, regardless of age. Next, prevalence of the 200 women aged 15–24 would be reported. The key is not to use the additional young women in calculating the overall prevalence for the site. Just as in calculating the sample size for all ages, if the sample sizes for young women are too small, the confidence intervals will be so wide that it will be difficult to measure whether HIV prevalence has truly changed over time among this age group or whether prevalence among women aged 15–24 is truly different from that among women aged 25–49.

3. Finally, a separate serosurvey for women aged 15–24 can be conducted. This approach will require additional financial and human resources and therefore is not typically recommended.

In many geographical areas, there may not be enough pregnant women aged 15–24 years to achieve the desired sample sizes. This may increasingly become the case if the age at first childbearing is increasing, because of successful reproductive health programmes. This does not mean that prevalence should not be reported by 5- or 10-year age group. Rather, even if individual clinics cannot reach the desired sample size, it is important to report prevalence data by age, since these data can, at the central level, be aggregated to create an overall measure of prevalence among age groups.

2.2 Other operational procedures

In most resource-constrained countries, most serosurveys among pregnant women use UAT because of its relatively low cost, easy access to specimens, and absence of participation bias. In this section, the emphasis is on serosurveys using UAT. For a more detailed description of linked and unlinked testing approaches, refer to *Guidelines for using HIV testing technologies in surveillance* (CDC/UNAIDS/WHO, 2001).

2.2.1 Serosurvey staffing and training

Required personnel for serosurveys include clinic staff, laboratory technicians, supervisory staff, data managers/statisticians, and survey coordinators. Responsibilities for all surveillance staff members (regardless of their position in the programme) should be clearly defined in the serosurvey protocol. Table 4 outlines appropriate responsibilities for serosurvey personnel.

Table 4. Appropriate responsibilities for serosurvey personnel

Level and title	Appropriate responsibilities
Local level (clinic)	
Clinic staff*	<ul style="list-style-type: none"> • Ensure that eligible women are included in the serosurvey at the time of their first antenatal visit • Complete data collection forms
Laboratory technician	<ul style="list-style-type: none"> • Obtain and process aliquots of residual blood specimens
Supervisory staff	<ul style="list-style-type: none"> • Provide adequate oversight at the clinic and ensure confidentiality
Regional level	
Laboratory technician	<ul style="list-style-type: none"> • Ensure provision of equipment, supplies and test kits • Conduct HIV testing
Survey coordinator	<ul style="list-style-type: none"> • Ensure provision of equipment, supplies and test kits • Ensure adequate oversight and confidentiality at the regional level • Ensure training takes place for local-level staff • Enter data in the programme's database • Manage data • Analyse data • Disseminate survey findings
National level	
Laboratory technician	<ul style="list-style-type: none"> • Ensure provision of equipment, supplies and test kits • Conduct HIV tests • Oversee quality assurance of testing procedures at regional and local levels
Data manager/statistician	<ul style="list-style-type: none"> • Enter data in the programme's database • Manage data • Analyse data
Surveillance coordinator	<ul style="list-style-type: none"> • Develop serosurvey protocol with programme staff • Ensure adequate funding • Provide adequate oversight and training at the regional and national levels • Ensure confidentiality • Interpret findings, in conjunction with regional-level survey coordinator, and prepare survey report for dissemination
National AIDS programme manager	<ul style="list-style-type: none"> • Protocols approved by national ethics committee

*For more detail on clinic-level staffing, see 2.2.1.1.

2.2.1.1 Staff required at clinic level for data collection

Staffing at the clinic level is critical to the success of prevalence serosurveys and should include:

- one clinic staff member (nursing supervisor or senior laboratory technician) responsible for ensuring the efficient operation of the serosurvey and for supervising other serosurvey staff on site;

- one clinic staff member (nurse or laboratory technician) responsible for ensuring that the data forms are complete and that aliquots of residual blood specimens (in the case of UAT) are obtained (as well as labeled, stored properly, and unlinked) in preparation for transport to the testing laboratory;
- one clinic staff member trained in serosurvey operations to assume duties in the absence of the person responsible for conducting the serosurvey;
- a laboratory technician, if available, to assist with processing and preparing serosurvey specimens for transport to the testing laboratory; and
- a courier. In some cases, someone from the Ministry of Health will be available to transport specimens to the testing laboratory. If not, the clinic laboratory technician may be required to do so, especially if specimens are tested in the same town or city.

2.2.1.2 Training of serosurvey personnel

To conduct serosurveys with adequate quality and oversight, all personnel involved must be trained. After appropriate individuals are identified and selected at the local, regional and national levels to conduct the serosurvey, the national surveillance staff should conduct training before every serosurvey. Participants should include supervisors, laboratory staff, and clinic staff. Training should include a review of operational procedures, field protocol, and previous serosurvey findings. During the training session(s), surveillance staff should have the opportunity to discuss concerns and obtain further clarification of serosurvey operations. Training sessions may be conducted either at the site or in a central location at the regional or national level. Sessions that involve multiple staff members from each site would give staff the opportunity to share experiences from their respective sites. Training should be offered on a regular basis, and take place at least once before every round of surveillance. Maintaining motivation among serosurvey personnel will facilitate completion of serosurveillance activities (see below).

Maintaining motivation among serosurvey personnel

- Develop a sense of serosurvey 'ownership'
- Clearly define responsibilities and roles for all staff involved, at all levels
- Emphasize the importance of each person's contribution to the serosurvey's success
- Provide adequate staff training
- Ensure that the necessary equipment is available to conduct the serosurveys
- Assign certain data management and analysis responsibilities to regional coordinators.
- Provide feedback on staff performance and serosurvey results

2.2.2 Data collection

2.2.2.1 Sociodemographic data elements

In serosurveys that use UAT, sociodemographic data collected for each pregnant woman must not be so comprehensive that they facilitate identification of a specific woman. Therefore, it is suggested that a minimum of sociodemographic information be collected during serosurveys using UAT. Interactions with patients concerning the serosurvey must be kept to a minimum, if there are any at all. All data collected for the serosurvey should be information routinely collected at the site—for example, during the registration process. In serosurveys using linked testing, more data may be gathered and a standardized questionnaire can be used. Regardless of the testing approach, confidentiality of the patient's information must be ensured.

Second-generation surveillance recommends collecting, at a minimum, the following data elements: age, education level, occupation, residence, gravidity, parity, HIV test results, and syphilis test results (see Appendix 3). Countries may wish to include additional sociodemographic data elements (e.g., duration of stay at the present residence) on the basis of programme needs, as long as this information does not result in the ability to identify serosurvey participants.

Sociodemographic data elements

Age: Prevalence trends should be monitored by age. Patients may not know their exact age, so an approximate year of age within a five-year range can be used. Use of a chronological list of important national and local events may aid in recall of birth dates.

Education level and occupation: Education level and occupation may provide information that could be related to use of HIV services and HIV status; education level may be directly associated with specific risky behaviours. (See Appendix 7.3 for examples of education and occupation categories.)

Residence: Residence information will help define the catchment population at a given site (e.g., rural or urban) and will aid in the interpretation of prevalence trends. In serosurveys using UAT, the variable for residence should not be so specific that the participant can be identified. For example, it would be better to record the participant's health district, village, or distance from the clinic in kilometres, rather than her exact address.

Gravidity and parity: Gravidity (total number of pregnancies) and parity (the number of full-term children borne by a woman, excluding miscarriages or abortions in early pregnancy but including stillbirths) are indicators for assessing the association between HIV infection and exposure to unprotected sex. For women who do not use contraceptives, number of births tends to be a better measure of sexual exposure than a woman's age (Zaba, Boerma and White, 2000).

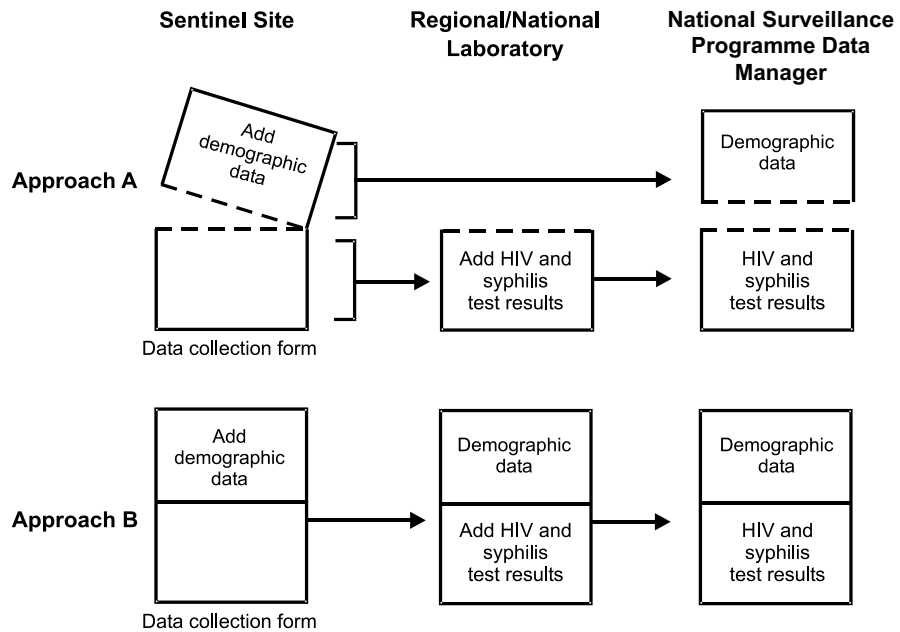
2.2.2.2 Data collection forms and data flow

The forms used for data collection should be designed so that surveillance staff can efficiently collect all of the sociodemographic data needed for the serosurvey.

In serosurveys using UAT, personal identifiers, which could be linked with serologic data, should not be included on the data collection form. Instead, only a survey code should be used. In serosurveys using linked testing, the form may include more sociodemographic information. In any case, the confidentiality of such information needs to be ensured.

In serosurveys using UAT, sociodemographic and serologic data can be collected and transmitted using one of two approaches (see Figure 1).

Figure 1. Flow of data collection forms for unlinked anonymous testing



- **Approach A.** Sociodemographic and serologic data are collected on separate sections of a perforated form. The sociodemographic data section is completed by clinic staff at the site, and the serologic data section is completed by a laboratory technician at the laboratory. Each section is then forwarded to the surveillance programme’s national data manager who then matches the section by the unique survey code. Each sociodemographic data form must have a corresponding serologic data form.
- **Approach B.** Both sociodemographic and serologic data are collected on a single form. After the sociodemographic information has been recorded on the form at the site, the form is then sent with the blood specimen to the laboratory responsible for testing the blood for the serosurvey (either at the regional or national level). The laboratory technician who tests the specimens records the HIV and syphilis test results on the form and sends the completed form to the regional or, more likely, the national data manager.

Although logistically the second approach is simpler, the first approach is preferable. Keeping sociodemographic and serologic data separate until entry into the system’s database may further protect the anonymity of the serosurvey participant.

