

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

Guidelines for Using HIV Testing Technologies in Surveillance



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Guidelines for Using HIV Testing Technologies in Surveillance: Selection, Evaluation and Implementation

2009 update

“It is anticipated that the recommendations in this guideline will remain valid until 2015. The Department of HIV/AIDS at WHO headquarters in Geneva will be responsible for initiating a review of this guideline at that time”.



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Global surveillance of HIV/AIDS and sexually transmitted infections (STIs) is a joint effort of the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS). The UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance, initiated in November 1996, is the main coordination and implementation mechanism for UNAIDS and WHO to compile the best information available, and to improve the quality of data needed for informed decision-making and planning at the national, regional and global levels.

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HIV Testing Technologies for Surveillance

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Acronyms

ART	antiretroviral therapy
ARV	antiretroviral
BSI	body substance isolation
DBS	dried blood spot
DHS	demographic health survey
DPS	dried plasma spot
DSS	dried serum spot
EIA	enzyme immunoassay
EQAS	external quality assessment scheme
EQC	external quality control
ICU	intensive care unit
IDU	injection drug user
IQC	internal quality control
MSM	men who have sex with men
PEP	postexposure prophylaxis
QA	quality assurance
QC	quality control
SGS	second generation HIV surveillance
STI	sexually transmitted infection
TB	tuberculosis
UNAIDS	Joint United Nations Programme on HIV/AIDS
UP	universal precautions
VCT	voluntary counselling and testing
WHO	World Health Organization

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Guidelines for Using HIV Testing Technologies in Surveillance

As the HIV/AIDS epidemic imposes an ever-larger burden globally, surveillance for HIV becomes more critical in order to understand the trends of the epidemic and make sound decisions on how best to respond to it. This is especially true in low- and middle-income countries, which account for a disproportionate share of new and long-standing infections. To help countries focus their surveillance activities in the context of their epidemic state (low-level, concentrated or generalized), the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) have developed a conceptual framework to improve HIV surveillance, known as Second Generation HIV Surveillance (SGS)¹ Guidelines for SGS suggest approaches to make better use of data so that the response to the HIV epidemic can be enhanced. As serosurveillance is an important component of most HIV surveillance activities, an understanding of current HIV testing technologies is important.

In the context of SGS, these guidelines suggest methods for selecting, evaluating and implementing HIV testing technologies and strategies based on a country's laboratory infrastructure and surveillance needs. The guidelines provide recommendations for specimen selection, collection, storage and testing, and for the selection and evaluation of appropriate HIV testing strategies and technologies to meet surveillance objectives. Quality assurance issues are also addressed.

These technical guidelines are written for HIV surveillance coordinators and laboratory professionals involved in HIV testing for surveillance purposes in low- and middle-income countries. They are part of a series of operational guidelines for SGS systems.

These guidelines were first published in 2001; this revision provides updated information on HIV testing for surveillance.

1.0 Introduction

summary

Topics addressed in these guidelines

- Specimen selection, collection, storage and testing
- HIV testing technologies and strategies
- Selecting and evaluating testing technologies
- Quality assurance measures

Aim and Objectives

Aim

The aim of “Guidelines for using HIV testing technologies in surveillance update 2008” is to support countries in ensuring that appropriate HIV testing strategies and selection of HIV tests are used when conducting HIV surveillance activities in countries with different epidemic situations and in different populations.

Objectives

This document is designed to support the strengthening and improvement of HIV surveillance programs in countries. The specific objectives are to:

Provide technical advice on:

- the types of specimens to be collected and how to store and test them
- HIV testing strategy and algorithms recommended by WHO/UNAIDS,
- HIV testing technologies used for biological surveillance
- the selection and evaluation of HIV testing strategies and technologies
- quality assurance of HIV testing in HIV surveillance systems

The recommendations and algorithms provided in this document are specific to the HIV surveillance and some of these are not designed for other applications such as diagnostic testing.

Target audience

This document is primarily intended for use in countries that conduct HIV sero surveillance in different populations and epidemic contexts. They have been developed for use by:

- Program managers responsible for national AIDS Programs
- Epidemiologist responsible for undertaking HIV surveillance activities in countries
- Laboratory technical staff in laboratories undertaken HIV testing
- Laboratory technical staff in reference laboratories.

The document may also be useful for other relevant stakeholders such as training institutions, bilateral or multilateral donors and programs supporting HIV surveillance activities.

Methodology

Who was involved?

In 2001 the The UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance developed and published a guidelines in HIV testing technologies in the context of Second Generation Surveillance. In the context of second generation HIV surveillance, these laboratory guidelines suggest methods for selecting, evaluating, and implementing HIV testing technologies and strategies based on a country's laboratory infrastructure and surveillance needs. The guidelines provided recommendations for specimen selection, collection, storage, and testing and for the selection and evaluation of appropriate HIV testing strategies and technologies to meet surveillance objectives. Quality assurance issues were also addressed.

In 2007 a group of experts from the Global HIV surveillance Working Group and CDC experts in the field undertook a revision of the 2001 guidelines in order to update them. The working group emphasized the need to ensure that the recommendations would be evidence-based to take into account new technologies and developments that are relevant for HIV surveillance activities.

The key professional staff and reviewers were Bharat Parekh, Kassim Sidibe, Sadhna Patel, John Nkengasong from the Centers for Disease Control and Prevention (CDC), Atlanta, Dr Gaby Vercauteren and Anita Sands (ETH), Jesus M Garcia-Calleja (HIV) from WHO, Peter Ghys, (UNAIDS)

The members of the The UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance we also responsible for reviewing the final drtaft.

How was the evidence identified?

During the period of implementation of Second Generation Surveillance guidelines countries have undertaken more often HIV surveillance activities in different population groups. At the same time technologies for HIV testing have evolved, with access to rapid tests, new ELISA and tools for collecting blood samples as the dried blood spots. Based in the expert experience on the field and the regular revisions made by WHO/Essential health technologies department and HIV testing technologies, a revision of the 2001 guidelines were undertaken. The initial draft of the updated guidelines were developed by CDC.

How were the recommendations developed, reviewed, revised and finalized?

The draft was thus subjected to an extensive consultative and review process by international experts, of the UNAIDS/WHO Working Group on Global HIV/AIDS/STI surveillance and was shared with experts in the WHO regional offices. Comments extensive revisions were made by the members of the working group.

Declaration of interests

Conflict of interest statements were collected from all of the above major contributors. No conflict of interest has been declared by any contributors to the document.

The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) recommend the use of second generation HIV surveillance (SGS) to improve collection, analysis and use of data essential for AIDS control programmes. Use of the SGS strategy is also promoted to help national and international institutions monitor the epidemic and guide their responses to it.¹ Using SGS approaches, surveillance systems should be flexible in order to change with a country's needs and state of the epidemic: low-level, concentrated or generalized (Box 1).¹ As countries develop or enhance their surveillance programmes using SGS principles, the surveillance data collected can be better used for the purposes described above.

Box 1. Three different epidemic states

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.

Concentrated epidemic

The epidemic state in which HIV has spread rapidly in a defined vulnerable subpopulation with high-risk behaviours but is not well established in the general population.

Generalized epidemic

The epidemic state in which HIV is firmly established in the general population.

These categories do not imply transition from one type of epidemic to another and are not linear; in the sense that it is possible for countries to have different epidemics at the same time in different geographical locations or populations, especially in large countries.

SGS relies on data collected from biological surveillance (HIV serosurveillance), behavioural surveillance and other sources (e.g. HIV/AIDS case surveillance, death registration, sexually transmitted infection [STI] surveillance, tuberculosis [TB] surveillance) to describe a country's HIV epidemic and respond effectively. It aims to improve integration of data from these sources. It also supports continuous research into new epidemiological tools; improved methods for building estimates and modelling the epidemic; and better ways for using data for advocacy, planning, monitoring and evaluation.

HIV serosurveillance data are used to estimate HIV prevalence rates and the geographical distribution of infection, monitor trends over time in specific population groups and identify subpopulations at increased risk for infection. This information is then used to assist countries' efforts to set HIV policy and priorities, plan and evaluate prevention programmes, and evaluate the effectiveness of countries' responses to the epidemic.

As HIV serosurveillance is the primary method for determining HIV prevalence locally or nationally, accurate HIV testing is critical for SGS. With advances in diagnostic immunology, HIV testing technologies have improved greatly. Currently available enzyme immunoassays (EIAs) are more accurate than earlier generations of EIAs, and the latest generation of HIV rapid tests can provide results similar to EIAs in less than 30 minutes with minimal or no need for equipment. HIV rapid tests can be performed by non-laboratory staff after adequate training and with the necessary quality assurance measures (including on-site supervision). These rapid tests enable testing for HIV surveillance activities in areas where testing could not previously be conducted (e.g. areas with limited laboratory resources) and among hard-to-reach populations (e.g. female sex workers) who may not be accessible through clinical services.

This document provides guidance on the types of specimens to be collected and how to store and test them. It describes the HIV testing strategy recommended by WHO, and existing HIV testing technologies used for biological surveillance. It also includes information regarding strategies and technologies for use in diagnostic testing since data from diagnostic testing may also be used as a source of surveillance information. These guidelines outline methods for selecting testing technologies appropriate to a country's epidemic state and needs. Because accurate results are important in biological surveillance for HIV, quality assurance (QA) measures are also addressed. The guidelines also include a glossary of terms used in the document. These technical guidelines have been written for HIV surveillance coordinators and other health professionals involved in HIV testing for surveillance purposes in low- and middle-income countries. They are part of a series of operational guidelines for SGS systems.

2.0 Overview of HIV testing in HIV serosurveillance

summary

Overview of HIV testing in HIV serosurveillance

- Objectives of HIV testing
 - Surveillance
 - Diagnosis
 - Blood screening
- HIV testing approaches
 - Unlinked testing
 - Linked testing
- Populations surveyed

In order to appreciate the context of these guidelines, it is important to understand the objectives of conducting HIV testing, the different testing approaches used in serosurveillance and populations tested.

