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

# Guidelines for Using HIV Testing Technologies in Surveillance

WHO

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CDC
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Distr.: General
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# *Guidelines for Using HIV Testing Technologies in Surveillance: Selection, Evaluation, and Implementation*




World Health Organization
(WHO)

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 national, regional and global levels.

WHO and UNAIDS express their gratitude to those who have contributed their time and experience and provided precious inputs and suggestions for the development of these Guidelines.

The Centers for Disease Control and Prevention (CDC), Atlanta, USA, deserves special credit for having prepared these Guidelines. The key professional staff, Dr. Christopher Murrill and Dr. Rebecca Martin, should be congratulated for their efforts in producing this document, with editorial assistance from Ms. Sadhna Patel and Ms. Beatrice Divine.

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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 to make sound decisions on how best to respond to it. This is especially true in developing 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—second generation HIV surveillance. Guidelines for second generation HIV surveillance suggest approaches to make better use of data to increase and improve the response to the HIV epidemic. As biological surveillance (serosurveillance) is an important component of most HIV surveillance activities, an understanding of current HIV testing technologies is important.

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 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 other health professionals involved in HIV testing for surveillance purposes in developing countries. They are part of a series of operational guidelines for second generation HIV surveillance systems.

# 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

The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) recommend the use of second generation HIV surveillance to improve collection, analysis, and use of data essential to AIDS control programs. The use of second generation HIV surveillance is also promoted to help national and international institutions monitor the epidemic and guide their responses to it (UNAIDS/WHO 2000). Using second generation HIV surveillance 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) (UNAIDS/WHO 2000). As countries develop or enhance their surveillance programs using the principles of second generation HIV surveillance, 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. (HIV prevalence has not consistently exceeded 5% in any defined subpopulation.)

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.)

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

Second generation HIV surveillance relies on data collected from biological surveillance (serosurveillance), behavioral surveillance, and other sources (e.g., HIV/AIDS case surveillance, death registration, sexually transmitted infection surveillance, tuberculosis 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 epidemiologic tools; improved methods for building estimates and modeling 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 geographic 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 programs, and evaluate the effectiveness of the countries' response to the epidemic.

As biological surveillance is the primary method for determining HIV prevalence rates locally or nationally, accurate HIV testing is critical for second generation HIV surveillance. 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 45 minutes with minimal experience and no equipment. These rapid tests enable testing for HIV surveillance activities in areas where testing could not previously occur (e.g., areas with limited laboratory resources) and for hard-to-reach populations (e.g., female sex workers) who may not be accessible through clinical services.

These guidelines provide guidance on types of specimens to collect and how to store and test them. They describe HIV testing strategies recommended by WHO, which are based on HIV prevalence rates, and existing HIV testing technologies used for biological surveillance. They also include 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. They outline methods for selecting and evaluating testing technologies appropriate to a country's epidemic state and needs. Because accurate results are important in biological surveillance for HIV, quality assurance measures are also addressed. The guidelines also include a glossary of terms used in the document. These technical guidelines are written for HIV surveillance coordinators and other health professionals involved in HIV testing for surveillance purposes in developing countries. They are part of a series of operational guidelines for second generation HIV surveillance systems.

# 2.0 Overview of HIV Testing in HIV Serosurveillance

summary

Overview of HIV Testing in HIV Serosurveillance

- HIV Testing Objectives
  - 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, 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 (UNAIDS/WHO 1998). In developing countries, most HIV testing for surveillance purposes is conducted as part of seroprevalence surveys among population groups such as women attending antenatal clinics, patients with sexually transmitted infections, female sex workers, or injection drug users. In addition, the results of testing for diagnostic purposes (e.g., in voluntary counseling and testing clinics) and for blood screening purposes (e.g., in blood donors), as well as 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, given the epidemic state, appropriate population groups 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) (Box 2). If feasible, such testing should be conducted in settings where referrals to voluntary counseling 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, for example, HIV surveillance in populations not accessible through clinic settings (e.g., hard-to-reach populations such as injection drug users, female sex workers, and men who have sex with men) (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.