2.2.3 Specimen collection, handling, processing and tracking

Specimens should be collected following routine procedures (most commonly venipuncture, but occasionally finger-stick) currently implemented at the sentinel site. For serosurveys using UAT, once routine testing for other purposes (e.g., syphilis) is complete, an aliquot (0.5 to 2.0ml) of leftover sera should be obtained and transferred to a sterile plastic tube or cryovial and labeled with the survey code that corresponds to that on the form with the sociodemographic data. HIV testing is then performed. However, if linked testing is conducted, the specimen is labeled with a survey code that can be linked to personal identifying information on the data collection form. In linked anonymous testing, similar collection and processing procedures are used, except that a code (not linked to a specific individual) is used to label the specimen. The code is provided to participants so that they may obtain their test results (see 1.1).

Survey codes may be written or printed on a label affixed to the tube or written with a permanent marker directly on the tube. If possible, surveillance coordinators should provide a series of labels or permanent markers and unique survey codes to field staff responsible for specimen collection.

If serum is to be tested for HIV more than three days after collection, it should be stored at -20°C in a non-frost-free freezer. For longer-term storage, serum should ideally be frozen at -70°C in a non-frost-free freezer. The number of freeze/thaw cycles, which may result from storage and transportation, should be limited to five.

Specimens not tested on site need to be transported to a regional or national laboratory for testing. Transport methods depend on a country's infrastructure. Frequently, field staff from the surveillance programme are responsible for transporting specimens from the local to national level. The specimens should be packed for transporting in a cooler that has an ambient temperature of 4°C . If cold packs are not available, serum specimens can remain at room temperature for up to three days.

To track specimens for the serosurvey, surveillance staff should maintain a separate laboratory logbook at the testing laboratory. HIV test results should be recorded in this logbook by corresponding survey code. The logbook should be accessible only to laboratory and surveillance staff and should be secured in a locked drawer or cabinet when not in use to help ensure confidentiality of test results. For unlinked and linked anonymous testing, no personal identifier should be included in the logbook—only the date and site of collection, survey code, and HIV result.

For linked confidential testing (see 1.1), codes are linked to personal identifiers in order to facilitate the provision of test results. Strict measures (see 2.2.7) must be followed so that such information is accessible only to laboratory and surveillance staff. After the patient has received her results, all personal identifying information should be destroyed. For the purpose of future data analyses, HIV test results should be linked only to survey codes.

2.2.4 Serologic testing

2.2.4.1 Tests and algorithms

The most appropriate type of test for surveillance purposes are tests that identify antibodies to HIV. Of these, enzyme immunoassays (EIAs) and rapid tests are the most cost-effective and accurate. EIAs require skilled technicians and special equipment and are commonly used in national or regional laboratories. Rapid tests have simpler requirements and so are useful in some settings (e.g., rural) that have limited laboratory infrastructure. Most EIAs and rapid tests contain antigens to both HIV-1 and HIV-2 and thus may be used to identify antibodies to either HIV type. Tests are also available to distinguish between HIV-1 and HIV-2. In situations where the participant receives test results, a confirmatory HIV test must be performed if the initial screening result is positive.

A single testing algorithm should be used nationwide for surveillance. The algorithm should be validated and approved in the country where it is to be used. The surveillance coordinator should document the HIV tests and algorithm used in the serosurvey.

2.2.4.2 Performing HIV testing

The manufacturer's instructions provided with the test should be followed when performing the test and interpreting test results. Depending on the HIV prevalence, testing strategy, and algorithm, a specimen may be considered HIV-positive if it is reactive on a single test in some settings, and if it is reactive on both the initial screening and the confirmatory tests in other settings. Please refer to *Guidelines for using HIV testing technologies in surveillance* (CDC/UNAIDS/WHO, 2001) for a more in-depth discussion on HIV testing.

2.2.5 Data management

After the sociodemographic and HIV test result data have been collected and recorded and the forms have been sent to regional and/or national levels, the data need to be entered into a database, checked for accuracy, and stored. This can be done on a continual basis during the serosurvey.

It is strongly recommended that a computer-based data management system be used. Epi Info, a software package commonly used in many countries, can be used to perform necessary data management functions, as well as to perform analyses. Data entered into Epi Info can also be exported for analysis in other statistical software packages (e.g., STATA, SPSS, SAS), if needed. The current version of Epi Info is available online ([http://www.cdc.gov/Epi Info](http://www.cdc.gov/Epi%20Info)).

Several procedures can help ensure accurate data entry:

- Design data entry screens to match the data collection form.
- Enter data from each form twice (referred to as 'double data entry') to check for accurate entry, define acceptable (legal) data values, and manually validate a minimum of 5% of randomly selected forms.
- Ensure that trained data entry clerks, who are supervised by the data manager, enter data into the database.

Other procedures can facilitate good data management:

- Data should be entered into the database on a continual basis to prevent a backlog of unentered data and to catch data collection errors.
- During data entry, data files should be regularly backed up, either on diskette or CD-ROM. When not being used, backed-up data files should be kept in a locked filing cabinet to help keep test results confidential.
- After data are entered, frequency tables should be produced for each data element (e.g., age, residence, HIV test result) to identify missing or inconsistent values that may have originated from incorrect entry of data into the computer (referred to as 'data cleaning'). Generating frequency tables for each site will aid in checking for errors in the data files and identifying missing values for specific variables.
- Completed data collection forms, which have been stored in a locked filing cabinet, should be available for verification during data cleaning.
- Preservation of data files over time for secondary analysis.

Because the anonymity of those participating in UAT must be protected, the persons who enter data and have access to the sociodemographic data and test results should not be the same persons who are responsible for data and specimen collection.

2.2.6 Quality assurance of serosurvey operational procedures

Quality assurance during serosurvey operations (e.g., during sampling, data collection, specimen testing, data management) is essential to ensure the overall accuracy of the data collected. The quality assurance for a serosurvey is the responsibility of all staff involved (see below). Quality assurance is also an important aspect of ethical compliance in serosurveillance.

Staff responsibilities for quality assurance

- **National surveillance coordinator:** Primary responsibility for monitoring compliance with the field protocol.
- **Regional survey coordinators and local (clinic) supervisory staff:** Play an active role in quality assurance during data collection and HIV testing.
- **Persons who select patients, collect data and specimens, and complete forms:** Ensure that procedures are being performed in accordance with the field protocol.

Regular supervisory visits to all sentinel sites are essential to ensure the quality of the survey. An example of a checklist to assist in the monitoring of operational procedures during such visits is provided in Appendix 4.

Enrolment of eligible patients at each serosurvey site should be periodically monitored to ensure that all eligible participants are included. The following are two approaches to monitoring enrolment:

- Routinely audit patient charts or intake logbooks to determine how many women visit the ANC for the first antenatal examination of their current pregnancy. Compare the number of pregnant women during a given period (e.g., 1-2 weeks) with the number of data collection forms completed for the serosurvey.
- Compare the number of routine blood tests ordered (e.g., syphilis tests) with the number of data collection forms completed.

Monitoring the completeness of enrolment also includes ensuring that an aliquot of residual blood is obtained from all eligible women. Residual blood may not be available for a specific woman for several reasons: insufficient quantity of blood after the routine test was conducted, inadvertent discard of residual blood before taking an aliquot of the specimen for the serosurvey, or failure to collect blood during the antenatal exam. Monitoring how often residual blood specimens are not obtained may help identify and correct a potential problem in the serosurvey operations. A systematic failure to obtain an aliquot of residual blood for HIV testing, for whatever reason, may result in biased seroprevalence results. As a means of assessing such a situation, a data collection form should be completed for all eligible patients, regardless of whether or not a specimen was obtained. The serosurvey form should document whether blood is obtained.

The accuracy of data being collected and entered into the data management system should also be monitored. If feasible, local supervisory staff could perform an audit of sociodemographic data collection forms (before UAT) by comparing recorded sociodemographic information on the data form with that from the clinic registry. Also, if feasible, the data manager could conduct a similar audit for data entry by comparing information recorded on the original data collection form with information in the serosurvey database. All staff involved with such audits should be provided audit results.

The quality assurance of laboratory procedures conducted during HIV testing is also important and is further discussed in *Guidelines for using HIV testing technologies in surveillance* (CDC/UNAIDS/WHO, 2001).

2.2.7 Measures to protect anonymity

Surveillance staff should ensure the anonymity of persons in serosurveys that use UAT. This is of critical importance and is ultimately the responsibility of the surveillance coordinator and other programme staff. For serosurveys using linked testing, mechanisms that ensure anonymity should also be employed, as appropriate, to ensure that test results are confidential.

Protecting participant anonymity

- **Collect only needed data.** If UAT is used, data should be restricted to selected sociodemographic data elements; they should not be detailed enough to identify an individual. When possible, socio-demographic data should be categorized (e.g., age in five-year age-groups).
- **Make no permanent record of serosurvey participation.** A copy of the survey code should not be kept in the medical chart or the woman's health card, nor should names be included in a laboratory logbook containing serosurvey HIV test results.
- **Do not link databases.** Serosurveys using UAT should not be linked to any other database (research, clinic patient, or laboratory database).
- **Be sure that laboratory staff taking aliquots of residual sera are not the same members of staff performing testing.**
- **Protect databases, laptops, and forms.** Databases must be password-protected so that only persons working in the surveillance programme will have access. Laptop computers and serosurvey forms should be locked in file cabinets or drawers when not being used by surveillance staff.
- **Restrict access to HIV test results and clinical records.** Data management staff should ensure that HIV test results are not available to staff who abstracted sociodemographic data and collected serum aliquots or to serosurvey clinical staff. Serosurvey staff members who work with the databases should not have access to patient clinical records.

3. DATA ANALYSIS AND INTERPRETATION OF FINDINGS FROM ANTENATAL CLINIC SEROSURVEYS

Summary box: Data analysis and interpretation of findings from antenatal clinic serosurveys

- Descriptive analyses
 - Calculating prevalence
 - Comparing prevalence
 - Identifying prevalence
 - Aggregating data
 - Estimating national prevalence
- Potential biases
- Interpretation of findings

3.1 Descriptive analyses

After the NAP data manager has cleaned and verified the data (see 2.2.5), the distribution of data elements by sentinel site should be analysed. This type of analysis—descriptive analysis—consists of describing the general characteristics of the serosurvey population (e.g., HIV prevalence, age distribution, and geographic distribution), which can be summarized in tables, graphs or maps (see Table 5 for an example of a frequency table). Frequently, such information is obtained from data files by running frequency tables in computer statistical software packages.

Table 5. Example of a frequency table: age distribution of patients sampled in Site A

Age	Number sampled at Site A	Percentage of total sample (%)
15–19	60	12
20–24	100	20
25–29	120	24
30–34	140	28
35–39	55	11
40–44	15	3
45–50	10	2
Total	500	

3.1.1 Calculating seroprevalence

The principal outcome measure of a serosurvey, the seroprevalence (P), may be calculated as $P = x/n$, where x is the total number of persons testing positive for HIV (based on a country-specific testing algorithm) and n is the total number of specimens tested at a given site or among subgroup members (e.g., 20–24-year-old ANC patients). Multiplying this proportion by 100% will express HIV prevalence as percentage positive. For example, if 93 of 500 specimens at a sentinel site are HIV-positive, the HIV prevalence at that ANC is 18.6% ($93/500 \times 100\%$).