2.1 Objectives of HIV testing

HIV testing can be conducted for surveillance, diagnosis or blood screening purposes.² In low- and middle-income countries, most HIV testing for surveillance purposes is conducted as part of seroprevalence surveys among population groups such as pregnant women attending antenatal clinics, patients with STIs, female sex workers, or injection drug users (IDUs). National population-based surveys have also been conducted for an increasing number of countries with a generalized epidemic; these provide an estimate of HIV prevalence among adults living in households.^{3,4,5} In addition, the results of testing for diagnostic purposes (e.g. in voluntary counselling and testing [VCT] clinics) and for blood screening purposes (e.g. for blood donation), as well as the results of regular testing of other groups (e.g. military recruits) can provide additional prevalence data for surveillance purposes. These data from diagnostic testing, blood screening and regular HIV testing of other groups should not be extrapolated to the general population because they contain inherent biases.

2.2 HIV testing approaches

The selection of HIV testing approaches for serosurveillance depends on contextual factors, such as country policies and depending on the epidemic state, appropriate population groups and settings for HIV testing. Unlinked anonymous HIV testing (without informed consent) for surveillance is only conducted in clinic settings where blood is collected regularly for other purposes (usually syphilis testing) (Box 2). These settings should offer referrals to VCT. Linked testing (confidential or anonymous) with informed consent is the preferred approach when the specimens are collected solely for HIV testing, for example, HIV surveillance in populations not accessible through clinic settings (e.g. population-based surveys, hard-to-reach populations such as IDUs, female sex workers, and men who have sex with men [MSM]) (Box 2). When specimens are collected solely for HIV testing, unlinked anonymous testing with informed consent may also be used, depending on the country's relevant policies and guidelines. Participants should be provided access to counselling and testing.

When informed consent is required (Box 2), it must be obtained before the specimen is tested for HIV, following the country's relevant policies and guidelines. Whenever informed consent is obtained, participation bias is an important issue, and should be assessed and taken into consideration in the analysis.

A study code should be assigned to every specimen tested for HIV. The study code is used to identify each specimen during the testing procedures. It is unique to a specimen, and it may or may not be linked to personal identifying information (e.g. name, clinic identification number), depending on whether the HIV testing is linked or unlinked. The study code can be linked to demographic (e.g. age, sex, marital status, geographical area of residence) (in linked and unlinked testing) and risk behaviour information (in linked testing) that is obtained at the time the specimen is collected.

Box 2. Linked and unlinked HIV testing

Unlinked anonymous testing (without informed consent)

- Testing of unlinked specimens collected for other purposes
- No informed consent required
- Test results are not returned so no counselling conducted
- No personal identifiers or names obtained
- Coded specimen

Unlinked anonymous testing (with informed consent)^a

- Testing of unlinked specimens collected solely for surveillance purposes
- Informed consent^b required
- Test results are not returned so no counselling conducted
- No personal identifiers or names obtained
- Coded specimen

Linked anonymous testing (with informed consent)

- Testing of specimens collected specifically for HIV testing
- Informed consent and pre- and post-test counselling required
- No personal identifiers or names obtained
- Coded specimen; code given to patient so that only patient can link himself or herself to results

Linked confidential testing (with informed consent)

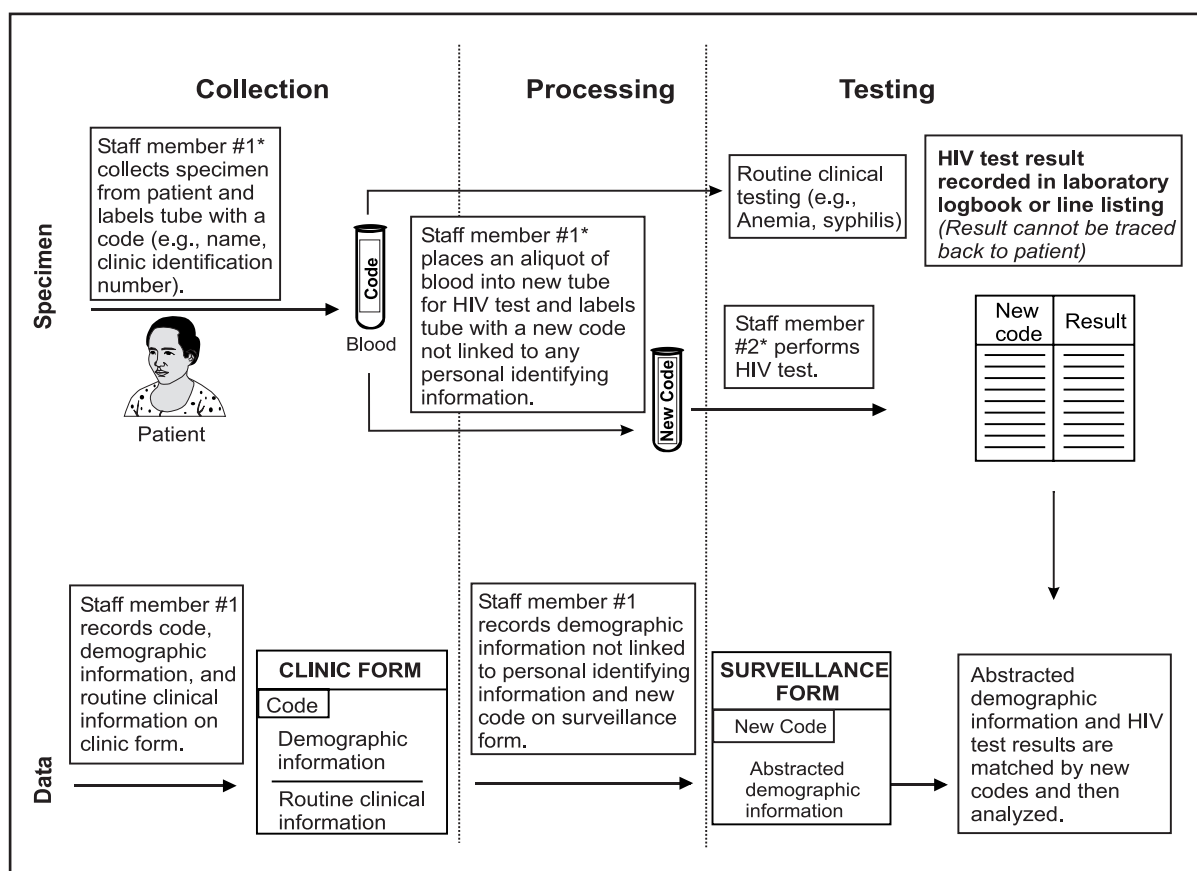
- Testing of specimens collected specifically for HIV testing
- Informed consent^b and pre- and post-test counselling required
- Personal identifiers or names obtained
- Coded specimen; code linked to personal identifying information

a The use of this method is described by the UNAIDS/WHO Working Group on Global HIV/AIDS/STI Surveillance; its use in HIV surveillance activities should be guided by the policies of the country in which these activities are carried out.

b 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. (Adapted from WHO/Global Programme on AIDS 1992)⁶

Unlinked anonymous testing (Figure 2.1) without informed consent is conducted only in clinical settings in which a specimen originally collected for other purposes, such as syphilis testing at antenatal clinics, is tested for HIV, after all information that could identify the source of the specimen is removed from the specimen tube. Thus, the test result may not be traced back to the patient nor the patient informed of the test results.

Figure 2.1 Unlinked anonymous HIV testing



*Staff members #1 and #2 should not be the same person so that the patient's anonymity is ensured.

To ensure patient anonymity at the clinic and laboratory, one staff member should collect the specimen and a different staff member should perform the test. One staff member (clinician or laboratory technician) should collect and process the specimen for routine clinical testing and unlinked anonymous HIV testing. Processing of the specimen for unlinked anonymous HIV testing requires removing an aliquot of the specimen, i.e. the specimen is split and placed it into a new tube that is labelled with a new study code not linked to any personal identifying information. It is good practice to ensure that study codes are randomly assigned to ensure complete delinking from any personal identifying information. Another staff member should perform unlinked anonymous HIV testing.

In some settings, one staff member (the laboratory technician) is responsible for both collecting the specimen and performing unlinked anonymous HIV testing. In these situations, it is suggested that another staff member process the specimen for unlinked anonymous testing (i.e. split the specimen and place it in another tube labelled with a new study code not linked to any personal identifying information).

At the time of specimen collection, a staff member collects demographic information (e.g. age, sex, marital status, geographical area of residence [e.g. urban/rural], parity, education) and the medical history from the patient. This information is recorded onto a clinic form along with the code on the specimen (e.g. clinic identification number). After the specimen