When informed consent is required (Box 2), it must be obtained before testing the specimen 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 code should be assigned to every specimen tested for HIV. The code is used to identify each specimen during 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 code can be linked to demographic (e.g., age, sex, marital status, geographic area of residence) (in linked and unlinked testing) and risk behavior 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 personal identifiers or names obtained, no informed consent, no counseling required
- Coded specimen

Unlinked anonymous testing (with informed consent)[a]
- Testing of unlinked specimens collected solely for surveillance purposes
- Informed consent[b] required
- No personal identifiers or names obtained, no counseling required
- Coded specimen

Linked confidential testing (with informed consent)
- Informed consent[b] and pretest and posttest counseling required
- Personal identifiers or names obtained
- Coded specimen; code linked to personal identifying information

Linked anonymous testing (with informed consent)
- Informed consent[b] and pretest and posttest counseling required
- No personal identifiers or names obtained
- Coded specimen; code given to patient so that only patient can link himself or herself to results

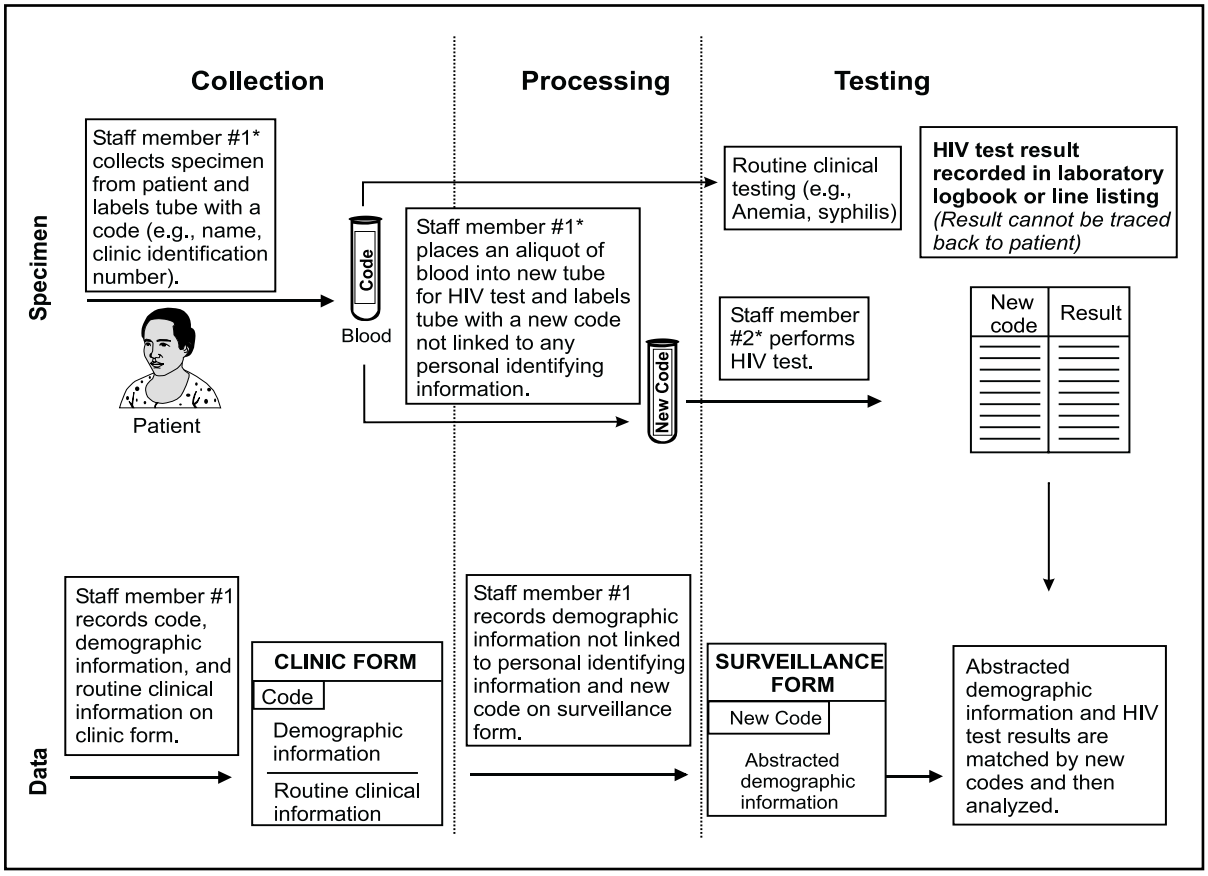
[a]The use of this method is described by the UNAIDS/WHO Working Group on Global HIV/AIDS/STI Surveillance; its use in HIV surveillance activities should be guided by the policies of the country in which these activities are carried out.