The confidence interval for seroprevalence estimates may be calculated using the following formula (Daniel, 1991), based on the binomial normal theory method (where P is the prevalence and n is the total number of specimens tested).

$$P \pm 1.96 \sqrt{\frac{(1-P)P}{n}} \times 100\%$$

Example: $0.186 \pm \sqrt{(0.814)(0.186)/500} \times 100\% = 15.2\%-22.0\%$. The 95% CI is interpreted as having a 95% probability of containing the true population prevalence. Therefore, we can say that we have 95% confidence that the interval from 15.2% to 22.0% includes the true population HIV prevalence.

HIV prevalence can be calculated by age group for each site. The age-specific prevalence should be calculated by five-year age groups (or by the way in which age is recorded on the data collection form). An example of calculating and presenting age-specific prevalence may be found in Appendix 5.

3.1.2 Comparing HIV prevalence using two samples

Samples from two populations may be used to test whether the HIV prevalence in those populations are significantly different from one another. The samples may be from two different clinics, in which case it is important to know whether one clinic has significantly lower or higher prevalence than the other. The samples may be from the same clinic but taken at two different points in time, in which case it should be determined whether there is sufficient evidence to say that the prevalence is increasing or decreasing.

Before HIV prevalence estimates from two or more sites are compared, the site-specific prevalence data should be adjusted appropriately for differences in age distribution. It is not advisable to compare sites' HIV prevalence estimates directly without adjustment because the sites may have different age compositions. See Appendix 5 for an example of how to weight infection levels across sites. After the prevalences have been appropriately weighted, the significance of the difference in prevalence estimates can be determined by calculating the z-statistic or chi-square statistic (see Appendices 6 and 7).

It is important that consistency in the selection of sentinel sites and the sampling methods used for inclusion of patients in the serosurvey be maintained over time in order to facilitate the detection and interpretation of differences in HIV prevalences.

3.1.3 Identifying and testing trends in HIV prevalence over time

Temporal HIV prevalence trends can be compared by site if standardized serosurveys have been repeated over time using the same strategies and methods and have been conducted within the same sentinel sites. Plotting HIV prevalence over time for specific sociodemographic groups (especially age groups) will help in identifying specific changes in levels of HIV infection. The most effective way to present such data and the resulting temporal trends is graphically (using either bar charts or line graphs). It is important to have at least four separate data points (i.e. annual prevalence) when presenting trends graphically.

If the serosurveys were conducted in the same clinic at two different time periods, then the two prevalence sample estimates may be used to test whether there is sufficient statistical evidence to determine whether the prevalence has increased or decreased between those two periods (see 3.1.2). If ANC surveillance serosurveys have been conducted over many consecutive years, a different approach should be used to determine whether the prevalence has significantly increased or decreased over time.

The simplest approach for comparing prevalence estimates for three or more points in time would be to use one of the two sample methods just presented in 3.1.2 and ignore the data in intervening years. The prevalence estimate value from the most recent year may be compared with the prevalence estimate value from the first year in which the serosurvey was conducted, or the prevalence estimate from the most recent year could be compared with the preceding year. However, ignoring prevalence estimates from earlier or from intervening years will make it more difficult to ascertain whether prevalence is increasing or decreasing.

One common method for utilizing all available data to determine changes over time is to use the chi-square test for linear trends (see Appendix 8). If the test for linearity is statistically significant, then there is sufficient evidence in the data to suggest that the prevalence trend is not linear and that the chi-square of slope will not provide valid results. In these situations, more complex statistical methods are needed to model the changing trends over time. Those methods, which include logistic regression, are not discussed in these guidelines.

3.1.4 Aggregating data and reporting summary prevalence

A serosurveillance estimate is truly representative only of the population that accesses the particular sentinel site for which the estimate is made. Therefore, aggregating data from all sentinel serosurvey sites and using these aggregated data as the seroprevalence for a country as a whole is not advisable because important differences by site or region are not taken into account. However, data may be aggregated for a province or region if certain conditions apply: relatively small sites (100–200 women per site) are used to obtain the overall sample size for a province or region, and the women's sociodemographic characteristics and clinic catchment areas are relatively homogenous.

To report a summary HIV prevalence from site-specific prevalence, it is recommended that the median, rather than the mean, of the site-specific prevalence be used, in order to minimize effects of outlying prevalence. The range for site-specific prevalence should also be reported to demonstrate that differences between sites may exist and are not hidden by the reported median value.

3.1.5 Estimating national prevalence

ANC prevalence data should not be directly extrapolated to the general population of the catchment area, nor should regional or national median ANC prevalence be extrapolated to the general adult population. However, a software package was recently developed by UNAIDS, based on recommendations of the UNAIDS Reference Group on Estimates, Modeling

and Projections (2002). This package can be used to estimate national prevalence rates, given data from ANCs in countries with a generalised epidemic, and is available at www.unaids.org and www.tfgi.com. Additional information about the models on which the package is based can be found in *Improved methods and assumptions for estimation of the HIV/AIDS epidemic and its impact: Recommendations of the UNAIDS Reference Group on Estimates, Modelling and Projections* (the UNAIDS Reference Group on Estimates, Modelling and Projections, 2002).

Estimation and Projection Package (EPP)

The Estimation and Projection Package (EPP) is used to estimate and project adult HIV prevalence from surveillance data. After surveillance data from various sites and years showing HIV prevalence among pregnant women from both urban and rural sites are input, the package then fits the best epidemic curve. Different sub-epidemics can be created. The resulting national estimated adult HIV prevalence can be transferred to a demographic package (Spectrum: a computer modelling for demographic projections) to calculate the number of people infected and other variables, such as AIDS cases, AIDS deaths and other information.

3.2 Potential biases

Several biases arising during sampling or collecting data may affect the validity of a measured HIV prevalence. As much as possible, these biases or errors should be minimized.

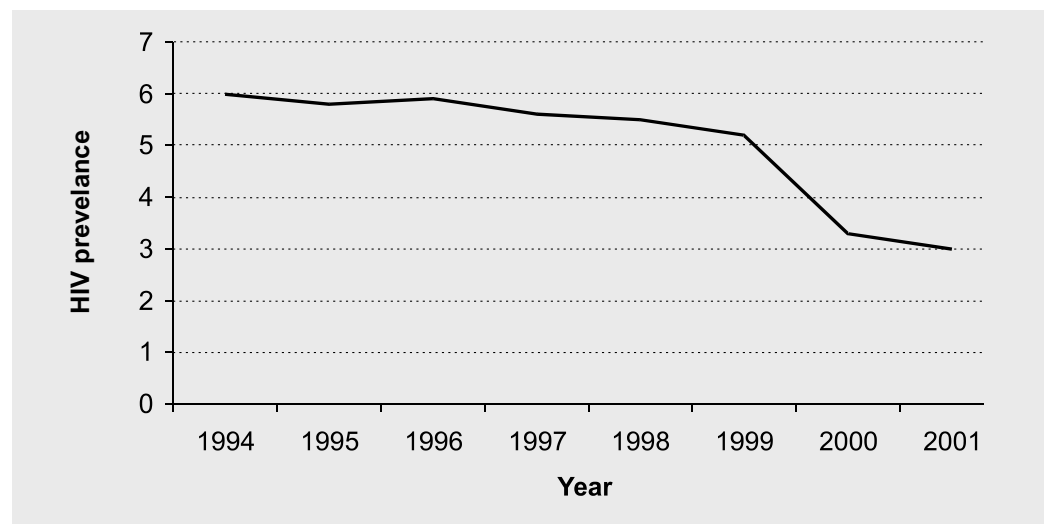
Two common biases are selection bias and information bias.

- Selection bias occurs when individuals selected for the serosurvey differ in an important way, based on sociodemographic characteristics or risky behaviours, from those not selected.
- Information bias is a systematic error in the collection of information for the serosurvey, such as inaccurate recording of age on the data collection form.

While selection bias is related to the way the participants are selected and depends on how the serosurvey is designed, information bias is related to the instruments used to collect the data and how the information is recorded.

An example of selection bias can be found in the results of an ANC HIV serosurvey conducted in a large urban ANC clinic in country X from 1994 to 2001 (see Figure 4).

Figure 2. HIV prevalence among antenatal clinic attendees, country X, 1994–2001



At first glance, HIV prevalence among pregnant women in this country seems to have decreased. However, further analysis of the data reveals that, in the 1994 survey, the targeted sample size of 800 was obtained, but that, in the 2000 and 2001 surveys, it was not. In 2000, the number of samples tested was 157, and, in 2001, only 91 samples were tested.

It was determined that deterioration of services at the ANC, introduction of user fees, and migration of patients to neighbouring facilities, among other factors, affected the number of pregnant women attending the ANC and thus the number available for inclusion in the serosurvey. The apparent decline in the observed prevalence rate did not represent a real decline. Instead, it was caused by decreased sample sizes in recent years, and women at higher risk of HIV infection having migrated away in recent years.

To minimize bias, the national surveillance programme should:

- dedicate time and effort to designing and supervising the HIV serosurvey;
- regularly monitor clinic attendance rates and possible variations in the characteristics of the population in the clinic's catchment area; and
- gather information about the policies in the selected clinics (e.g., patient care policies and other policies affecting patient access to services).

3.3 Interpretation of findings

3.3.1 Considerations affecting the interpretation of results

Two important issues must be taken into account when interpreting prevalence estimates from serosurveys:

- HIV prevalence is the percentage of persons who are HIV-infected, and their infections may or may not be recent. Thus, a relatively high prevalence among a specific subgroup may be the result of many recent infections or an increased survival rate of HIV-infected persons, or both. A decline in observed HIV prevalence over time may reflect a low number of recent infections among a specific group or an increase in deaths among HIV-infected persons.
- Although HIV prevalence is not a measure of new infections, HIV prevalence among younger age groups (< 25 years) may be more representative of new infections than is prevalence among persons \geq 25 years. See 2.1.6.

3.3.2 Generalizability of results

Population-based studies and ANC serosurveys concurrently conducted in the same area in several sub-Saharan African countries have provided similar prevalence data (Changalucha et al., 2002; Fylkesnes et al., 1998; Neequaye et al., 1997; Kwesigabo et al., 1996), supporting the use of ANC attendees as good proxies for the general population. Prevalence estimates from ANC attendees can also be used as an indicator of the potential number of infected infants and can be useful for assessing the need for mother-to-child HIV transmission intervention programmes.

However, caution is suggested for the following reasons:

- HIV prevalence among younger ANC attendees (15–24 years) may overestimate the prevalence among women of similar ages in the general population, given their riskier sexual behaviour (unprotected sex and thus potential exposure to HIV).
- Extrapolating prevalence data from this younger age group to men of similar ages in the general population may also overestimate HIV prevalence because men are more likely to become infected at an older age than women.

- HIV prevalence among older ANC attendees (≥ 25 years) is likely an underestimate of HIV prevalence among nonpregnant women of similar ages because HIV infection is associated with decreased fertility. Thus, older women who have been infected for a relatively longer time will be less likely to become pregnant and consequently will not attend ANCs.

In addition, HIV prevalence from ANCs will not be representative of prevalence among women who regularly use contraceptives or who are older when they have their first sexual experience. In areas where contraceptive use is high—more than 50%—caution should be used in interpreting HIV prevalence data.