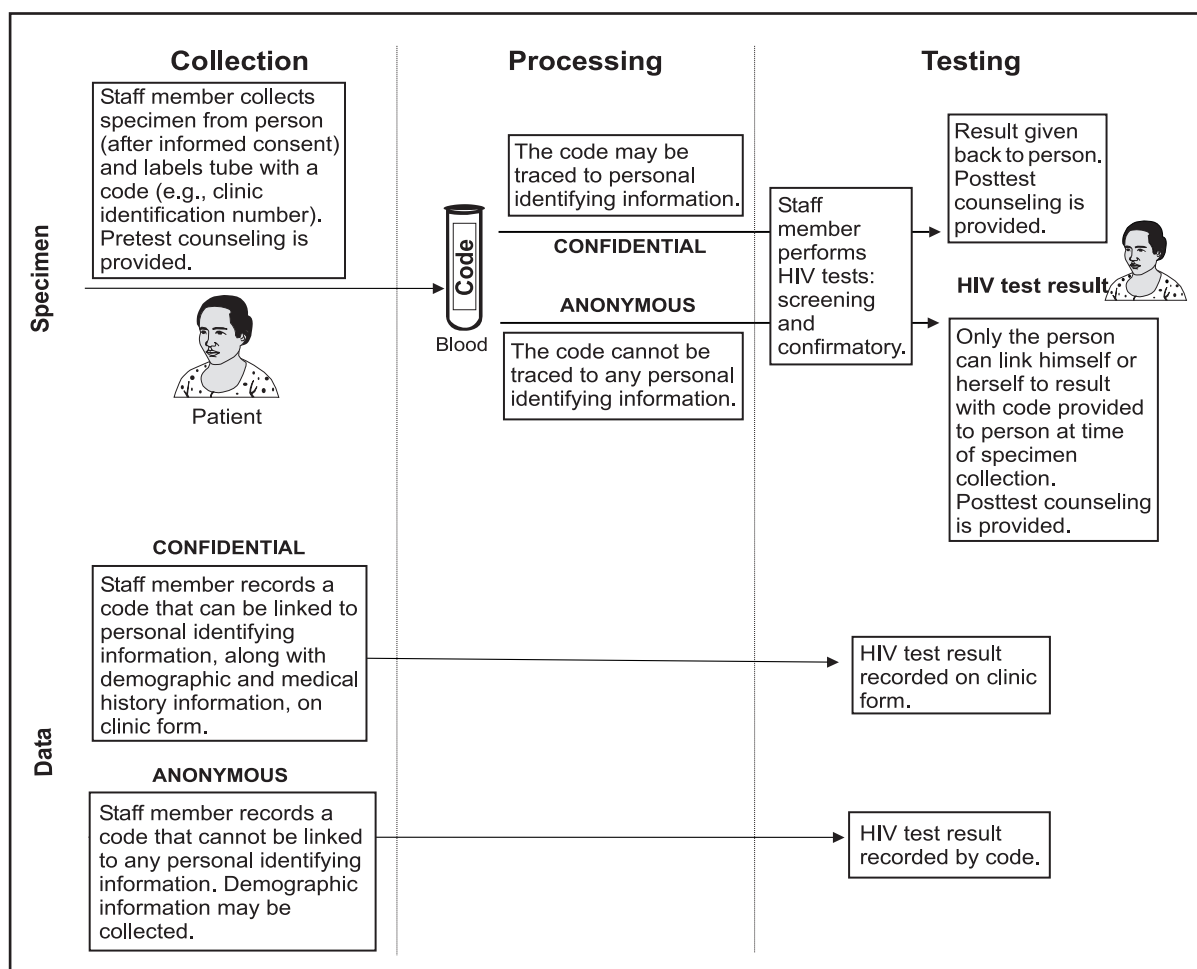
is processed for unlinked anonymous HIV testing and the aliquot is labelled with a new study code, the same staff member records the new study code onto a surveillance form and abstracts the needed demographic information (e.g. age, sex, marital status, geographical area of residence [e.g. urban/rural], parity, education) onto the form. This abstracted information is therefore not linked to any personal identifying information. The unlinked anonymous HIV test results can then be merged with the demographic information for analysis by the new study code. It is important to not compromise the anonymity of the specimen by collecting too much demographic information that may allow identification of individuals.

In settings where specimens are not routinely collected for other purposes, unlinked anonymous testing without informed consent is considered unethical. Therefore, in these situations, linked testing with free access to VCT should be provided or, if country policy permits, unlinked anonymous testing with informed consent should be conducted. In unlinked anonymous testing with informed consent, the participant is asked whether he or she would agree to participate in the HIV surveillance study and is informed that the results of the HIV test performed will be unlinked by removing all personal identifying information from the specimen. Therefore, it will not be possible to trace which participants have positive test results. The person can refuse to participate in the study, thereby introducing a possible participation bias. Unlinked anonymous testing is typically used in national population-based surveys.^{3,4} It should be noted that the use of unlinked anonymous testing in national surveys means that survey participants do not benefit from knowing their serostatus within the survey. This is compensated for by providing the participants with access to confidential HIV testing and counselling outside the survey.

Linked testing (Figure 2.2) involves linking the results of the HIV test with the person tested and allows the person to receive his or her HIV test results. This method requires obtaining informed consent and providing pre- and post-test counselling. Linked testing may or may not occur in a clinical setting, and can be either confidential or anonymous. Since the person tested may receive the test results, as has been done in some demographic health survey (DHS) studies in Africa, linked testing requires additional confirmatory HIV testing using the HIV diagnostic testing strategy. In linked confidential testing, a person agrees to have an HIV test with the assurance that the test result will be kept confidential and only selected health-care providers may be informed on a need-to-know basis. HIV test results can be matched to the person by a specimen code linked to personal identifying information. For linked confidential testing, a staff member obtains informed consent and provides pre-test counselling prior to specimen collection. The specimen is then labelled with a specimen code that can be linked to personal identifying information (e.g. name, clinic identification number). After linked confidential HIV testing is performed, the test results are given to the person, along with post-test counselling.

In linked anonymous testing, a person consents to having an HIV test. The methods for collecting and processing the specimen are the same as for linked confidential testing. However, the specimen is labelled with a study code not linked to any personal identifying information, and only the person can link himself or herself to the test result.

Figure 2.2 Linked confidential and anonymous HIV



2.3 Populations surveyed

The state of the epidemic determines which population groups need to be surveyed. Countries with generalized epidemics conduct sentinel serosurveillance primarily among pregnant women at antenatal clinics as the basis of their surveillance systems. Serosurveillance is typically conducted in large clinics but in the past few years countries have added a number of rural clinics to the national serosurveillance system to collect more accurate information on HIV levels and trends among the rural population, outpatient clinics at hospitals or health centres. In addition, national population-based surveys are conducted with a recommended frequency of approximately once every five years.⁵ Countries with concentrated or low-level epidemics focus primarily on specific population groups perceived to be at high risk for infection, for example, female sex workers and their clients, IDUS or MSM. Therefore, venues attended by such persons are selected as sentinel sites; these may include clinics that treat STIs, drug treatment centres, correctional facilities, brothels, bars and clubs. Increasingly, these populations are sampled in their communities, with the aim of achieving samples that are as representative as possible within the selected geographical area. Other populations surveyed can include (1) military recruits or conscripts and (2) occupational groups at increased risk for infection, such as factory workers,

miners or migrant workers. The prevalence rates determined for these specific population groups should be applied only to the group studied, not to the general population.

Further discussion of the methods used for the selection and sampling of appropriate subgroups and sites for conducting HIV surveillance activities can be found at <http://www.who.int/hiv/pub/surveillance/en/> and <http://www.unaids.org/en/KnowledgeCentre/HIVData/Epidemiology/epipublications.asp>.

recommendation

- If the epidemic is generalized, antenatal clinics are the preferred sites for regular serosurveillance and national population-based surveys are recommended periodically.
- If the epidemic is concentrated or at a low level, the surveillance system should focus on populations at increased risk for infection (e.g. female sex workers and their clients, IDUs and MSM).

2.4 Prospects for estimating HIV-1 incidence using laboratory-based tests

HIV-1 incidence can provide valuable information about the current dynamics of HIV infection in the population but is difficult to measure. The development of tests to detect recent HIV-1 infections offers the prospect of allowing estimation of the incidence.^{7,8,9,10,11}

Since surveillance activities already collect specimens for the determination of HIV-1 prevalence, testing for recent HIV-1 infections could be added provided specimens are processed and stored in optimal conditions. Advances in testing for recent HIV infections are expected to permit the use of serum, plasma, dried blood spot (DBS), dried plasma spot (DPS) or dried serum spot (DSS) specimens on filter-paper, which facilitates specimen processing, storage and transport.¹²

It is important to note that tests for recent HIV-1 infections are quantitative antibody tests and are therefore more easily affected by the quality of the specimen, which may have a lesser impact on HIV diagnostic tests. Performance of recent HIV-1 infections tests may also be affected by HIV-1 subtypes, different sub population, AIDS or low CD4 cell count, antiretroviral therapy (ART) or other factors. Moreover, there are individual differences in antibody development, which suggest that these tests should be used only for population incidence estimates and not for identifying individuals with a recent infection. Other HIV tests are available for detecting recent HIV infections at individual level.

Recent developments suggest that current approaches to estimating the incidence rate using the available tests may overestimate the incidence rate in certain populations. To address this overestimation for BED assays, adjustment factors have been proposed.^{13,14,15,16,17,18} The need to validate current and future HIV incidence tests has been recognized, and WHO in collaboration with technical experts is developing guidance in this area.

Newer HIV incidence tests have been developed to detect recent HIV infections. These tests use antibody avidity or differential development of antibodies to various proteins in an EIA format. All recent HIV infection tests need to be reviewed for optimization, and evaluated and validated before they can be deployed for estimation of incidence.

3.0 Specimens used for HIV testing

summary

Specimens used for HIV testing

- Selecting specimens
 - Advantages and disadvantages of serum and plasma
 - Advantages and disadvantages of whole blood
 - Advantages and disadvantages of dried blood spots (DBS)
 - Advantages and disadvantages of oral fluids
- Collecting, processing and storing blood specimens
- Collecting and storing DBS
- Collecting and storing oral fluids
- Labelling and logging specimens

Many types of specimens can be used for biological surveillance of HIV: plasma, serum, whole blood, DBS and oral fluid. The choice of specimen collected depends on the logistics, populations and sites selected, and the HIV testing strategy and algorithm. Specimens must be collected, tested and stored in an appropriate manner in order to obtain accurate and reliable results.

For serosurveillance activities, specimens are usually collected and stored prior to HIV testing at a regional or national laboratory. Serum, plasma and DBS can be stored and tested at a later date; specifications for storage will depend on the type of specimen collected.

Specimens not tested on site at the local level will need to be transported to a regional or national laboratory for testing. The methods by which specimens are transported will depend on the country's infrastructure. Few countries may have courier systems linking health-care facilities and laboratories. More frequently, the field surveillance staff members themselves transport the specimens from the local to the national laboratory. However, other options such as public transportation can be explored.