[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)

Unlinked anonymous testing (Figure 2.1) without informed consent is only conducted in clinical settings in which a specimen of blood 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 blood is removed from the specimen. Thus, the test result may not be traced back to the patient nor may he or she be 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 testing. One staff member (clinician or laboratory technician) should collect and process the specimen for routine clinical testing and for unlinked anonymous HIV testing. The processing of the specimen for unlinked anonymous HIV testing requires removing an aliquot of blood and placing it into a new tube that is labeled with a new code not linked to 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., remove an aliquot of blood and place it in a new tube labeled with a new 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, geographic area of residence) and medical history from the patient. This information is recorded onto a clinic form along with the code on the specimen (e.g., name, clinic identification number). After the specimen is processed for unlinked anonymous HIV testing and the aliquot is labeled with a new code, the same staff member records the new code onto a surveillance form and abstracts the needed demographic information (e.g., age, sex, marital status) onto the form. This abstracted information is therefore not linked to any personal identifying information. The unlinked anonymous HIV test results can then be matched with the demographic information for analysis by the new code. It is important that the anonymity of the specimen not be compromised by the collection of too much demographic information that may allow identification of individuals.

In settings where blood is 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 voluntary counseling and testing should be provided, or, if country policy permits, unlinked anonymous testing with informed consent should be conducted.

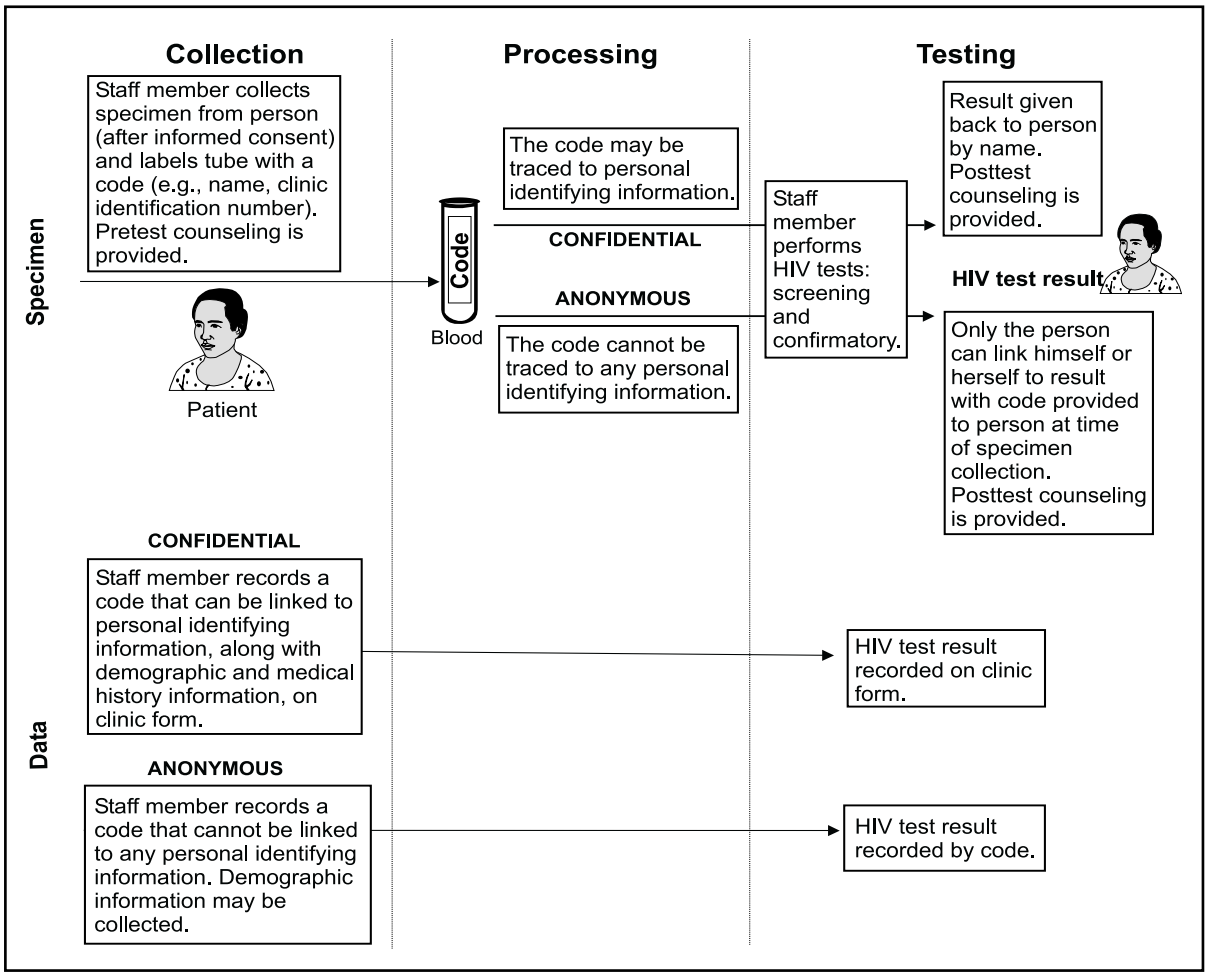
In unlinked anonymous testing with informed consent, the participant is asked whether he or she would agree to participate in the HIV surveillance study and informed that the results of the HIV test performed will be unlinked by removing all personal identifying information from the specimen. Therefore, it will not be possible to trace which participants have positive test results. The participant should be provided free access to HIV testing and counseling. The person can refuse to participate in the study, thereby introducing a possible participation bias.