3.3.3 Further issues of representativeness

The primary purpose of antenatal-clinic-based surveillance is the assessment of *trends in HIV prevalence*. Therefore, the consistency of methods and instruments used (especially the continuing participation of the same clinics) is an essential feature of good surveillance systems. However, since there are no other major sources of data to estimate the *level of HIV prevalence* in countries, antenatal clinic-based surveillance data are often used for that purpose.

In addition to those raised in 3.3.2, there are several other factors that may affect the extent to which pregnant women attending antenatal clinics of the surveillance system are representative of all pregnant women in the country. These include non-attendance of antenatal clinics, attendance of private clinics, and location of clinics.

Non-attendance of antenatal clinics

If large proportions of pregnant women do not attend antenatal clinics, one has to be more cautious in generalizing the findings of the surveillance system to all pregnant women. In most countries with generalized epidemics, more than 80% of women attend antenatal clinics. Women who do not attend antenatal clinics are often more rural, less literate and older than women attending antenatal clinics. HIV prevalence among such non-attending women is likely to be lower than among those attending, but the situation may vary from country to country.

Attendance of private clinics

Another limitation of surveillance systems is the exclusion of private clinics. In most countries, the overwhelming majority of women attend public antenatal clinics and the impact of not including private clinics is small. However, in urban areas with large numbers of women using antenatal clinics, it may make an important difference in HIV prevalence. South Africa is an example of a country where a significant proportion of women with a higher economic status use private clinics.

Under-representation of rural clinics

Because HIV prevalence tends to vary between urban and rural areas, the geographic location of the antenatal clinics becomes very important. National surveillance systems are usually based on a convenience sample of clinics. The country is stratified into administrative or other type of region and urban and rural clinics are selected from the different strata for the national surveillance system. Such a system cannot be considered as representative for the whole antenatal population.

In this process of selecting clinics, one of the most important issues is the location of the rural clinics. Most clinics referred to as rural in national surveillance systems are located in small towns or large villages and are not typical of rural settings. Midsize health facilities are selected as rural antenatal clinics, as the goal of surveillance is often to obtain 200–300 new antenatal attenders in a short time span, usually 8–12 weeks. These midsize facilities are mostly

rural hospitals or large health centres. Such facilities are often located in places with higher levels of economic activity and mobility and probably are also associated with higher HIV prevalence, as has been shown in several population-based studies. However, most of the countries have been expanding the number of sentinel sites in order to include more rural areas.

3.3.4 Interpretation of trends

If a significant change in HIV prevalence is observed, the following issues need to be considered first:

- Is there any reason to doubt the quality of the data? Training, supervision, transport of samples, etc. all need to be considered.
- Was the HIV testing strategy the same? The quality of testing at the laboratory needs to be evaluated and it should be established whether or not the same testing strategy was used (see *Guidelines for Using HIV Testing Technologies in Surveillance: Selection, Evaluation and Implementation*, 2001).
- Were there any changes in the population that the clinic served? Selective out-migration of women with higher HIV infection rates may occur if there are major economic changes such as a collapse of the tourism sector.

If there are no reasons to believe that any of the above changes have led to a decline, then one could assume that mortality among HIV-infected women has been higher than HIV incidence. If data show a decline in HIV prevalence, it must be ensured that the tools, methods and populations used are consistent and that the declining trends are confirmed by more than two data points. Constant HIV prevalence may indicate that deaths and new cases are in balance. And an increase in prevalence means that incidence likely exceeds mortality. The association between HIV and fertility may complicate the interpretation of results for pregnant women, but, as noted above, no study has shown a dramatic increase in female infertility over time. Differential increases in contraceptive use (a greater increase among HIV-infected women than HIV-negative women or the other way around) may affect trends. Therefore, it would be useful to check contraceptive use by HIV status but such data are rarely available.

Focusing on those aged 15–24 has the advantage that a greater proportion of infections are more recent than among women aged 25 years and over or among all women. Among 15–19-year-olds, almost all women would be recently infected. Yet, it is not advised to rely too much on the 15–19-year-olds for the assessment of trends (Zaba et al., 2000). Sample size may be a problem, but, more importantly, prevalence among 15–19-year-olds as an indicator of population incidence and prevalence patterns is sensitive to changes in sexual behaviour (especially age at first sex) and contraceptive use. Prevalence among pregnant women aged 15–24 is more robust and has been selected as the main impact indicator for monitoring of the goals of the UNGASS Declaration of Commitment on HIV/AIDS.

4. SEROSURVEYS AMONG OTHER SELECTED GROUPS: METHODS AND DATA INTERPRETATION

Summary box: Serosurveys among other selected groups: methods and data interpretation

- Military
- Occupational groups
- Blood donors
- Patients with tuberculosis
- Hospital inpatients

HIV prevalence rates among other populations are also needed to monitor the epidemic and to plan, implement and evaluate prevention activities and clinical services. While surveillance for HIV infection among ANC attendees is an important and frequent means of monitoring HIV infection, it does not capture information on infection in the male population. To provide a more complete picture of infection within the general population, infection data on other groups, and particularly on males, should be collected.

Members of the military, occupational groups, blood donors, TB patients, and hospital inpatients may help define the epidemic in the general population. These populations may be similar to, or different from, the general population (e.g., in terms of their risky behaviour or the HIV seroprevalence in the geographic area in which they live).

Many countries routinely collect blood from men—for example, during screening physicals for specific occupations, including the military, and during blood donor screening. If the men are representative of the general population or if there are systematic, identifiable differences between them and the general population, they may serve as an important complementary source of information for HIV surveillance.

4.1 Military

Most countries systematically screen new entrants to the armed forces for specific health problems. Blood is collected for routine tests, which may or may not include HIV. New entrants provide a useful birth cohort of young men, and prevalence in this group may be monitored over time.

Methods: Military

If recruits are selected by draft or lottery (or if military service is compulsory for all), the information provided by each new group on a yearly basis is sufficient to show trends in HIV infection. If recruits are voluntary, cross-sectional serosurveys at the recruitment centres can be conducted annually. The number of sites will depend on the number of recruitment centres in the country. The sample size must be sufficient to provide an accurate baseline estimate of HIV prevalence and to detect significant differences over time. The minimum data elements for the serosurvey would be education level, age, sex (most will be male), and residence.

Using information from routinely performed HIV tests among recruits would be more cost-effective than conducting cross-sectional serosurveys. It may be difficult to obtain routinely collected information; strong diplomacy and negotiation skills may be needed to convince the military authorities to release the information. If this information can be obtained, attention should be given to the methods used to measure HIV prevalence.

Interpretation of data: military

When a country requires young men to perform military service or when men are selected randomly by lottery or draft, military recruits may be considered representative of the general male population. If the young men are voluntary recruits, they are less likely to be representative of the general population of men of recruitable age. Therefore, basic sociodemographic information (at a minimum, age, education level, and residence before recruitment) for all recruits should be collected for comparison with the general male population.

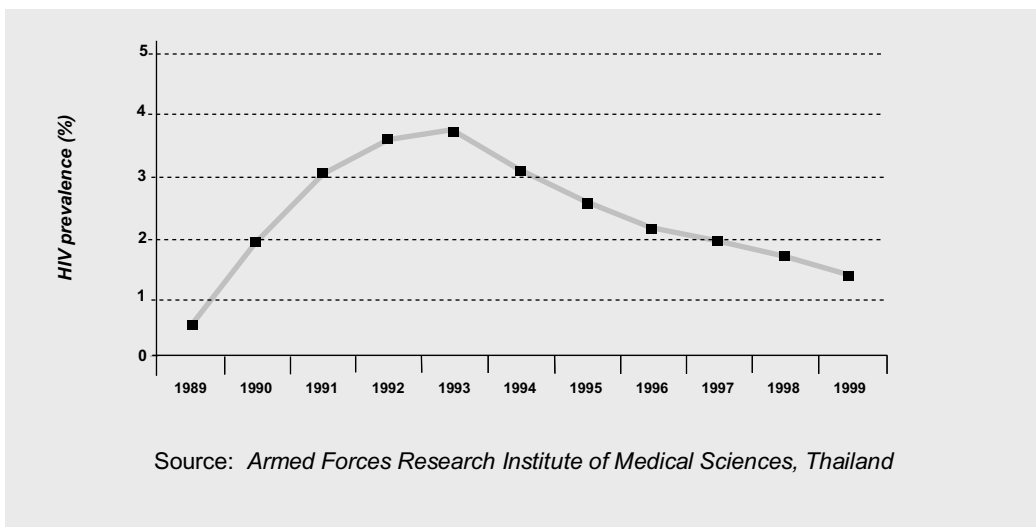
Because the ages of military recruits are generally younger (18–21 years) than the age of peak HIV incidence in men, extrapolating data from this group to the general male population will likely underestimate HIV prevalence. However, because of the recruits' relatively young age, prevalence data among them could be a proxy for incidence, depending on the age at which men are first sexually active. Over time, information on HIV infection in recruits could be used to help monitor the impact of interventions targeting young people.

Voluntary recruits may avoid being recruited if they know they will be tested for HIV. Certain countries may also actively discourage or disqualify men from certain risk groups (e.g., men who have sex with men, injecting drug users) from entering, resulting in an underestimate of HIV prevalence.

Members of the armed forces may continue to have their blood taken annually for regular health checks, but they rapidly lose their value as a proxy for the general male population because their risk of exposure to HIV may be generally higher than that of men in the population at large.

Thailand, for example, has been monitoring HIV prevalence among the military since the late 1980s (see Figure 5). This information, when contrasted with other sources of information, such as sexually transmitted infection prevalence, condom use, or other indicators, will contribute to the understanding of trends in HIV prevalence.

Figure 3. HIV prevalence trends in 21-year-old military conscripts, Thailand, 1989–1999.



4.2 Occupational groups

Many countries regulate the health of occupational groups, and some require that companies employ a medical doctor if the labour force is larger than 100 employees (e.g., miners, electricity companies). In addition, each sector may require regular check-ups once or twice a year to monitor adverse health effects associated with the workplace. Sometimes large companies or industries conduct health screenings and collect blood from potential and/or current employees for HIV testing. Information collected from occupational groups can be used as another source of data for serosurveillance. Ethical issues (e.g., confidentiality) should be taken into consideration when contemplating using information from these sources.

Methods: occupational groups

Blood samples taken for occupational health screening can be used for UAT for HIV. Linked testing could be offered if counselling services are available. If resources are available, a cohort serosurvey could be established; however, repeated cross-sectional serosurveys over time are preferred because they are easier to implement and less expensive to conduct.

Interpretation of data: occupational groups

Persons with late-stage HIV or symptomatic infection are unlikely to be included in the workforce. Thus, workers may be less likely to be HIV-infected than the general population, given that they are able to work (i.e., 'healthy-worker effect'). Prevalence data from these groups probably underestimate HIV prevalence in the general population.

In addition, many companies carry out pre-employment screening for HIV, and insurance companies may require a negative HIV test in order to provide health or life insurance. It can be difficult to ascertain if companies are using such screening practices, which would result in selection bias if these populations are used for surveillance.

4.3 Blood donors

Most countries have blood donation policies and intend for all blood units to be screened for HIV before they are used in transfusions. As part of these policies, blood donor programmes test donors for HIV infection, as well as for other transfusion-transmissible infections. HIV prevalence data among blood donors are thus easily available and not costly for the surveillance programme to obtain and use.