3.1 Selecting specimens

3.1.1 Advantages and disadvantages of serum and plasma

HIV testing of serum and plasma, which can be collected by venepuncture (see section 3.2), have the following advantages and disadvantages:

Advantages

- Have higher concentrations of HIV antibodies than oral fluids¹⁹
- Have the potential for additional routine testing (e.g. syphilis, hepatitis B, hepatitis C) from a single specimen
- Have the potential for special studies (e.g. testing for recent infections, HIV typing [HIV-1 vs HIV-2], HIV subtyping, antiretroviral [ARV] resistance)
- Are easy to collect and test in clinical settings with a trained phlebotomist and a laboratory

Disadvantages

- Require invasive collection technique
- Require skilled staff (for collecting and processing serum or plasma)
- Compared with oral fluid, require more equipment (e.g. needles, tubes or lancets) and biohazard waste facilities
- Serum or plasma are difficult to collect in non-clinical settings as venepuncture is required
- Pose a greater risk to health-care workers and staff through inadvertent exposure, both because of higher HIV concentrations and the use of sharp collection devices

3.1.2 Advantages and disadvantages of whole blood

Whole blood, which can be collected by venepuncture or finger-stick (see section 3.2), has the following advantages and disadvantages in HIV testing:

Advantages

- Has higher concentrations of HIV antibodies than oral fluids¹⁹
- Has the potential for additional routine testing (e.g. syphilis, hepatitis B, hepatitis C) from a single specimen
- Is easy to collect and test in clinical settings (with trained phlebotomist, if venous whole blood)
- Is easy to collect in non-clinical settings (if finger-stick)

Disadvantages

- Requires invasive collection technique
- Requires skilled technician (for collection)
- Compared with oral fluid, requires more equipment (e.g. needles, tubes or lancets) and biohazard waste facilities

- Difficult to collect in non-clinical settings if venepuncture is required
- Poses a greater risk to health-care workers and technicians through inadvertent exposure, both because of higher HIV concentrations and the use of sharp collection devices

3.1.3 Advantages and disadvantages of dried blood spots

Dried blood spots can be prepared by collecting venous whole blood or finger-stick whole blood and dropping an amount onto a filter-paper. DBS have the following advantages and disadvantages:

Advantages

- Are easy to collect in a clinical or non-clinical setting, depending on whether venepuncture is available
- Do not require a centrifuge or other equipment for processing the blood specimen
- Once dried, can be stored at room temperature for a short period of time
- Can be transported easily to the central laboratory for further testing
- Facilitate testing for prevalence, incidence and special studies such as resistance testing

Disadvantages

- Require specific filter-paper (see Section 3.2) for preparation
- Modified procedure required for DBS elution and HIV testing
- Potential for less accuracy (false-positives and/or false-negatives) if the test is not optimized for DBS
- Make the testing process more lengthy as an elution step needs to be performed
- Limited number of tests validated for use with DBS specimens

3.1.4 Advantages and disadvantages of oral fluid

Other specimens besides blood and blood products can be used for HIV testing. For linked testing, where informed consent must be obtained, oral fluid may be used.

Advantages

- Does not require a trained laboratory technician for specimen collection and processing, can be collected by a trained health worker
- Does not require contact with possibly contaminated laboratory materials, e.g. used needles or lancets that need biohazard waste facilities for sharps disposal
- Can be collected in a variety of field settings, including non-clinical settings
- Collection of oral fluid may be more acceptable to hard-to-reach populations than specimen collection requiring venepuncture or finger-stick. Therefore, a greater percentage of the target population may agree to be tested.

Disadvantages

- May require special collection devices
- Currently available testing technologies used for oral fluid specimens are limited but additional new tests are being validated.
- Cannot be used to perform additional testing for special studies (e.g. testing for recent infections, HIV subtyping, ARV resistance)
- Same specimen cannot be used to confirm initial reactivity with a second test; therefore, a second specimen must be taken, i.e. whole blood, serum, plasma for further testing (this is specific to the OraQuick HIV rapid test as the oral fluid collection device and test are one and the same)
- Should not be used for confidential linked testing (i.e. with the return of results to the individual)

recommendation

Blood (serum, plasma, DBS) is the preferred specimen for testing because it has a higher concentration of HIV antibodies than oral fluid. It also allows for additional testing, including for syphilis, hepatitis B and hepatitis C, and for special studies of HIV type and subtype, and ARV resistance.

3.2 Collecting, processing and storing blood, serum and plasma specimens

blood

Blood needed for an HIV test can be collected either by venepuncture (whole blood, serum, plasma) or by finger-stick (whole blood).

3.2.1 Processing blood collected by venepuncture

To collect blood by venepuncture, follow local clinical or laboratory procedures. See the Appendix and Section 6.3 for information on safety procedures.

The following steps are recommended for processing blood collected by venepuncture²⁰: Collect up to 10 ml of blood from the patient's vein into a sterile 10 ml tube.

For serum, blood is collected in a red-top tube (without anticoagulants).

For plasma, blood is collected in a purple-top tube (with anticoagulants, e.g. EDTA).

For safety reasons, the use of an evacuated blood collection system (e.g. Vacutainer® tube) is recommended. (Note: Obtaining an additional tube of blood during routine blood collection solely for the purpose of unlinked anonymous testing is considered unethical and is not advised.)

1. If the blood specimen will not or cannot be processed immediately (e.g. no centrifuge is available), collect the blood in a red-top tube and allow it to stand at room temperature

for at least 20–30 minutes, and then remove the serum. Usually, plasma takes longer (~1 hour) to separate without centrifugation than serum collected in a red-topped tube. Process (see Step #3) and test within 24 hours to avoid haemolysis of the specimen.

2. Centrifuge the specimen to separate the serum (without EDTA) or plasma (with EDTA). If blood is collected for serum, allow the blood to stand for at least 20–30 minutes so that a clot forms before the specimen is centrifuged. In general, the specimen should be centrifuged at 300–400 *g* or 1200–1500 rpm for 10 minutes.²¹
3. After the specimen is centrifuged or has had time to separate, use a clean plastic pipette (do not pour) to remove an aliquot of 0.5–2.0 ml off the top layer. Transfer this to another sterile labelled tube (plastic, not glass) or cryovial (1.5–2.0 ml with a screw cap) and tighten the cap. The specimen is ready for storage and testing.

3.2.2 Storing serum and plasma collected by venepuncture

To store serum and plasma, consider the following:^{22,23}

- Make sure the cap is tight on the labelled cryovial or plastic tube. (Do not use glass tubes for storing specimens.) Place the cryovials in a cardboard freezer box with a partitioned insert.
- If the specimens are to be transported to the testing laboratory, store the specimens at 4–8 °C for up to a maximum of 1 week. For longer-term storage, the specimens should be frozen at –20 °C or below.
- Pack the specimens upright in a cooler containing cold packs for transport to the testing facility.
- Limit the number of freeze/thaw cycles because it may impact the HIV test results and subsequent additional testing.

3.2.3 Collecting blood by finger-stick

Blood collected by finger-stick can be used to perform rapid tests or make DBS on filter-paper. A DBS may be preferred in rural and non-clinical settings, which often do not have trained phlebotomists or laboratory facilities with appropriate equipment (e.g. centrifuges).

1. To obtain a finger-stick specimen, massage the finger (preferably the middle or ring finger), which will cause blood to accumulate at the tip of the finger.
2. Cleanse the finger pad (not just the tip or side of the finger) with 70% isopropyl (rubbing) alcohol. Wipe away the alcohol with a sterile gauze pad.
3. Use a sterile lancet to firmly prick the finger pad. Wipe the first drop of blood off the finger with sterile gauze before collecting subsequent blood using a collection device to place on the rapid test device or on the filter-paper for the DBS. If the original puncture is inadequate, the same site should not be reused; another site or finger should be used. Avoid milking or squeezing the puncture as this may cause haemolysis of the specimen and could invalidate the test result.¹⁹ The ear lobe may be pricked instead of the finger.

3.2.4 Preparing and storing a dried blood spot for an HIV test

Blood from a finger- or ear-lobe stick can be used to make DBS.²⁴ Although finger-stick is the most typical method, DBS can also be obtained by using blood collected in a tube with an anticoagulant.²⁵ DBS have the advantage of being easy to transport, without the need for a cold chain.

1. Apply blood directly from a finger or a pipette onto special filter-paper (Schleicher and Schuell Grade 903 filter paper). The paper may come with preprinted circles that will contain approximately 100 µl blood when completely filled. If the paper does not have preprinted circles, place blood on the paper so that it makes a circle with a 1.5 cm diameter. Allow the blood to soak through and fill the entire circle. Caution: If the blood does not saturate the filter-paper, do not use that paper.
2. Label the side of the filter-paper with a specimen reference code after the filter-paper is saturated with blood (circle is filled).
3. Suspend filter-paper strips containing the filled circles during the drying process to allow air to circulate around the paper. Stands for holding the strips are commercially available. However, strips may also be dried by placing them between two books (taping the edges of the strips to the books with sticky tape) on a table or a laboratory bench top so that the blood-containing part of the paper is not in contact with the surface of the table or laboratory bench top. Be sure not to get tape on the blood spot.
4. Let the blood spots air dry at room temperature for at least 4 hours (and for at least 24 hours in humid climates). Do not heat or stack blood spots, and do not allow them to touch other surfaces while they are drying.
5. After blood spots have been adequately dried, wrap the strip in one sheet of glassine paper or plastic to prevent carryover of specimen from one sheet to another.
6. Place the wrapped strips in a gas-impermeable bag (zip-lock bag) with desiccant and humidity indicator cards. Approximately 20 strips may be placed in each bag. Bags may be kept at room temperature for up to 30 days and then stored at 4 °C for up to 90 days. If the DBS in their plastic bags are to be stored for more than 90 days, they should be maintained at -20 °C.²⁵ Properly stored DBS have been shown to be stable for at least two years. The bags should be placed in a sturdy envelope for shipment.²⁶ If additional testing, such as resistance testing, is anticipated, DBS must be stored at -20 °C or below immediately after the DBS specimens are dry.

3.3 Collecting and storing oral fluid

3.3.1 Collecting oral fluid

For specimen collection, follow test instructions as part of a standard operating procedure. Oral fluid collection devices are available and may be used, if indicated. Some rapid test devices contain an oral fluid collection pad at one end which facilitates collection and testing. See Section 6.3 for information on safety procedures.

Oral fluid can only be used with certain EIAs and rapid tests designed for oral fluid specimens, such as the OraQuick brand. Additional rapid tests using oral fluid are currently under field evaluation.

The following are the general steps for collecting a specimen:

1. Use a specially treated absorbent pad attached to a plastic stick (usually provided by the test kit manufacturer).

2. Collection procedures are specified by the manufacturers of collection devices, which must be carefully followed. Then place the pad into a vial containing a preservative solution (usually provided by the test kit manufacturer).
3. If an oral fluid-specific rapid test (e.g. OraQuick) is performed, storage and transport are not necessary.