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 pretest and posttest counseling. Linked testing may or may not occur in a clinical setting. Linked testing can be either confidential or anonymous. Since the person tested may receive the test results, linked testing requires additional confirmatory HIV testing.

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. HIV test results can be matched to the person by a code linked to personal identifying information. For linked confidential testing, a staff member obtains informed consent and provides pretest counseling prior to specimen collection. The specimen is then labeled with a 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 posttest counseling.

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 labeled with a 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 Testing




## 2.3 Populations Surveyed

The state of the epidemic determines which population groups are 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, outpatient clinics at hospitals, or health centers. Countries with concentrated epidemics or low-level epidemics focus primarily on specific population groups who are perceived to be at high risk for infection, for example, female sex workers and their clients, injection drug users, or men who have sex with men. Therefore, venues attended by such persons are selected as sentinel sites; these may include clinics that treat sexually transmitted infections, drug treatment centers, correctional facilities, brothels, bars, and clubs. 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.

**recommendation**

- If the epidemic is generalized, antenatal clinics are preferred sites for serosurveillance.
- If the epidemic is concentrated or low-level, the surveillance system should focus on populations at increased risk (e.g., female sex workers and their clients, injection drug users, and men who have sex with men).

Further discussion of the methods used for the selection and sampling of appropriate subgroups and sites for conducting HIV surveillance activities are beyond the scope of this document.

## 2.4 Measuring HIV Incidence

The recent modification of a type of HIV test termed "detuned assay" or Standardized Testing Algorithm for Recent HIV Seroconversion (STARHS) is now available only as a research tool for measuring HIV incidence (rate of new HIV infections). Its use in nonresearch settings has not been approved in any country. However, because further development is under way for its use in the field as a surveillance tool, it is briefly described here.