However, the use of blood donor HIV prevalence data has some limitations. Approximately 4 in 10 resource-constrained countries have not implemented a national blood donation policy, and more than 40% of donated blood is not screened for transfusion-transmissible infections (including HIV) (WHO, 2001). Even if a country has a policy and implements it, the policy may not be consistently implemented within all regions of the country. Local differences in blood-screening regulations, availability of test kits, or special campaigns, for example, may also affect the quality of data. In addition, where screening of the blood supply is decentralized, maintaining a centralized database of test results from blood screening programmes may be difficult.

Methods: blood donors

The most cost-effective method for determining HIV prevalence in blood donors is to regularly collect data from health facilities that have transfusion services. The minimum data required are age, sex, type of donor (voluntary, paid, or replacement), and HIV status. Information on occupation, donation location (rural, urban), and the standard donation screening and blood use practices and policies would help improve the quality and interpretation of the data.

Information should be collected on HIV prevalence among blood donors only during a three-month period each year to avoid possible repetition of donors in the same year. In general, a blood donor cannot give blood more than four times per year. The surveillance programme should collect information during a three-month period on a yearly basis to determine prevalence trends.

Interpretation of data: blood donors

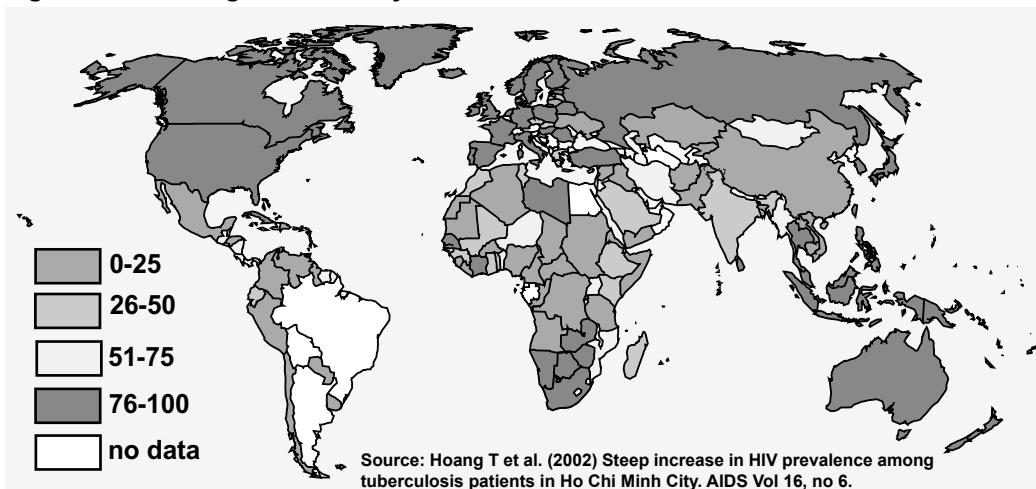
Since, in principle, 100% of all blood donors or blood units are screened, the sampled units should represent all blood donors. However, the extent to which blood donors represent the general population may be difficult to assess.

The types of donors and the kinds of pre-donation services available affect the degree to which HIV prevalence among a country's blood donors reflects HIV prevalence in its general population.

- Voluntary donors are generally at low risk of HIV infection and thus prevalence among voluntary donors may be lower than that in the general population.
- Despite recommendations to the contrary, some countries use paid blood donors, who are considered at high risk of HIV and often give blood more than once every three months. The prevalence in this group may overestimate HIV infection in the general population. However, in countries with high generalized prevalence, prevalence from paid donors may approximate that in the general population.
- Replacement donors (family and friends of hospital patients who need transfusions, generally for non-HIV-related reasons) are often provided by male family members, because of cultural practices. In such situations, the denominator for replacement donors is skewed towards men. However, replacement donors may be a fairly good proxy for the general adult population.

According to WHO, a significant part of the world's population is receiving blood transfusions from voluntary non-remunerated blood donors, as presented in Figure 6 below.

Figure 4. Percentage of voluntary non-remunerated blood donors worldwide



Many countries screen potential donors for risky behaviours, excluding or deferring those who are at elevated risk of HIV infection. In these cases, the prevalence among blood donors probably under-represents the true HIV prevalence in the general population. The bias may change over time, as the donor-screening programme grows stronger. If, however, criteria for self-exclusion and self-deferral are not explained well or used, HIV prevalence in blood donors may be approximately the same as in the general adult population.

In some countries, most donors are male, because of cultural practices, while in others, most are young people. It is useful to monitor both the male-to-female ratio and the age of blood donors to see if HIV prevalence changes over time or remains constant.

Despite these limitations, HIV infection levels reported from blood donors can provide useful information for advocacy, monitoring of blood transfusion services, and measuring the effectiveness of policies and strategies to decrease the risk of HIV infection in the blood supply.

4.4 Patients with tuberculosis

The interaction between HIV and tuberculosis (TB) is well documented, with HIV infection the most powerful known risk factor for tuberculosis disease (WHO, 2002, Ref WHO-CDS-TB-2002.296). HIV fuels the tuberculosis epidemic, increasing both the risk of reactivation of latent mycobacterium tuberculosis infection and the risk of rapidly progressive tuberculosis developing soon after infection or re-infection.

A higher HIV seroprevalence among smear-negative and extra pulmonary tuberculosis has been found and confirmed in other studies. This was explained by the difficulty in diagnosing TB in patients with HIV infection and the higher risk for HIV-infected persons to develop these forms of tuberculosis.

TB rates may not increase and HIV prevalence may not be elevated among TB patients early in an HIV epidemic or in low-level HIV epidemics. This may also be the situation in countries where HIV infection is concentrated in high-risk populations.

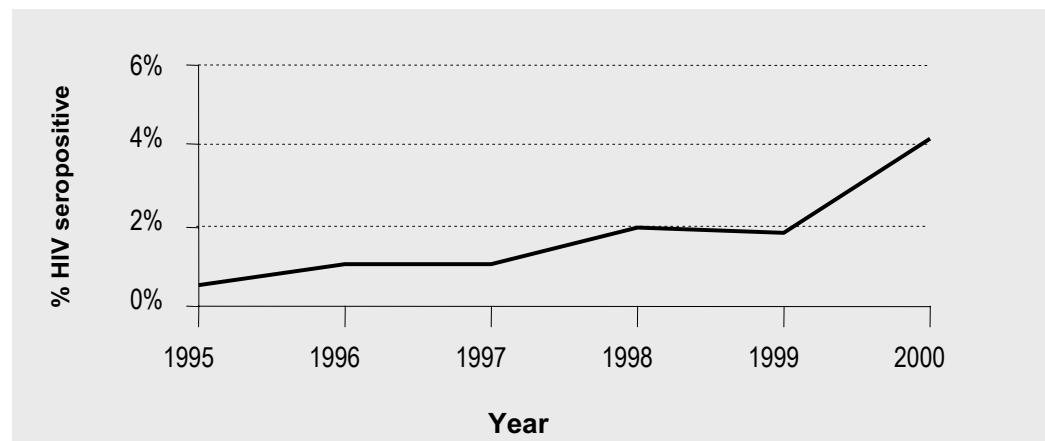
However, in generalized HIV epidemics, more active TB cases will occur, and the prevalence of HIV among TB patients will increase. For example, the prevalence of HIV in smear-positive pulmonary tuberculosis patients is up to 70% in some countries in sub-Saharan Africa (Ref WHO-CDS-TB-2002-296). The HIV prevalence rate among TB patients is an indicator of the level and maturity of the HIV epidemic, as well as an indicator of the effect of HIV on health-care services. Information from TB patients can be used in conjunction with other HIV serosurveillance data among population groups representing the general population and high-risk groups to help show TB trends.

Methods: TB patients

HIV serosurveys among patients in TB outpatient clinics and among TB inpatients can be conducted using methods similar to those described in Section 2. TB/HIV co-infection serosurveys can be conducted by consecutively sampling patients at TB outpatient clinics (based on the date of visit noted in the clinic registry) and performing HIV testing. For example, in Viet Nam in 12 urban districts of HoChi Minh City, representative samples of tuberculosis patients have undergone HIV testing since 1995. HIV prevalence increased from 0.5% in 1995 to 4% in 2000. This study has led to an intensification of the HIV-prevention programme (*AIDS* 2002, Vol 16, Research letters).

Where blood is not routinely collected from TB outpatients, patient consent will be required in order to collect and test the blood for HIV.

Figure 5. HIV prevalence among patients with active tuberculosis in Ho Chi Minh City



Increasingly, HIV testing is being offered to TB patients, partly because TB clinics are recognised as good entry points for the provision of antiretroviral treatment. HIV prevalence data resulting from these activities are increasingly being used for surveillance.

As with HIV serosurveys among ANC attendees, eligibility criteria will need to be defined and the length of the survey determined. Sociodemographic and clinical data can be collected using standardized forms. If HIV serosurveys are being conducted in a hospital or clinic and the hospital or clinic also has a TB clinic, it may be useful to conduct TB/HIV co-infection serosurveys at the same facility in order to provide further information about the HIV epidemic in that geographic area.

Interpretation of data: TB patients

HIV surveillance in TB outpatient clinics and inpatient wards not only provides information on the HIV prevalence among TB patients, but also reveals trends in HIV-related TB morbidity.

HIV surveillance among tuberculosis patients may help a country assess the impact of HIV on the tuberculosis epidemic and this information can inform the targeting of resources and the planning of health-care services for people who are co-infected with HIV and TB. This information also allows countries to monitor the effectiveness of joint strategies aimed at reducing the TB-HIV burden.

4.5 Hospital inpatients

Periodic HIV prevalence serosurveys among hospital inpatients (both children and adults) can provide important information for developing national, regional, and hospital HIV counselling and testing policies; planning and allocating resources; and providing information on the burden of HIV to the national health system. For example, in hospitals and their catchment areas where HIV prevalence is determined to be high, routine counselling and testing should be available.

Methods: hospital inpatients

Cross-sectional serosurveys can be conducted among inpatients, outpatients, and emergency room patients, as well as in hospital morgues. In hospitals where blood is routinely collected, UAT serosurveys using consecutive sampling of newly admitted patients are the fastest and easiest to conduct (see 2.1). The sample can be stratified by age group and sex, if analyses by these factors will be conducted. Laboratory registries can be used to identify the specimens to be tested. It will be important to analyse specimens collected and sent to the laboratory at different times of the day, as some wards may send specimens to the laboratory in the morning, while others send specimens in the afternoon. Eligibility criteria will need to be developed, and data elements to be collected will need to be defined. In addition to cross-sectional serosurveys using consecutive sampling, a serosurvey using a simple random sample of inpatients in specific wards can also be conducted.

Interpretation of data: hospital inpatients

When serosurveys are conducted among hospital inpatients, both the type of services provided at the hospital and the ward from which the specimens came will need to be known in order to interpret HIV prevalence; it is expected that the prevalence will vary among the different wards. Analysis by sex, age groups, and other variables are important for an understanding of the epidemiology of HIV in hospital patients.