Due to the complexity of the test, oral fluid specimens collected for EIAs are sent to a laboratory performing EIAs for analysis.

3.3.2 Storing oral fluid

Oral fluid specimens should be stored at 4 °C for a short period of time. They should be refrigerated during shipment. Specimens should be frozen (–20 °C or below) if stored for an extended period of time. Once thawed, they can be refrozen once. Consult the test kit insert prior to testing for more specific storage information.

3.4 Labelling and recording collected specimens

3.4.1 Labelling specimens

The plastic tube, cryovial or filter-paper containing the specimen must be labelled with a specimen code at the time of collection and processing. If labels are used, make sure the label is placed on the side of the tube, not on the cap. Pre-printed cryolabels designed to adhere during freezer storage should be used when specimens are stored in cryovials. It is important that freezing does not affect the visibility of the printing on the label. Surveillance coordinators should provide the field staff responsible for specimen collection with a series of labels or permanent markers and the codes to be used.

For unlinked anonymous testing, label the tube only with a new specimen code unlinked to personal identifying information (see Figure 2.1).

3.4.2 Recording specimens and test results

A separate laboratory logbook or line-listing for surveillance activities should be maintained to record HIV test results by the corresponding code. The logbook should be accessible only to laboratory and surveillance staff; it should be secured in a locked drawer or cabinet when not in use to ensure confidentiality of the persons' test results as well as their participation in surveillance activities (see Figures 2.1, 2.2).

For unlinked anonymous testing, the logbook or line-listing should contain only the new specimen codes and corresponding HIV test results; no personal identifying information on the patients whose specimens are tested should be included. HIV test results can be matched by the new specimen code to the demographic information abstracted earlier on the surveillance form (see Figure 2.1).

4.0 Current HIV Testing Technologies and Strategies Used in Surveillance

summary

Current HIV testing technologies and strategies

- HIV testing technologies for surveillance
 - Enzyme immunoassays (EIAs)
 - Rapid tests
- HIV testing strategies for surveillance

4.1 HIV testing technologies for surveillance purposes

4.1.1 Overview of HIV testing

For surveillance as well as diagnostic purposes in low- and middle-income countries, technologies that identify HIV antigen are expensive and technically more difficult. In most industrialized countries, current diagnostic testing procedures use an EIA to screen a specimen and, if it is reactive, the result is confirmed by testing the specimen with a western blot test. However, studies have shown that the testing algorithms using EIAs and rapid tests are as reliable for confirmation as western blots. In addition, compared with western blots, EIAs and rapid tests are less expensive, do not require as high a level of technical expertise to perform and interpret, and produce fewer indeterminate results.^{27,28} Therefore, UNAIDS and WHO recommend alternative testing strategies using combinations of EIAs or rapid tests to confirm initial reactive test results.²⁹ The tests should be highly sensitive and specific to provide reliable detection of antibodies in a specimen.³⁰

EIAs and rapid tests contain antigens to both HIV-1 and HIV-2, and can therefore detect antibodies to both HIV types. Some tests are capable of discriminating between HIV-1 and HIV-2. EIAs and rapid tests are recommended for both HIV surveillance and diagnostic purposes because they are the most accurate and cost-effective.

recommendation

Of the available testing technologies, EIAs and rapid tests are the most practical and cost-effective for both surveillance and diagnostic purposes, and provide results comparable to an EIA/western blot algorithm.

4.1.2 Enzyme immunoassays

4.1.2.1 General description

EIAs rely on a primary antigen–antibody interaction and can use whole viral lysate of HIV or one or more antigens from the virus. Early EIAs produced more false-positive HIV antibody results than the current third- and fourth-generation of EIAs.²⁷ Therefore, specimens initially found reactive when screened with an EIA were further tested with a western blot for confirmation. However, second- and third-generation EIAs have shown marked improvement in specificity as well as sensitivity, making them candidates for confirmatory tests. The use of recombinant proteins and synthetic peptides also improved the sensitivity of EIAs. The development of fourth-generation EIAs further improved detection of HIV infection due to their ability to detect both antibody towards HIV and HIVp24 antigen, which reduced the non-reactive (window) period after initial infection with HIV.³⁰ Most HIV EIAs contain antigens to HIV-1 and HIV-2, and have been optimized to detect antibodies to both.^{27,30} EIAs that detect antibodies to both HIV-1 and HIV-2 are better suited for surveillance.

4.1.2.2 Characteristics of EIAs

EIAs are best performed at a regional or national laboratory since they require well-trained and skilled laboratory technicians, technologically advanced equipment (incubators, washers and spectrophotometers) that needs maintenance, and a constant source of electricity. EIAs are most efficient for laboratories that process a large number of specimens (90 or more) daily or for batch testing, which is common in HIV sentinel surveillance activities. Because of test design, they are not cost-effective or suitable for use on a small number of specimens. However, if a laboratory processes at least 40 specimens a day on a regular basis, EIAs may still be more appropriate than rapid tests. Because laboratories often batch specimens and run them at one time, the time before results are available may be from days to 2 or 3 weeks after collection. While this delay might be of concern for diagnostic purposes, it is not a disadvantage for unlinked anonymous testing since results are not provided back to the person, and same-day testing could lead to identification of the person tested. Since EIAs are usually performed at the regional or national level, QA and quality control (QC) measures need to be implemented in only a few laboratories (see Section 6.0). EIAs may have limited application in rural settings where the laboratory infrastructure and equipment may be insufficient (Table A)

With the UN bulk procurement scheme, the cost of an EIA ranges from US\$ 0.35–1.2 per test. When considering both direct and indirect costs (technology, human resources and equipment requirements), the actual cost may exceed US\$ 15–20 per test.³¹ However, if the direct and indirect costs are shared across all programmes (blood screening, diagnosis and surveillance), a more realistic cost for surveillance activities would be US\$ 4–6.

4.1.2.3 Performing an EIA

EIAs can be performed with serum, plasma, oral fluid or DBS specimens (once eluted). They normally take between 30 minutes to up to 2 hours to perform. The manufacturer's instructions provided with the specific EIA kit should be followed.

The following are critical to the success of conducting an EIA:

- Use of test kits that have not expired
- Calibrated and well-maintained equipment
- Adherence to dilutions and incubation times described in the manufacturer's instructions
- Use of a spectrophotometer to read results accurately and objectively
- Adequate training of the technologists
- Consistent source of power without outages as this would affect the storage of reagents and functioning of equipment
- Use of appropriate positive and negative controls as internal quality control (IQC) reagents and use of external quality control (EQC) reagents.

recommendation

EIAs are efficient for laboratories that process large numbers of specimens daily, as is common when conducting HIV sentinel surveillance.

4.1.3 Rapid tests

4.1.3.1 General description

Interest in HIV antibody tests that provide same-day results and do not require additional reagents or equipment led to the availability of HIV rapid tests. Current rapid tests are based on four immunological principles: particle agglutination, immunodot (EIA), immunofiltration (flow-through device) and immunochromatography (lateral flow).² Most HIV rapid tests contain antigens to HIV-1 and HIV-2, and detect antibodies to both strains of the virus.^{27,30} A reactive test result is indicated by clumping, a spot, dot or line, depending on the test format. The sensitivity and specificity of the latest generation of rapid tests are similar to those of EIAs. Many rapid tests are under evaluation or are currently in use in low- and middle-income countries for screening, diagnostic and surveillance purposes.

4.1.3.2 Characteristics of rapid tests

Rapid tests are useful for small laboratories that routinely perform less than 40 HIV tests per day, for laboratories without electricity or equipment, and for geographical areas with limited laboratory infrastructure. In some instances, even if a laboratory performs more than 40 tests per day but only during a limited period in a year, rapid tests may be more appropriate than EIAs. A well-organized testing facility may be able to perform up to 100 rapid tests per day (7.5 working hours) for surveillance purposes (e.g. by performing rapid tests in batches of 5–10 specimens). A result can usually be obtained in less than 30 minutes, and it is relatively easy to interpret. However, adequate training is required to correctly perform the test and interpret the results. HIV rapid test kits generally contain all the reagents needed to run the test: no additional reagents or equipment are required. Most rapid tests do not require electricity, special equipment, refrigeration or highly skilled staff, although a few that contain heat-sensitive reagents require refrigeration.

**Table A. Comparison of HIV testing technologies:
enzyme immunoassays and rapid tests**

HIV testing technology	Specimens	Advantages	Limitations	Cost (US\$)	Complexity (from 1 [simple] to 4 [highly complex])
Enzyme immunoassays (EIA)	Serum Plasma Dried blood spots Oral fluid	<ul style="list-style-type: none"> • Can be batched: good for ≥ 90 specimens at a time • Can be automated • QC; easier to control • Identifies seroconverters earlier: highly sensitive, reduces window period if fourth-generation EIA 	<ul style="list-style-type: none"> • Requires skilled, trained technicians to perform testing and calculate results • Can take ± 2 hours • Requires special equipment • Requires maintenance of equipment • Kits require refrigeration 	0.5–1	4
Rapid test	Serum Plasma Whole blood Oral fluid	<ul style="list-style-type: none"> • Requires minimal equipment and reagents • Can be performed outside a laboratory (on-site testing) • Test results easy to interpret • Results in 30 min or less • Most kits can be stored at up to 30°C 	<ul style="list-style-type: none"> • Not suitable for large numbers of specimens • Positive and negative control specimens often not included in the kit • May cost more per test than EIA 	1–3	For tests based on: Immunochromatography 1 Dipstick and flow-through devices 1–2 Agglutination 2–3

Rapid tests may be appropriate for linked confidential testing among hard-to-reach populations (e.g. injecting drug users IDUS, female sex workers, MSM) or geographically remote populations. In these populations, opportunities for provision of results may be limited after the initial encounter; therefore, testing (screening and confirmatory) may need to be performed on site on the same day as specimen collection. If HIV test results are to be returned to the person tested, a confirmatory test is required (e.g. another rapid test or EIA).