STARHS allows for determining whether an HIV-infected person has been recently infected. Specifically, it indicates whether the person has seroconverted (produced HIV antibodies) within an average of about 130 days prior to being tested (Janssen et al. 1998). This ability to distinguish recent from older infections permits estimation of recent HIV incidence based on a single cross-sectional survey. Such estimates of HIV incidence can be extremely valuable for evaluating the current trends of HIV infection.

# 3.0 Specimens Used in HIV Testing

summary

## Specimens Used in HIV Testing

- Selecting Specimens
  - Advantages and Disadvantages of Whole Blood, Serum, and Plasma
  - Advantages and Disadvantages of Urine and Oral Fluids
- Collecting, Processing, and Storing Blood Specimens
- Collecting and Storing Urine and Oral Fluids
- Labeling and Logging Specimens

Many types of specimens can be used with HIV testing technologies for HIV biological surveillance: whole blood, plasma, serum, oral fluids, and urine. The choice of specimen collected depends on logistics, populations and sites selected, and the HIV testing strategy. 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 dried blood spots 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.

## 3.1 Specimen Selection

### 3.1.1 Advantages and Disadvantages of Whole Blood, Serum, and Plasma

Whole blood, serum, and plasma, which can be collected by venipuncture or finger stick (see 3.2), have the following advantages and disadvantages in HIV testing:

Advantages

- Have higher concentrations of HIV antibodies than urine or oral fluids (Rose et al. 1997).
- Have potential for additional routine testing (e.g., syphilis, hepatitis B, hepatitis C) from a single specimen.
- Have potential for special studies (e.g., HIV typing [HIV-1 vs. HIV-2], HIV subtyping, antiretroviral resistance).
- Are easy to collect and test in clinical settings with a laboratory and a trained phlebotomist.
- Are easy to collect in nonclinical settings (whole blood from finger stick).

Disadvantages

- Require invasive collection technique.
- Require skilled technician (for collecting and processing serum or plasma).
- Compared with urine and oral fluids, require more equipment (e.g., needles, tubes, or lancets) and biohazard waste facilities.
- Is difficult to collect serum or plasma in nonclinical settings if venipuncture is required.
- Compared with oral fluids, pose a greater risk to health-care workers and technicians through inadvertent exposure, both because of higher antibody concentrations and the use of sharp collecting devices.

## 3.1.2 Advantages and Disadvantages of Urine and Oral Fluids

Other specimens besides blood and blood products can be used for HIV testing. For linked testing, where informed consent must be obtained, oral fluids may be used. Urine is the only other specimen (besides blood) routinely collected at some clinics where unlinked anonymous testing could be conducted.

Advantages

- Do not require a trained technician for specimen collection and processing.
- Do not require contact with possibly contaminated laboratory materials, e.g., used needles or lancets that need biohazard waste facilities.
- Oral fluids can be collected in a variety of field settings, including nonclinical settings.
- Oral fluid collection may be more acceptable to hard-to-reach populations than specimen collection requiring venipuncture or finger stick. Therefore, a greater percentage of the target population may agree to be tested.

Disadvantages

- Urine EIA tests are less sensitive and specific than blood-based tests (Martinez et al. 1999).
- Urine specimens must be tested at a laboratory (EIA); no rapid test is currently available.
- Oral fluid collection may require special equipment.
- Currently available testing technologies for use with these specimens are limited.

- Are likely to cost more than using serum or plasma.
- May require an additional specimen (e.g., blood) for confirmation.
- Cannot be used to perform additional testing for special studies (e.g., HIV-1 vs. HIV-2, HIV subtyping, antiretroviral resistance).

**recommendation**

Blood (whole blood, serum, plasma) is the preferred specimen for testing because it has a higher concentration of HIV antibodies than urine or oral fluids. It also allows for additional routine testing, including syphilis, hepatitis B, and hepatitis C, and for special studies of HIV type and subtype and antiretroviral resistance.

## 3.2 Collecting, Processing, and Storing Blood Specimens

### 3.2.1 Whole Blood, Serum, and Plasma

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

#### 3.2.1.1 Processing Blood Collected by Venipuncture

To collect blood by venipuncture, 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 venipuncture (National Committee for Clinical Laboratory Standards [NCCLS] 1998):

1. 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.)