5. DISSEMINATION OF SEROSURVEY FINDINGS

Summary box: Dissemination of serosurvey findings

Key components of the communication cycle

- Determine objective
- Establish message
- Select audience
- Select channel
- Select tools
- Evaluate impact

Public health surveillance for HIV has been defined as “the collection, analysis and dissemination of epidemiologic information of sufficient accuracy and completeness regarding the distribution and spread of HIV infection to be relevant to the planning, implementation, and monitoring of HIV/AIDS prevention and control programs” (adapted from CDC definition of public health surveillance, 1988). It is critical that information be turned into action.

Information in many countries is collected and analysed by the surveillance section of the Epidemiology Department of the Ministry of Health. Often key players, NGOs, public and private sectors, and others involved in the field of AIDS are invited to a consensus meeting in which data are analysed and interpreted in context. Information regarding HIV infection should be shared widely and freely with all partners and the community.

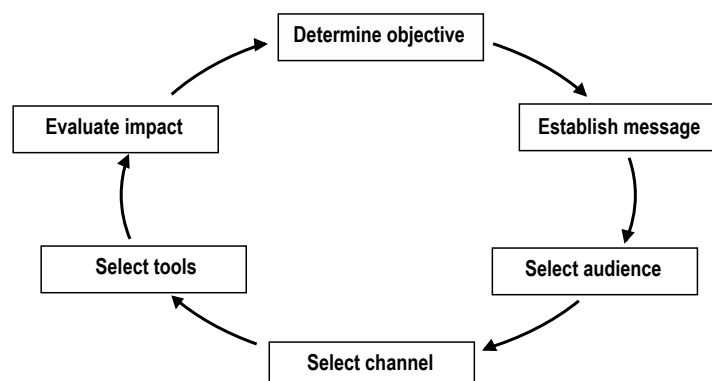
Sometimes NAPs send epidemiologic information to collaborating donors or international agencies. However, few surveillance programmes have developed a systematic, standard approach for disseminating surveillance results, and even fewer programmes have developed an approach for communicating successfully to target audiences (those who generate the information, general public or subpopulations, and decision-makers). HIV second generation surveillance will provide an increasing amount of information from different sources, including behavioural data, HIV and STI prevalence data, and AIDS case data. The NAP will need to decide which information to communicate and to whom, how, when and evaluate where, and whether this information has been successfully understood.

Communication is a two-way process; communication is complete when the receiver of the information acknowledges receipt and comprehension of the sender’s message.

Key components of the communication cycle

The main components of the communication cycle need to be considered when planning to disseminate findings and communicate messages (see Figure 8).

Figure 6. The communication cycle



- **Determine the objective**

Before a NAP communicates information, the programme must first decide on the objective of the communication. For example, does the NAP want to raise awareness of increasing HIV prevalence, promote national response to the epidemic in other sectors (e.g. policy-making, advocacy), or boost financial resources for HIV-prevention programmes? The communication objective will determine the message, audience, content and format.

- **Establish the message**

What information does the NAP want to transmit? Does the NAP want to transmit the message, for example, that interventions in schools are succeeding or that a rapid increase of HIV infection has been detected in certain subpopulations and so prevention activities need to be implemented in those subpopulations? The information should be clearly stated and expressed at an appropriate level for the target audience.

- **Select the audience**

Once the objective has been established, it is easier to define the target audience. With the multisectoral response promoted in all countries, the audience for information and communication is much broader than health professionals. The general public, policy- and decision-makers, media, other sectors, nongovernmental organizations, and other national or international organizations are current partners and important target audiences of NAP. The objective, content of the message, and target audience should be linked. The message sent to the recipient will differ if the NAP wants to mobilize the population for World AIDS Day or if the NAP wants to secure more commitment within the Ministry of Education.

The target audience should include surveillance staff members at the national and local levels who help conduct the serosurveys. They need to receive feedback about how the serosurvey was conducted and to be apprised of the latest serosurvey findings. Feedback enhances and helps maintain the system.

- **Select the channel**

The selection of an appropriate channel increases the probability that the message will reach the target audience and achieve the objective. Examples of channels that may be used are television, radio, newspapers, scientific journals, conferences, newsletters, press releases, Internet, and epidemiologic bulletins. If the NAP wants to raise awareness about an increase in HIV, a press release addressed to the most widely read newspapers may be appropriate. On the other hand, if the NAP wants to influence decision-makers at the local level, a brief summary report with explanatory graphics could be used in a meeting.

- **Select the tool**

It is essential to use the appropriate tools to transmit the message in order to capture the attention of the audience (see Table 6). For example, if decision-makers are the target audience, visual tools, graphs and maps with a short text explanation will be much more efficient than technical reports, since this audience is pressed for time and may be less technically oriented.

Table 6. Examples of essential channels and tools

Audiences	Channels	Tools
Technical professionals involved in NAP and monitoring and evaluation	Dissemination or evaluation workshops	Full technical report on HIV/sexually transmitted infections/behavioural surveillance
Nongovernmental organizations, other sectors or partners	Conferences	A nontechnical review of data from different sources
Media, journalists, communities	Press conferences	A press release highlighting the main findings
Other sectors involved in HIV prevention: communities, decision-makers	Coordination or planning meetings	Brief summaries of main findings

The tools used to transmit information should convey the information clearly. Whether a full technical report or a brief summary is used, graphs may facilitate the transmission of the message.

- **Evaluate impact**

An evaluation of the impact of the communication will assess whether and/or to what extent the objectives have been achieved.

6. MONITORING AND EVALUATION OF NATIONAL HIV SURVEILLANCE SYSTEMS

Summary box: Monitoring and evaluation of national HIV serosurveillance systems

- Surveillance systems
- Serosurveys

The monitoring and evaluation of a newly-developed or well-established serosurveillance system is an important component of successful serosurveillance activities. Information gained from such an exercise should help improve serosurveillance programmes. As stated in the introduction, second-generation surveillance systems should be dynamic and adaptable to the needs of a country and should try to improve their performance. To achieve this, it is essential that the HIV surveillance system be monitored regularly and evaluated to identify the gaps, constraints and limitations in order to improve its quality. Other guidelines are available and provide more comprehensive information on the evaluation of public health surveillance programmes (CDC, 2001 and WHO/CDS/CSR/ISR/2001.2).

Evaluation involves the regular assessment of the HIV surveillance system's efficiency. It explains which components of the system are providing good information and which ones need to be strengthened in order to improve the quality of data collected. Evaluation is aimed at determining whether the goals of the surveillance system were achieved. It involves in-depth analysis of the HIV information system.

An evaluation should address the following key issues:

- Have the goals and objectives of the HIV surveillance system been clearly stated and met?
- Are there gaps in surveillance activities?
- Are there flow charts and task descriptions?
- Are there standard protocols for the mechanisms used to collect information?
- Are surveillance staff members trained to implement the protocol?
- How effective are the various components of the system (e.g., data collection, laboratory, questionnaires used)?
- Is a laboratory assessment tool in place, including a check list for laboratory assessment?
- What are the human and financial resources used, and the direct and indirect costs involved?

Important indicators for evaluating a serosurveillance system include:

- frequency and timeliness of surveys;
- appropriateness of population groups and geographic coverage of the sites included in the surveys;
- use of the same sites in each data collection cycle in order to measure trends in the epidemic;
- representativeness of coverage (i.e., whether the sites in the surveillance programme provide results for a representative sample of pregnant women) (Walker et al., 2001).
- Is the acquired information being analysed and adequately disseminated?

As discussed in 2.2.6, conducting quality assurance during serosurvey operations to monitor the accuracy of data collection and other field activities will help ensure accurate survey results and reliable data. Appendix 4 provides an indicator checklist that can be used to assess operational procedures by staff responsible for conducting quality assurance and programme monitoring during their visits to sites conducting serosurveys. Two important indicators are:

- the proportion of eligible patients actually included in the survey at each serosurvey site (completeness of enrolment of all eligible patients); and
- the proportion of enrolled patients from whom residual blood for unlinked HIV testing was obtained.

The accuracy of the data being collected and entered into the surveillance programme's database should also be monitored.

Appendix 1.

Recommended outline for a protocol for HIV serosurveillance among pregnant women

1. Introduction: review of existing HIV/AIDS epidemiological situation and justification for surveillance of HIV infection
 - 1.1. Objectives of the HIV serosurvey
2. General serosurvey methods
 - 2.1. Selection of sentinel populations
 - 2.2. Selection of sites for sentinel surveillance
 - 2.2.1. Site selection criteria
 - 2.2.2. Number of sites
 - 2.2.3. Distribution by sentinel population and location
 - 2.3. Sampling methods
 - 2.3.1. Periodicity of sampling
 - 2.3.2. Duration of sampling per sampling period
 - 2.3.3. Minimum sample size required per sentinel site
 - 2.3.4. Patient eligibility criteria
 - 2.4. Sociodemographic data and specimen collection
 - 2.4.1. Sociodemographic data collection
 - 2.4.1.1. Data collection form
 - 2.4.2. Blood sample collection and processing for HIV testing
3. Confidentiality
 - 3.1. Measures to ensure confidentiality
 - 3.2. Roles of staff members
4. Laboratory HIV testing
 - 4.1. Testing protocol
 - 4.2. Quality control of laboratory HIV testing
5. Data management: Methods for data entry, analysis and presentation of collected data
6. Personnel requirements/role of surveillance staff
 - 6.1. Blood collection, serum separation, storage
 - 6.2. Data and transport of specimens to the laboratory
 - 6.3. Laboratory HIV testing
 - 6.4. Data entry, analysis, presentation and interpretation
 - 6.5. District, regional and national supervision
7. Training and briefing requirements
 - 7.1. Sentinel site staff
 - 7.2. Laboratory staff

- 7.3. Staff assigned supervisory responsibility
- 7.4. Data management personnel
8. Quality assurance of serosurvey operations
 - 8.1. Supervision of sentinel site staff
 - 8.2. Data and specimen collection operations
 - 8.3. Data entry, analysis and presentation
 - 8.4. Roles and responsibilities of district, regional and national staff
9. Dissemination of serosurvey findings
10. Time line for implementation of the protocol for HIV surveillance
11. Budget

Appendix 2.

How to use Epi Info's STATCALC to determine sample sizes in simple random samples

1. From the main menu, select *Programs*.
2. Select STATCALC
3. Select *Sample size and power*
4. Select *Population serosurvey*
5. A screen will appear where one is required to enter the following information:
 - *Size of population from which the sample will be selected*
 - *Expected frequency of the factor under study*
(*err towards 50%*) – true rate in the population
 - *Worst acceptable rate*
(*furthest from the rate you would accept in your sample, high or low*)
6. Press *F4 Calculate*
7. The sample size is listed by confidence intervals.

The current version of Epi Info is available from www.cdc.gov/Epi Info.

Appendix 3.

Sample HIV surveillance data collection form for antenatal clinics

The following sample data collection form may be used to record sociodemographic and test result data.