Rapid tests generally cost about US\$ 1–3, a slightly higher cost per test than an EIA. However, rapid tests may be more cost-effective than EIAs if the additional costs of conducting an EIA are considered (e.g. equipment, laboratory infrastructure, technician training). Since there are very few steps in performing a rapid test, there is less chance for error than with

an EIA. The most common error with rapid tests is not following the instructions for use as issued by the manufacturer, especially with respect to the time spent reading the results. In addition, most rapid tests include an IQC. However, when using rapid tests, QA and EQC measures need to be developed and implemented at all sites that are using them (Table A).

4.1.3.3 Performing a rapid test

Rapid tests can be performed with serum, plasma, venous or finger-stick whole blood and oral fluid. For some rapid tests, specimen preparation may take as long as 10–20 minutes, while the simplest rapid tests require no specimen preparation. The manufacturer's instructions provided with the test kit should be followed. Most results are easy to read and are indicated by clumping, a spot, dot or line, depending on the test format.

The following are critical to the success of conducting a rapid test:

- Use of test kits that have not expired
- Training in the technology being used
- Adherence to manufacturer's instructions
- Correct interpretation of results by person reading them

recommendation

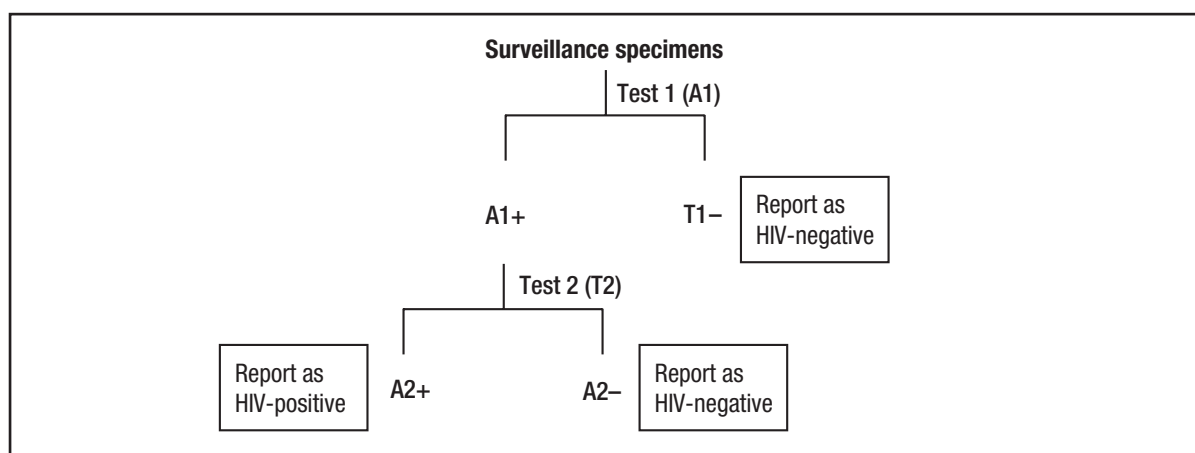
Rapid tests are useful in settings where EIAs are not feasible or practical and in geographical areas with limited laboratory infrastructure. Rapid tests may be appropriate for hard-to-reach populations (e.g. IDUS, female sex workers, MSM) or geographically remote populations for whom HIV test results may need to be provided on site on the same day as specimen collection.

4.2 HIV testing strategies for surveillance

4.2.1 Strategy for unlinked anonymous surveillance

For unlinked anonymous surveillance, the revised guidelines from WHO recommend a two-test strategy, used in a serial algorithm.³² Specimens that are reactive by both tests are considered HIV-positive for surveillance purposes. If tests are properly selected and performed, discordant results are rare. Since unlinked anonymous surveillance data are not used for individual diagnosis, no additional testing is recommended to confirm HIV seropositivity in low-prevalence epidemic states or for discordant specimens, if any. Discordant specimens (Test 1 reactive/Test 2 non-reactive) are considered as non-reactive.

Figure 4.1 WHO/UNAIDS -recommended testing strategy for unlinked anonymous surveillance



The selection of testing technologies and the order in which they are used (testing algorithm) are important for obtaining accurate test results. The tests should contain different antigens. They should be highly sensitive ($\geq 99\%$) and highly specific ($\geq 99\%$). The first test is the screening test, so it is ideal to have a more sensitive test to detect all positive results. Because some false-positives do occur, the second test needs to be highly specific to ensure that all truly negative test results are identified as negative. In addition, tests that can use serum and plasma are recommended so that multiple tests can be performed from a single specimen, avoiding the need for collection of additional specimens.

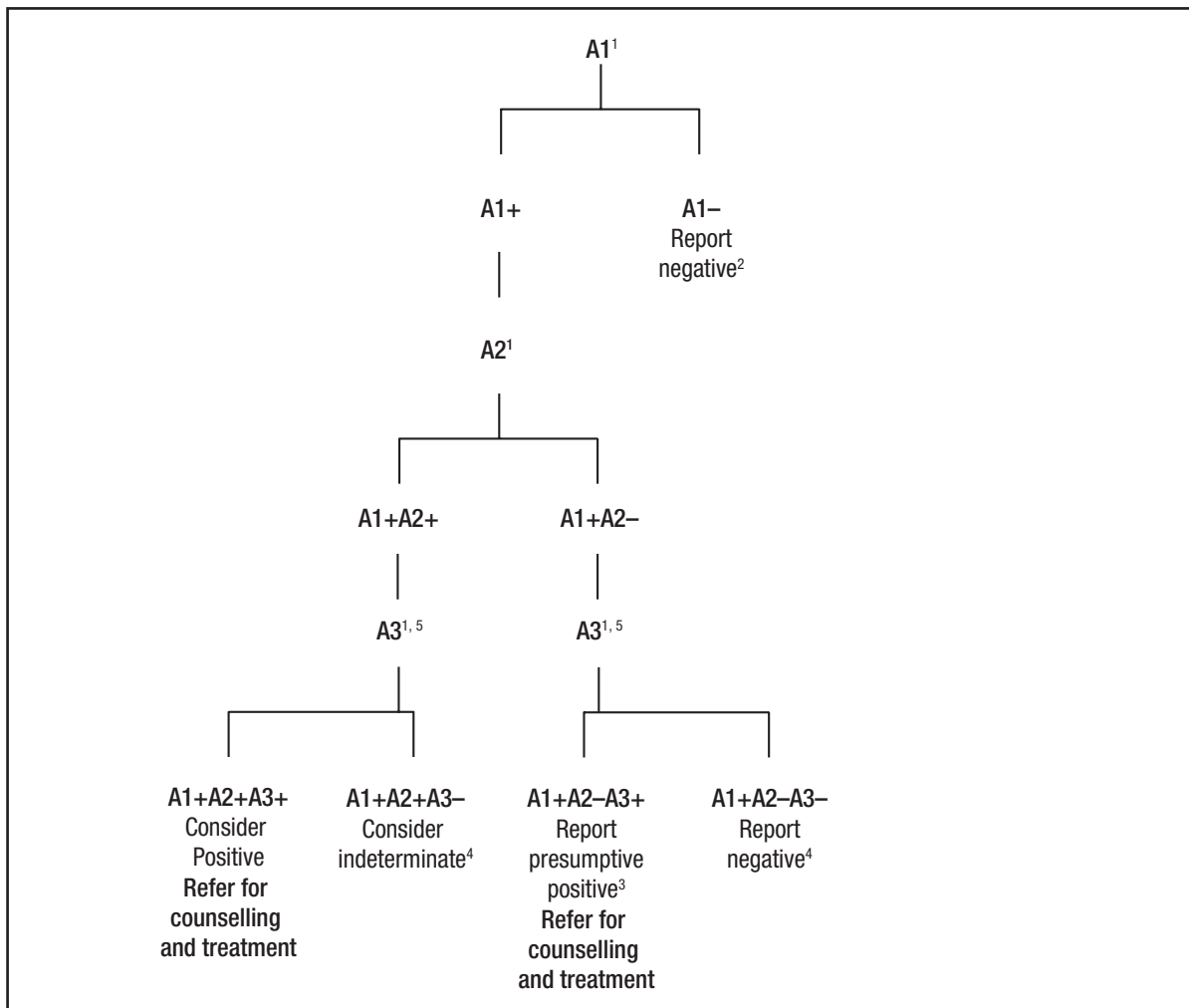
For rapid tests that use oral fluid or whole blood from a finger-stick, the specimen is collected with a collection device and placed directly on the rapid test apparatus; thus, a second specimen must be collected for confirmation if the first test is reactive. Therefore, in such situations, two rapid tests should be performed simultaneously and then analysed as if performed sequentially.

4.2.2 Strategy for linked surveillance

When surveillance makes use of linked testing or unlinked anonymous testing with informed consent (see Box 2) (e.g. among hard-to-reach populations and in national population-based surveys where participants' HIV test results are linked to many characteristics), it is recommended to use the HIV testing strategy for diagnosis.

In many surveys, either within most-at-risk populations or the general population, the strategy selected for HIV testing is linked. The participants are required to provide informed consent and the HIV test results are provided to them. Therefore, in such cases, confirmatory testing is needed according to the national guidelines or HIV testing algorithms used for HIV diagnosis. In these situations, a three-test strategy should be used regardless of the HIV prevalence level. Figure 5 presents the scheme for HIV testing in linked surveillance.

Figure 4.2 Schematic representation of HIV-linked surveillance



- ¹ Assay A1, A2, A3 represent 3 different assays.
- ² Report: result may be reported.
- ³ For newly diagnosed individuals, a positive result should be confirmed on a second sample.
- ⁴ Testing should be repeated on a second specimen taken after 14 days.
- ⁵ If the third test is not available at the testing site, then the individual or specimen should be referred to another testing service for further testing.

If rapid tests are used for same-day return of results, there is only one opportunity during which to obtain informed consent, collect the specimens and conduct the required tests. As above, all necessary blood samples should be collected at the same time. If blood is the specimen, DBS can be prepared for QA purposes.³³

recommendation

HIV tests that can use different types of specimens (serum, plasma or DBS) are recommended so that multiple testing can be performed on the same type of specimen, avoiding the need for collection of additional specimens.