   If the blood specimen will not or cannot be processed immediately (e.g., no centrifuge is available), collect the blood in a purple-top tube with EDTA. Let the blood stand for at least 20-30 minutes, and then remove plasma carefully with a pipette so as not to get too many red blood cells. Process (see Step #3) and test within 24 hours to avoid hemolysis of the specimen. A dried blood spot can also be prepared at this time using anticoagulated blood.

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 will form before centrifuging. In general, the specimen should be centrifuged at 300-400 g or 1,200 to 1,500 RPM for 10 minutes (Lennette et al. 1985).
3. After the specimen is centrifuged or has had time to separate, use a clean pipette (do not pour) to remove an aliquot of 0.5 to 2.0 ml off the top layer. Transfer it to another sterile labeled tube (plastic, not glass) or cryovial (1.5-2.0 ml with screw cap) and tighten the cap. The specimen is ready for storage and testing.

### 3.2.1.2 Storing Serum and Plasma Collected by Venipuncture

To store serum and plasma, consider the following (NCCLS 1990):

- Make sure the cap is tight on the labeled 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, immediately pack the box upright in a cooler containing cold packs to produce an ambient temperature of 4º C within the cooler. If cold packs are not available, serum specimens can remain at room temperature for up to 3 days. Longer periods at room temperature may result in bacterial overgrowth and breakdown of the specimen.
- If specimens are held at the collection site for longer than 3 days prior to shipment to the laboratory where testing will be performed, freeze specimens at -20º C in a non-frost-free freezer. For long-term storage, specimens should ideally be frozen at -70º C in a non-frost-free freezer.
- Limit the number of freeze/thaw cycles to five because multiple thaws will affect antibody levels and therefore test results.

### 3.2.1.3 Collecting Blood by Finger Stick

Blood collected by finger stick can be used to perform a rapid test or make a dried blood spot on filter paper. A dried blood spot may be preferred in rural settings and nonclinical settings, which often do not have trained phlebotomists and 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 alcohol with 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 to place on the rapid test apparatus or on the filter paper for the dried blood spot. 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 hemolysis of the specimen and could invalidate the test result (NCCLS 1999). The ear lobe may be pricked instead of the finger.

#### 3.2.1.4 Preparing and Storing a Dried Blood Spot for an HIV Test

Blood from a finger or ear-lobe stick can be used to make dried blood spots (George et al. 1989). Although finger stick is the most typical method, dried blood spots can also be obtained by using blood collected in a tube with an anticoagulant (NCCLS 1997). Dried blood spots have the advantage of being easily transported, 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 or Whatman BFC 180 paper). The paper may come with preprinted circles that will contain approximately 100 $\mu$L 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, that paper should not be used.
2. Label the side of the filter paper with a 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 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 dried blood spots in their plastic bags are to be stored longer than 90 days, they should be maintained at -20°C (George et al. 1989). Properly stored dried blood spots have been shown to be stable for at least 2 years. The bags should be placed in a sturdy envelope for shipment (Knudsen et al. 1993).

## 3.3 Collecting and Storing Urine and Oral Fluids

### 3.3.1 Collecting Urine and Oral Fluids

For specimen collection, follow test instructions, as well as local laboratory procedures. See Section 6.3 for information on safety procedures.

#### 3.3.1.1 Urine

A variety of EIA protocols exist for using urine specimens; however, no rapid tests are available at this time for use with urine specimens.

A few important considerations when collecting urine are

- A minimum of 200 μL of urine is usually required for use with a urine-based EIA test kit.
- Specimen can be collected at any time of day and need not be midstream.
- A preservative may be added to the specimen for storage, although it is not required.

#### 3.3.1.2 Oral Fluids

Oral fluids can be used with both modified EIAs and rapid tests. Rapid tests using oral fluids are currently under field evaluation.

The following general steps to collect a specimen are provided:

1. Use a specially treated absorbent pad attached to a plastic stick (usually provided by the test kit manufacturer).
2. Place the pad in the person's mouth against the inner cheek for the length of time specified in the manufacturer's instructions. Then place the pad into a vial containing a preservative solution (usually provided by the test kit manufacturer).