Ministry of Health

HIV Surveillance Data Collection Form for Antenatal Clinics

Site: _____ District: _____

Demographic Information:

Survey ID code: <input type="text"/>	
Date of patient visit (dd/mm/yyyy): ___/___/___	Age (in years): <input type="text"/>
Residence: <input type="checkbox"/> URBAN <input type="checkbox"/> RURAL <input type="checkbox"/> Missing	
Highest level of school attended: <input type="checkbox"/> None <input type="checkbox"/> Primary <input type="checkbox"/> Secondary <input type="checkbox"/> Higher <input type="checkbox"/> Missing	
Occupation (Primary): (optional)	
<input type="checkbox"/> Business	<input type="checkbox"/> Housewife
<input type="checkbox"/> Police/military	<input type="checkbox"/> Domestic help
<input type="checkbox"/> Student	<input type="checkbox"/> Laborer
<input type="checkbox"/> Farmer	<input type="checkbox"/> Professional
Total number of pregnancies, including this pregnancy: <input type="text"/>	
Total number of live births: <input type="text"/>	

Test Result Information:

HIV	Screening (Initial Test) date: ___/___/___ dd mm yyyy	Positive <input type="checkbox"/> Negative <input type="checkbox"/>
	Confirmatory date: ___/___/___ dd mm yyyy	Positive <input type="checkbox"/> Negative <input type="checkbox"/>
Syphilis	RPR date: ___/___/___ dd mm yyyy	Positive <input type="checkbox"/> Negative <input type="checkbox"/>

Appendix 4.

Checklist for quality assurance of surveillance operations

Supervisory surveillance staff may use the following checklist as they monitor the quality of operational activities conducted at the sentinel site during supervisory visits.

Site name: _____	Site number: _____
Supervisor's name: _____	Date: _____
SAMPLING	
Total no. women visiting ANC since surveillance began: _____	
Total no. women sampled since surveillance began: _____	
No. of women sampled on last ANC day: _____	
Sampling consecutive?	()Yes ()No: _____
ANC staff present: ()Yes ()No: _____	Lab tech present: ()Yes ()No: _____
No. data forms: _____	No. blood samples: _____
No. data forms without ID#: _____	No. blood samples without ID#: _____
Comments:	

EQUIPMENT	
Cryovials stored in fridge: ()Yes ()No: _____	Fridge temperature: _____
Fridge working uninterrupted since last visit? ()Yes ()No: _____	
Centrifuge working?	()Yes ()No: _____
Comments:	

SAMPLE and DATA FORM TRANSPORT:	
No: _____	
No. data forms taken: _____	ID# range taken: _____
No. samples taken: _____	ID# range taken: _____
Site staff name: _____	Signature: _____
Comments:	

Appendix 5. Weighting infection rates (direct standardization)

Sentinel sites often serve clinic populations with different sociodemographic characteristics (e.g., age distributions). For example, clinic A may have a slightly younger patient population than clinic B. It therefore may have a higher overall HIV prevalence because younger women may engage in riskier sexual behaviour, and older women may have increased infertility associated with HIV. When the overall HIV prevalence of the two clinics is compared, it is best to weight, or directly standardize, the two prevalences based on a standard population's age-distribution (such as a country's census or total number of births by mother's age). Weighting, also called direct standardization, takes into account the different age-structures of the two clinics or sentinel sites and makes it possible to compare prevalence in these different sites. However, if the purpose is not comparison but rather resource allocation or evaluation, it is best to use the crude, unadjusted prevalence per site as this will represent the actual situation at a given site.

An example of weighting for age follows for two clinics (A and B) in country X. Clinic A is located in an urban zone, whereas clinic B is located in a more rural part of the country.

HIV prevalence in clinic A among women aged 15–49, 2000

Age (years)	No. tested	No. HIV-positive	Age-specific HIV prevalence (%)
15-19	75	12	16.0
20-24	100	20	20.0
25-29	125	31	24.8
30-34	80	21	26.3
35-39	60	10	16.7
40-44	30	1	3.3
45-49	30	1	3.3
Total	500	96	---

Overall crude prevalence for clinic A = $96 / 500 \times 100\% = 19.2\%$

HIV prevalence in clinic B among women aged 15–49, 2000

Age (years)	No. tested	No. HIV-positive	Age-specific HIV prevalence (%)
15-19	25	5	20.0
20-24	50	10	20.0
25-29	75	20	26.7
30-34	150	25	16.7
35-39	100	12	12.0
40-44	50	4	8.0
45-49	50	4	8.0
Total	500	80	---

Overall crude prevalence for clinic B = $80 / 500 \times 100\% = 16\%$

Clinic A's crude overall HIV prevalence (19.2%) is higher than that of clinic B (16%). However, the clinics have different age distributions: 60% of clinic A's population is younger than 30 years, but only 30% of clinic B's is younger than 30 years. To compare prevalence in the two clinics, the overall crude prevalence should be weighted by using a standard population (e.g., country-specific census data or data from one of the sampled clinics [A or B]). This procedure will adjust for the age differences between the two sites and remove any confounding of the overall prevalence that may be attributed to age. The following table shows census data for country X.

2000 census data for women in country X

Age (years)	Population
15–19	90,000
20–24	100,000
25–29	110,000
30–34	100,000
35–39	80,000
40–44	60,000
45–49	50,000

Multiplying the number of persons in each age category in the country by age-specific prevalence in the clinics will weight the overall crude prevalence for clinics A and B. This calculation will give the expected number of HIV-positive test results for each age group if the populations being compared had identical age distributions. This calculation should be performed for each age group in each clinic. For example, in clinic A, the expected number of infections for 15–19-year-olds would be as follows: 90,000 (number of women in the country aged 15–19) \times 0.16 (age-specific prevalence for 15–19-year-olds) = 14,400 expected infections. The following tables show the results of such calculations for clinics A and B.

Clinic A

Age (years)	2000 population	Age-specific prevalence in clinic A (%)	Expected number of HIV infections
15–19	90,000	16.0	14,400
20–24	100,000	20.0	20,000
25–29	110,000	24.8	27,280
30–34	100,000	26.3	26,300
35–39	80,000	16.7	13,360
40–44	60,000	3.3	1,980
45–49	50,000	3.3	1,650
Total	590,000		104,970

Clinic B

Age (years)	2000 population	Age-specific prevalence in clinic B (%)	Expected number of HIV infections
15–19	90,000	20.0	18,000
20–24	100,000	20.0	20,000
25–29	110,000	26.7	29,370
30–34	100,000	16.7	16,700
35–39	80,000	12.0	9,600
40–44	60,000	8.0	4,800
45–49	50,000	8.0	4,000
Total	590,000		102,470

The overall age-weighted prevalence for clinics A and B may thus be calculated as: expected number of HIV infections / total population of 15–49-year-olds in the country) × 100%

Clinic A:

$$104,970 / 590,000 \times 100\% = 17.8\%$$

Clinic B:

$$102,470 / 590,000 \times 100\% = 17.4\%$$

After taking into account the different age structures in clinics A and B, the difference in weighted prevalence between the two clinics is much smaller than the nonweighted prevalence.

For further information on the methodology of weighting, consult Hennekens CH and Buring JE, *Epidemiology in Medicine*, Boston/Toronto: Little, Brown and Company, 1987.

Appendix 6. Calculating the z-statistic

One commonly used test for determining significant differences between two prevalence estimates (either from two different sites or from the same site at two points in time) is to calculate a z-statistic for a difference in two proportions. Suppose the following data were collected from the same ANC at two different points in time:

Sample	Number of persons tested	Number of persons HIV-positive	Estimated HIV prevalence
#1	n_1	x_1	$p_1 = x_1 / n_1$
#2	n_2	x_2	$p_2 = x_2 / n_2$
Combined	$n (= n_1 + n_2)$	$x (= x_1 + x_2)$	$p = x / n$

Then the significance of the difference between p_1 and p_2 is determined from the z statistic, defined as:²

$$z = \frac{|p_2 - p_1| - \frac{1}{2} (1/n_1 + 1/n_2)}{\sqrt{p(1-p) (1/n_1 + 1/n_2)}}$$

To determine whether the HIV prevalence rates in the populations are different, compare the z statistic to the standard normal distribution, and calculate a p value for that statistic. A p value of less than 0.05 is generally considered statistically significant and provides evidence that the true population prevalences are indeed different from one another.

To illustrate the calculations with an example, the following data are proposed:

Sample	Number of persons tested	Number of persons HIV-positive	Estimated HIV prevalence
#1	400	20	$20/400 = .05 = 5\%$
#2	600	60	$60/600 = .10 = 10\%$

To calculate the z statistic, first calculate p for the combined samples:

Sample	Number of persons tested	Number of persons HIV-positive	Estimated HIV prevalence
Combined	1000 (= 400 + 600)	80 (= 20 + 60)	$80/1000 = .08 = 8\%$

² See for example Fleiss, 1981.

and then substitute all of the values into the formula for z itself:

$$z = \frac{|.10 - .05| - \frac{1}{2} (1/400 + 1/600)}{\sqrt{.08 (1-.08) (1/400 + 1/600)}} = \frac{.05 - .00208333}{\sqrt{.00030667}}$$

or $z = 2.74$. The p value for z , obtained from a table of standard normal values, is 0.0062. Since this p value is less than 0.05, one may conclude that there is a significant difference in the HIV prevalence for the populations represented by the two samples.

Appendix 7. Calculating chi-square statistics

Differences in HIV prevalence rates may also be tested using software that calculates chi-square statistics for 2-by-2 frequency tables. In these programs, the frequency tables should be defined as follows:

<u>Sample</u>	<u>Number of persons HIV- positive</u>	<u>Number of persons HIV- negative</u>
#1	x_1	$N_2 - x_1$
#2	x_2	$N_2 - x_2$

For the example above, the table would look like this:

<u>Sample</u>	<u>Number of persons HIV- positive</u>	<u>Number of persons HIV- negative</u>
#1	20	380
#2	60	540

These figures may then be entered into software for calculating chi-square statistics for 2-by-2 frequency tables. For example, if the Epi Info version 6 STATCALC program is used, the four numbers (20, 380, 60, 540) would be entered as shown, and the results would be as follows:

An example of calculating chi-square statistics by using Epi Info version 6 STATCALC

```

EpiInfo Version 6          Statcalc          November 1993
+ Disease -
+ 20      380      400
+ 60      540      600
+ 80      920      1000
E
X
D
o
S
H
P
e

          Analysis of Single Table
          Odds ratio = 0.47 (0.27 <OR< 0.82)
          Cornfield 95% confidence limits for OR
          Relative risk = 0.50 (0.31 <RR< 0.82)
          Taylor Series 95% confidence limits for RR
          Ignore relative risk if case control study.

          Chi-Squares      P-values
          Uncorrected      :      8.15      0.0043810 ←
          Mantel-Haenszel:      8.14      0.0043283 ←
          Yates corrected:      7.49      0.0062147 ←

          F2 More Strata; <Enter> No More Strata; F10 Quit

F1-Help  F2-Stratum  F5-Print  F6-Open File  F10-Done
  
```

The appropriate test statistic for comparing two prevalence rates is the Yates-corrected chi-square (X^2), which is calculated to be 7.49. This statistic has a p value of 0.0062, which is less than the traditionally-used significance level of $p=0.05$, so one may again conclude that the prevalence in the populations represented by the two samples is indeed different.

The p value displayed by STATCALC for the Yates-corrected chi-square is exactly the same as the p value for the z statistic calculated earlier. Note: the square root of the Yates-corrected chi-square is the same value as z (i.e., the square root of 7.49 is 2.74). This relationship between the chi-square and the z statistic will always be true. The Yates-corrected chi-square for a 2-by-2 table and the z statistic for a difference in prevalence are essentially the same, the former simply being the square root of the latter³.