5.0 Selection and Evaluation of HIV Testing Technologies Used in HIV Surveillance

summary

Selection and evaluation of HIV testing technologies

- Selection of tests
- Country evaluation of selected tests

The number of HIV testing technologies and testing algorithms must be limited. Because of differences in performance of HIV tests, it is important for the national surveillance coordinator to document all of the testing technologies and testing algorithms that are used in the country. In addition, in order to ensure reliable and comparable surveillance data over time, the same tests should be used over time for consistency, with the caveat that technologies are upgraded regularly and after 10 years.

recommendation

Use of same testing technologies and testing algorithms allow better comparison of surveillance data over time.

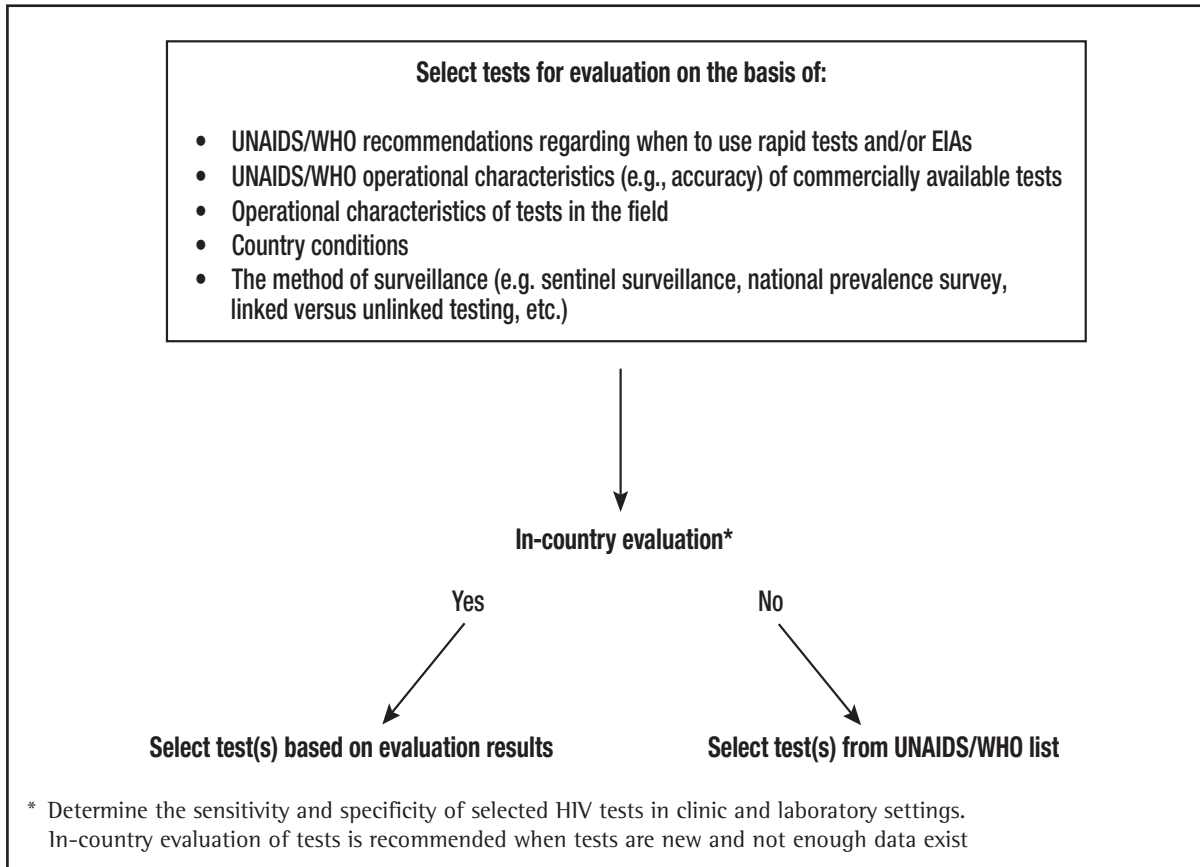
5.1 How to select tests

Identifying appropriate combinations of HIV testing technologies to be used in testing strategies for low- and middle-income countries may be a challenge since there may not be a national technical advisory group to review and evaluate the tests. Therefore, the decision about which test(s) to select may often be made by a donor agency, it may also be based on price, or the result of a manufacturer's influence.

If the laboratory infrastructure at the national level has sufficient capacity, then countries should evaluate the performance of HIV testing technologies in their population. If it is not feasible to conduct a country evaluation, a country should review the selection factors below and then either select test(s) from the UNAIDS/WHO list of currently available HIV tests that have been evaluated by WHO (http://www.who.int/diagnostics_laboratory/evaluations/hiv/en/index.html) or select an assay based on evaluations, preferably in the region, by another independent, non-commercial source (Figure 5).

In recent years, some countries have elected to use fourth-generation combined antigen-antibody tests for surveillance, partly because these tests are procured in-country for transfusion safety in blood bank settings. In most instances, use of fourth-generation EIAs will have a minimal impact on prevalence because very few antigen-positive specimens are antibody-negative.

Figure 5. How to select an HIV test



recommendation

Before tests are selected for use in a country, the country should evaluate the tests' accuracy and operational characteristics in that country or, if this is not possible, tests for potential testing algorithms should be selected on the basis of evaluation by an independent, non-commercial source.

5.2 Country evaluation of selected tests

The selected HIV testing technologies should be evaluated in the country to (1) determine commercially available HIV tests' sensitivity and specificity in the country, and (2) validate the testing algorithm to be used based on the results of the sensitivity and specificity of the tests evaluated, and their subsequent predictive values.³⁴ According to the *Guidelines for appropriate evaluations for HIV testing technologies in Africa*,³⁵ a three-phase implementation approach may be used for country evaluation.*

- Phase I is a laboratory-based evaluation to provide preliminary results on test performance characteristics (sensitivity, specificity) on the same set of samples. Having evaluated the same sample set that may consist of 4–7 rapid tests, an algorithm of 2–3 tests may then be proposed based on the performance of the combination of test methods.
- Phase II involves evaluation of the selected algorithm under field conditions, which may include test performance and interpretation by non-laboratory clinic staff. Phase II is often referred to as the field trials, and typically is conducted in at least 2–3 possible sites. Tests under evaluation in this phase should be performed in the same manner in which it is to be used, e.g. finger-stick specimens.
- Phase III represents ongoing evaluation of performance through EQA programmes that not only monitor the performance of individual clinic and/or staff, but also provide aggregate data for ongoing assessment of test performance.

The country evaluation of HIV testing technologies will need to be repeated at appropriate intervals to take into account the development of newer technologies.

* Adapted from *Guidelines for appropriate evaluations for HIV testing technologies in Africa* (WHO AFRO/CDC, 2001). Testing technologies that use only finger-stick whole blood or oral fluid can be evaluated only phase I and phase II evaluations.

6.0 Laboratory Quality Assurance and Safety

summary

Laboratory quality assurance and safety

- National Quality Assurance Programme
- Laboratory quality assurance
 - Preanalytical phase
 - Analytical phase
 - Postanalytical phase
- Safety procedures

Reliable and reproducible HIV testing over time is an important component of HIV serosurveillance. Having highly accurate HIV tests does not necessarily guarantee reliable laboratory results. Many processes take place from the time the specimen arrives in the laboratory until the results are recorded, during which time errors can occur.³² Therefore, the ongoing process of monitoring the laboratory system, both internally and externally, is essential. QA is the dynamic and ongoing process of monitoring a system for reliability and reproducibility of results, and permits corrective action when established criteria are not met.

6.1 National quality assurance

Countries should require that testing services performing HIV testing for surveillance purposes at all levels (e.g. national reference laboratory, hospital laboratories, private laboratories, and those testing services outside the traditional laboratory setting) operate within a quality management system otherwise known as QA. The national reference laboratory, in collaboration with the national AIDS control programme, should monitor the effectiveness of the participating laboratories' quality management systems to identify any testing service that might require further training or other corrective action.

QA is the total process which guarantees that the final results reported by a laboratory are as accurate as possible. This involves inspecting specimens, reviewing transcriptional measures, using the most reliable tests and verifying the final reports. A QA system ensures that the necessary checks and balances are made by the testing service in order to assure the quality of HIV testing for surveillance and creates good laboratory practice. The use of nationally standardized testing algorithms, as well as standard operating procedures and QC procedures, is vital to assuring the quality of testing.³⁶ Elements of the QA system should be included in procedure manuals used by laboratory technicians and other laboratory staff. Compliance with such programmes will maximize the reliability and accuracy of test results.

Quality control comprises those measures that must be included during each test run to verify that the test is working properly. This includes ensuring correct temperature conditions, kit controls, etc. Thus, QC indicates whether the test run was valid and has produced acceptable results. QC does not, however, indicate that the results are accurate, or that they have been reported properly.

Quality assessment is a means of determining the quality of results. It is usually an external evaluation of a laboratory's performance using proficiency panels. Quality assessment is undertaken to evaluate the effectiveness of a QA programme. A good QA/QC programme may make quality assessment less important in some situations; however, quality assessment is never a substitute for good QA/QC. Failure with quality assessment specimens usually indicates that there is a problem with QA/QC procedures. Furthermore, quality assessment schemes are much more efficient at detecting differences in performance between participating laboratories than between test methods and techniques.

One component of QA is external assessment of a laboratory's performance through proficiency testing (also known as an external quality assessment scheme [EQAS]).³² The national reference laboratory should send to all participating testing services a proficiency panel of approximately six to ten specimens for testing. This panel should contain HIV-negative and HIV-positive (weak to strong) specimens that are representative of the HIV strains circulating in a country.³⁶ Proficiency testing should be done at regular intervals (twice each year). EQA for the national reference laboratory may be provided by an independent laboratory (e.g. a university) or by one of WHO's EQA schemes.

recommendation

Countries should require that all testing services performing HIV testing for surveillance purposes at all levels (e.g. national reference laboratory, hospital laboratories, private laboratories, and testing services outside the traditional laboratory setting) operate within the principles of QA.

6.2 Laboratory quality assurance

Laboratories at all levels (national, regional and local) which conduct HIV testing should have a functioning QA programme. Each laboratory or testing service conducting HIV testing should routinely monitor and assess the quality of the testing process in the preanalytical, analytical and postanalytical phases.