³ The Epi Info EPITABLE procedure may also be used to compare two proportions. In some ways, this procedure is more intuitive to use than is an analysis based on a 2-by-2 table. EPITABLE will provide the same results as the 'uncorrected' chi-square of STATCALC. Most statisticians prefer that the Yates-corrected chi-square be used. Therefore the STATCALC procedure has been recommended for this particular analysis. The formula for the z statistic presented at the beginning of this section incorporates the Yates correction.

Appendix 8. Calculating chi-square tests for linear trends

A common method for determining whether or not changes (i.e. increases or decreases) have occurred in annual HIV prevalence over time is to calculate chi-square tests for linear trends. Consider the following example of hypothetical prevalence data collected from an ANC in 1998–2002:

Year	Number of persons tested	Number HIV-positive	Number HIV-negative	Estimated HIV prevalence
1998	400	60	340	0.150
1999	400	68	332	0.170
2000	400	73	327	0.183
2001	400	77	323	0.193
2002	400	80	320	0.200

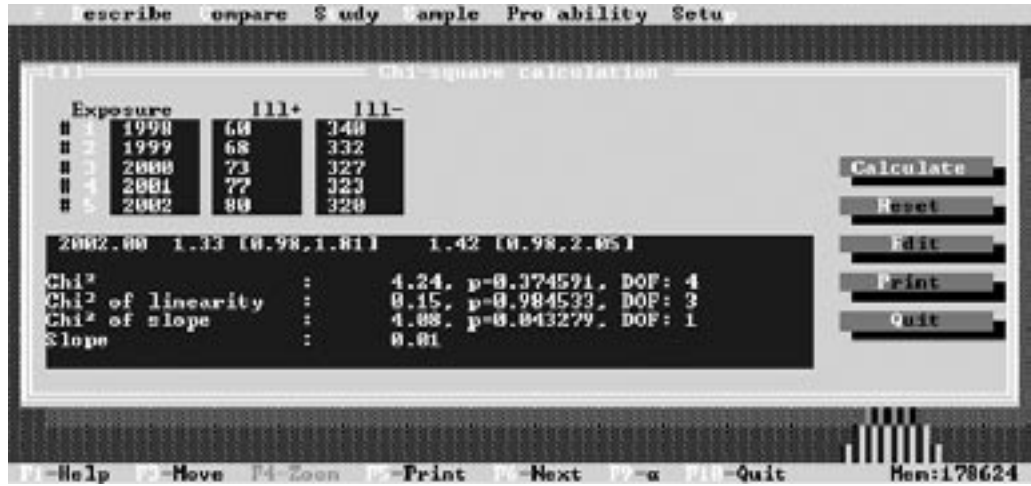
From the table, it appears that the prevalence rates are increasing from year to year. However, not all two-sample comparisons that are made are necessarily statistically significant at the 0.05 level. For example, a two-sample comparison of the 1998 data with those for 2002 gives a z statistic of 1.77. That value of z has a p value of 0.077. A comparison of the 2001 data with the 2002 data gives a z statistic of 0.178, which has a p value of 0.859. Based on these two comparison samples alone, the increases in prevalence do not appear to be statistically significant.

If data from each year are used, a different answer is obtained. The chi-square test statistic of a linear trend may also be calculated using the Epi Info EPITABLE procedure (see footnote 4). To select the test for trend, do the following:

1. Run EPITABLE
2. Select the 'Compare' option
3. Select the 'Proportion' option
4. Select the 'Trend – Quantitative Data' option

The sample data would be entered as shown in the figure opposite.

An example of calculating significance in trends by using Epi Info's EPITABLE



The resulting statistic for testing whether the trend in prevalence is increasing or decreasing over time is called the chi-square of slope by EPITABLE. In the example, the chi-square of slope is 4.08. This statistic has a *p* value of 0.043, which is statistically significant at the 0.05 level. It can therefore be concluded that the prevalence among all ANC attendees is indeed changing with time.

The chi-square test for linear trend is based on the assumption that the increase (or decrease) in the prevalence is indeed linear over time – hence the use of the term ‘linear trend’ in the name of the test. It is possible to test whether the linearity assumption is false⁴. In EPITABLE⁵, this test statistic is called the chi-square of linearity. In this example, the test statistic is 0.15, which has a *p* value of 0.984. This *p* value is not significant, indicating that the assumption of linearity is reasonable. Plotting the prevalence estimates by year can also provide a visual indication of whether this assumption of linearity is a reasonable one.

⁴ See, for example, Armitage, 1955.

⁵ The Epi Info STATCALC procedure will also calculate the chi-square of slope. However, it does not calculate the chi-square of linearity, so EPITABLE is preferred.

Glossary

Bias: A systematic error in the conduct of a research study. Any trend in the collection, analysis, interpretation, publication, or review of data that can lead to conclusions that are systematically different from the truth.

Concentrated epidemic: The epidemic state in which HIV has spread rapidly in a defined subpopulation but is not well established in the general population. (HIV prevalence is consistently >5% in at least one defined subpopulation and is <1% in pregnant women in urban areas.)

Confidence interval: A range of values that has a specified probability of including the true value of the variable—e.g., HIV prevalence. The specified probability is called the confidence level, and the end points of the confidence interval are called the confidence limits.

Cross-sectional study: A study or screening test conducted in a defined population at a single point in time.

Descriptive analysis: A summary of data for the entire serosurvey sample and for each subgroup for which sociodemographic data have been collected.

Enzyme immunoassay (EIA): A type of HIV test that identifies antibodies to HIV. EIAs rely on a primary antigen-antibody interaction and can use whole viral lysate of HIV or one or more antigens from the virus.

Generalized epidemic: The epidemic state in which HIV is firmly established in the general population. (HIV prevalence is consistently >1% in pregnant women.)

Incidence: The number of new cases of infection or disease in a defined population within a specified time period (i.e., the rate of incidence).

Information bias: A systematic error in the collection of information for the serosurvey, such as inaccurate recording of age. A flaw in measuring exposure or outcome that results in different levels of accuracy of information between compared groups.

Informed consent: Informed consent is based on the principle that competent persons are entitled to make decisions regarding their participation in, or acquiescence to, certain events in the context of a professional relationship between health-care provider and patient/client. Informed consent protects the person's freedom of choice and respects his/her autonomy, particularly with regard to decisions affecting his/her body and health.

Linked anonymous testing: In linked anonymous testing, a person agrees to have an HIV test, but the specimen is labeled with a code without a name or identifiers that could reveal the person's identity. This method is voluntary and requires obtaining informed consent and making the test results available (with appropriate counselling) to the person tested.

Linked confidential testing: In linked confidential testing, a person agrees to have an HIV antibody test with the assurance that the test result will be kept confidential and only selected health-care providers may be informed. This method is voluntary and requires obtaining informed consent and discussing the test results with the person. Linked confidential testing also allows for the collection of more detailed sociodemographic and risky-behaviour information.

Low-level epidemic: The epidemic state in which HIV has never spread to significant levels in any subpopulation, although HIV infection may have existed for many years. (HIV prevalence has not consistently exceeded 5% in any defined subpopulation.)

Mother-to-child transmission (MTCT): Transmission of HIV from the mother to the fetus or infant during pregnancy, delivery, or breastfeeding.

Participation bias: A systematic error due to differences in characteristics between those who are willing to participate in a study and those who are not.

Period prevalence: The percentage of persons known to have had a disease or condition at any time during a specified period.

Point prevalence: The percentage of persons with a disease or condition at a specified point in time.

Prevalence: The percentage of persons in a given population with a disease or condition at a given point in time.

Quality assurance: The dynamic and ongoing process of monitoring a system for reproducibility and reliability of results that permits corrective action when established criteria are not met.

Rapid test: An HIV antibody test that is simple, does not require any reagents or equipment other than what is contained in the kit, and provides fast results.

Sample: A selected subset of a population.

Second generation HIV surveillance: Developed by the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS), second generation HIV surveillance is a conceptual framework for improving HIV surveillance. Guidelines for second-generation HIV surveillance include approaches for making better use of data to increase and improve the response to the HIV epidemic.

Selection bias: A systematic error due to differences in characteristics between those selected for study and those not selected for study.

Sentinel surveillance: Surveillance conducted through 'watchpost' sites that provide access to populations that are of particular interest or representative of a larger population.

Serosurveillance: Epidemiologic study or activity based on the detection through serologic testing of the presence or absence of HIV antibodies. Latent, subclinical infections and carrier states can thus be detected, in addition to clinically overt cases.

Stratification: The separation of a sample into several subsamples based on specified criteria, such as age range, residence.

Testing strategy: The use of an appropriate HIV test or combination of HIV tests. The choice of testing strategy used is based on the objective of the test, the sensitivity and specificity of the test, and HIV prevalence in the population being tested. HIV testing strategies were created to maximize accuracy and minimize cost.

Unlinked testing: In unlinked testing, a sample of blood originally collected for other purposes is tested for HIV after all the information that could identify the source of the blood is eliminated from the sample.

Unlinked anonymous testing with informed consent: In unlinked anonymous testing with informed consent, the participant gives consent for HIV testing and is then given an HIV test, in which all personal identifying information is removed from the specimen being tested.

Voluntary counselling and testing (VCT): Voluntary Counselling and testing for HIV that is offered free of coercion. With VCT, patients have the option to accept or refuse HIV testing.

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The Joint United Nations Programme on HIV/AIDS (UNAIDS) brings together nine UN agencies in a common effort to fight the epidemic: the United Nations Children's Fund (UNICEF), the World Food Programme (WFP), the United Nations Development Programme (UNDP), the United Nations Population Fund (UNFPA), the United Nations Office on Drugs and Crime (UNODC), the International Labour Organization (ILO), the United Nations Educational, Scientific and Cultural Organization (UNESCO), the World Health Organization (WHO), and the World Bank.

UNAIDS, as a cosponsored programme, unites the responses to the epidemic of its nine cosponsoring organizations and supplements these efforts with special initiatives. Its purpose is to lead and assist an expansion of the international response to HIV/AIDS on all fronts. UNAIDS works with a broad range of partners – governmental and nongovernmental, business, scientific and lay – to share knowledge, skills and best practices across boundaries.

Produced with environment-friendly materials

Surveillance systems are an essential component of public health activities. They serve as early-warning systems for the detection of changes in diseases or patterns, which allow for the necessary interventions to be put into practice to prevent new infections. Since 1999, the UNAIDS/WHO HIV Surveillance Working Group has been promoting a new approach to monitoring HIV infection, known as second-generation surveillance. A key component of such surveillance are HIV prevalence surveys (showing whether and by how much prevalence rates are increasing or decreasing, and which populations are affected), and this contributes to effective monitoring of the epidemic, while also helping to enhance the planning and evaluation of prevention programmes.

These guidelines are aimed at National AIDS Programme managers and epidemiologists who work in HIV surveillance, and are intended to clarify the use of appropriate methods and strategies in setting up a sentinel surveillance system among antenatal clinic attendees and other groups. It describes the ethical context, data collection procedures and the analysis and use of information collected in different population groups.



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