6.2.1 Preanalytical phase

The preanalytical phase encompasses the following components:

- Training
- Laboratory safety
- Number of trained personnel available and capable of performing HIV testing

- Specimen collection, labelling and transport conditions
- Processing of specimens before testing and storage
- Sources and types of specimens tested
- Number of specimens tested
- Selection of test kits
- Checking the expiry dates of test kits. Kits need to be used before their expiry dates, which are typically indicated on the side of the test kit packaging. Careful rotation of test kit stocks (older kits should be used before their expiry dates and before newer kits) will result in more efficient use of test kit supplies.
- Storage of HIV test kit reagents. Reagents must be stored at the appropriate temperature as specified in the product insert provided by the manufacturer. Certain reagents (e.g. conjugates for EIAs) are likely to require refrigeration; other test kit components may require only storage at room temperature.
- Recording of information.

6.2.2 Analytical phase

The analytical phase encompasses the testing process itself. Some of the components to be reviewed by the QA programme include

- Written standard operating procedures manual
- Reagent preparation
- Testing performance
- Performance and preventive maintenance of equipment (e.g. spectrophotometers, washers)
- Correct use of reagents
- Inclusion of internal and/or external QCs in the test kits
- QC monitoring procedure

6.2.3 Postanalytical phase

The postanalytical phase encompasses all that occurs after testing including

- Interpreting results
- Transcribing results, e.g. recording results on the correct identifier code
- Reporting results (not necessarily to the individual, would depend on the method of surveillance, e.g. linked or not)
- Entering data into the tracking system (computer or hard copy)
- Maintaining records
- Reviewing QC

6.3 Safety procedures

Safety precautions are essential and should be followed at all points in the testing process – from specimen collection to testing, storage and disposal of biohazard wastes – so as to minimize occupational risk. Further recommendations on safety precautions can be found in various documents.^{37,38} Precautions for the prevention of transmission of HIV and other bloodborne pathogens are summarized in the Appendix. For information about postexposure prophylaxis (PEP), consult the country's national AIDS control programme and refer to the national guidelines.

Proper disposal of all contaminated laboratory waste is essential.³⁷ All contaminated waste in the clinic and laboratory should be decontaminated before disposal; this includes specimens of body fluids, broken glassware and containers of contaminated needles.³⁹ Methods to decontaminate all contaminated waste (e.g. autoclaving, chemical disinfection, incinerating) should be in place. Materials that are decontaminated or disposed of outside the laboratory should be placed in a strong, leak-proof container prior to transporting them outside the laboratory.

Appendix. Standard Precautions for Prevention of Transmission of HIV, Hepatitis B Virus, Hepatitis C Virus and Other Bloodborne Pathogens in Health-Care Settings^{37,38}

Standard precautions combine the major features of universal precautions (UP) and body substance isolation (BSI), and are based on the principle that all blood, body fluids, secretions, excretions (except sweat), non-intact skin and mucous membranes may contain transmissible infectious agents. Standard precautions include a group of infection-prevention practices that apply to all patients, regardless of suspected or confirmed infection status, in any setting in which health care is delivered. These include: hand hygiene; use of gloves, gown, mask, eye protection or face shield, depending on the anticipated exposure; and safe injection practices. Also, equipment or items in the patient environment likely to have been contaminated with infectious body fluids must be handled in a manner that prevents transmission of infectious agents (e.g. wear gloves for direct contact, contain heavily soiled equipment, properly clean and disinfect or sterilize reusable equipment before use on another patient). The application of standard precautions during patient care is determined by the nature of the health-care worker-patient interaction and extent of anticipated blood, body fluid or pathogen exposure. For some interactions (e.g. performing a venepuncture), only gloves may be needed; during other interactions (e.g. intubation), use of gloves, gown, and face shield or mask and goggles is necessary. Standard precautions are also intended to protect patients by ensuring that health-care personnel do not carry infectious agents to patients on their hands or via equipment used during patient care.

recommendation

Assume that every person is potentially infected or colonized with an organism that could be transmitted in the health-care setting and apply the following infection control practices during the delivery of health care.

Hand hygiene

1. During the delivery of health care, avoid unnecessary touching of surfaces in close proximity to the patient to prevent both contamination of clean hands from environmental surfaces and transmission of pathogens from contaminated hands to surfaces.
2. When hands are visibly dirty, contaminated with proteinaceous material, or visibly soiled with blood or body fluids, wash hands with either a non-antimicrobial or an antimicrobial soap and water.
3. If hands are not visibly soiled, or after removing visible material with non-antimicrobial soap and water, decontaminate hands in the clinical situations described in 3.a-f The preferred method of hand decontamination is with an alcohol-based hand rub. Alternatively, hands may be washed with an antimicrobial soap and water. Frequent use of alcohol-based hand rub immediately following handwashing with non-antimicrobial soap may increase the frequency of dermatitis. Perform hand hygiene:
 - 3.a. Before having direct contact with patients
 - 3.b. After contact with blood, body fluids or excretions, mucous membranes, non-intact skin, or wound dressings
 - 3.c. After contact with a patient's intact skin (e.g. when taking a pulse or blood pressure or lifting a patient)
 - 3.d. If hands are likely to move from a contaminated body site to a clean body site during patient care
 - 3.e. After contact with inanimate objects (including medical equipment) in the immediate vicinity of the patient
 - 3.f. After removing gloves.
4. Wash hands with non-antimicrobial or antimicrobial soap and water if contact with spores (e.g. *Clostridium difficile* or *Bacillus anthracis*) is likely to have occurred. The physical action of washing and rinsing hands under such circumstances is recommended because alcohols, chlorhexidine, iodophors and other antiseptic agents have poor activity against spores.
5. Do not wear artificial fingernails or extenders if duties include direct contact with patients at high risk for infection and associated adverse outcomes (e.g. those in intensive care units [ICUs] or operating rooms).

Gloves

1. Wear gloves when it can be reasonably anticipated that contact with blood or other potentially infectious materials, mucous membranes, non-intact skin, or potentially contaminated intact skin (e.g. of a patient with incontinence of stool or urine) could occur.

2. Wear gloves with fit and durability appropriate to the task.
 - 2.a. Wear disposable medical examination gloves for providing direct patient care.
 - 2.b. Wear disposable medical examination gloves or reusable utility gloves for cleaning the environment or medical equipment.
 - 2.c. Remove gloves after contact with a patient and/or the surrounding environment (including medical equipment) using a proper technique to prevent hand contamination. Do not wear the same pair of gloves for the care of more than one patient. Do not wash gloves for the purpose of reuse since this practice has been associated with the transmission of pathogens.
 - 2.d. Change gloves during patient care if the hands are likely to move from a contaminated body site (e.g. perineal area) to a clean body site (e.g. face).

Safe injection practices

Take care to prevent injuries when using needles, scalpels and other sharp instruments or devices; when handling sharp instruments after procedures; when cleaning used instruments; and when disposing of used needles.

1. Use an aseptic technique to avoid contamination of sterile injection equipment.
2. Needles, cannulae and syringes are sterile, single-use items; they should not be reused for another patient or to access a medication or solution that might be used for a subsequent patient.
3. Do not recap, bend, break or hand-manipulate used needles; if recapping is required, use a one-handed scoop technique only; use safety features where available; place used sharps in a puncture-resistant container.

Source: Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee, 2007. *Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings*, June 2007. Available at: <http://www.cdc.gov/ncidod/dhqp/pdf/isolation2007.pdf>⁴⁰

Aide-Memoire for a strategy to protect health workers from infection with bloodborne viruses. Available at: http://www.who.int/injection_safety/toolbox/docs/en/AM_HCW_Safety.pdf⁴¹

Glossary

Concentrated epidemic: The epidemic state in which HIV has spread rapidly in a defined subpopulation but is not well established in the general population

Enzyme immunoassay (EIA): A type of HIV test that identifies antibodies to HIV. EIAs rely on a primary antigen–antibody interaction and can use recombinant antigens and/or synthetic peptides of HIV.

Generalized epidemic: The epidemic state in which HIV is firmly established in the general population

Incidence: The number of new cases of infection or disease that occurs in a defined population within a specified time period

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 labelled 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 demographic and risk-behaviour information.

Low-level epidemic: The epidemic state in which HIV has never spread to a significant level in any subpopulation, although HIV infection may have existed for many years

Negative predictive value: In HIV testing, this means the probability that a person with a negative test result is not infected.

Positive predictive value: In HIV testing, this means the probability that a person with a positive test result is infected.

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

- Proficiency panel:** Panels containing HIV-negative and HIV-positive (weak to strong) specimens representative of the HIV strains circulating in a country. The panel, of approximately six to ten specimens, should be sent to participating laboratories at least twice each year for testing.
- Quality assurance:** The dynamic and ongoing process of monitoring a system for reproducibility and reliability of results, which permits corrective action when established criteria are not met
- Rapid test:** A rapid HIV antibody test that is simple, does not require any preparation or equipment other than what is supplied in the kit, and provides the results in 30 minutes or less.
- Second generation HIV surveillance (SGS):** Developed by the World Health Organization and the Joint United Nations Programme on HIV/AIDS, SGS is a conceptual framework to improve HIV surveillance. Guidelines for SGS suggest approaches to make better use of data to increase and enhance the response to the HIV epidemic.
- Sensitivity of an HIV test:** A measure of the ability of a test to correctly identify a specimen that contains antibody to HIV
- Sentinel surveillance:** Surveillance conducted through “watchpost” sites that provide access to populations that are of particular interest or representative of a larger population
- Serosurveillance:** Epidemiological study or activity based on the detection through serological testing of the presence or absence of HIV antibody. Latent, subclinical infections and carrier states can thus be detected, in addition to clinically overt cases.
- Specificity of an HIV test:** A measure of the ability of a test to correctly identify a specimen that does not contain antibody to HIV
- Specimen reference number:** A code that is used to identify a specimen. It is unique to a specimen and may or may not be linked to any personal identifying information.
- Testing strategy:** The use of an appropriate combination of HIV tests. The choice of testing strategy used is based on the objective 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 specimen of blood originally collected for other purposes is tested for HIV after all information that could identify the source of the blood is eliminated from the sample.
- HIV-1 western blot (immunoblot):** A supplemental confirmatory test for diagnosis of HIV infection. Western blot permits detection of antibodies to specific HIV proteins on a strip of nitrocellulose membrane.

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