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

### 3.3.2 Storing Urine and Oral Fluids

Urine specimens should be placed in plastic cryovials containing a preservative. Urine specimens with a preservative may be stored up to 1 year at 4º-8º C. Urine specimens for HIV testing must not be frozen.

Oral fluid specimens can be stored from 4º to 37º C for a maximum of 21 days (including the time for shipping and testing). Oral specimens should be refrigerated during shipment. Specimens can be frozen (-20º C) for a limited time (approximately 6 weeks). Once thawed, they can be refrozen once. Consult the test kit insert prior to testing for more specific storage information.

## 3.4 Labeling and Logging Collected Specimens

### 3.4.1 Labeling Specimens

The plastic tube, cryovial, or filter paper containing the specimen must be labeled with a 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. Preprinted cryolabels designed to adhere during freezer storage should be used when specimens are stored in cryovials. 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 code unlinked to personal identifying information (see Figure 2.1).

### 3.4.2 Logging Specimens

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 the 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 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 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 Testing Technologies and Strategies

- Testing Technologies (Enzyme Immunoassays and Rapid Tests)
  - Test Characteristics and Applications
  - General Steps for Performing Tests
- UNAIDS and WHO Testing Strategies I, II, III

## 4.1 HIV Testing Technologies

### 4.1.1 Overview of HIV Testing

HIV testing technologies can currently identify

- Antibodies to HIV (e.g., EIA, rapid test, Western blot, immunofluorescence assay, particle agglutination)
- Specific HIV antigens (e.g., EIA, antigen testing)
- HIV viral nucleic acid (e.g., by polymerase chain reaction or other techniques)
- HIV (by viral culture)

For surveillance as well as diagnostic purposes in developing countries, technologies that identify HIV antigen, viral nucleic acid, or HIV itself are expensive, technically more difficult, and more often affected by laboratory error. 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. However, studies have shown that the latest generation of 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 (Tam 1999; Stetler et al. 1997). Therefore, UNAIDS and WHO recommend alternative testing strategies using combinations of EIAs or rapid tests to confirm initial positive tests (UNAIDS 1997). The first test (screening test) should be highly sensitive to provide reliable detection of antibodies in a specimen. The second test (confirmatory test) should be highly specific to confirm that the specimen truly contains antibodies specific to HIV (UNAIDS/WHO 1999).

Most EIAs and rapid tests contain antigens to both HIV-1 and HIV-2 and therefore can detect antibodies to both HIV types. However, most tests do not distinguish 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. UNAIDS and WHO have defined four categories of complexity for EIAs and rapid tests: (1) No additional equipment or laboratory experience is required; (2) Reagent preparation or a multistep process is required; (3) Specific skills such as diluting are required; and (4) Equipment and skilled laboratory technicians are required (UNAIDS/WHO 1998).

**recommendation**

Of the available testing technologies, EIAs and rapid tests are the most accurate and cost-effective for both surveillance and diagnostic purposes.

## 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 generation of EIAs (Tam 1999). The antigen preparations in early EIAs were more cross-reactive with components from the host cell (the human lymphocyte cell lines in which the virus was grown). Therefore, specimens initially found reactive when screened with an EIA were always confirmed with a Western blot. However, second and third generation EIAs have shown marked improvement in specificity, as well as sensitivity, making them candidates for confirmatory assays. The use of recombinant proteins and synthetic peptides also improved the sensitivity, which reduced the nonreactive period after initial infection with HIV and permitted earlier detection of an infection (UNAIDS/WHO 1999). Most HIV EIAs contain antigens to HIV-1 and HIV-2 and have been optimized to detect antibodies to both (Tam 1999; UNAIDS/WHO 1999).

### 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 requires maintenance, and a constant source of electricity. EIAs are most efficient for laboratories that process a large number of specimens (100 or more) daily or for batch testing, which is common in HIV sentinel surveillance activities. Because of test design, they are not suitable or cost-effective to run on a small number of specimens. However, if a laboratory processes at least 50 specimens each 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, quality assurance and quality control 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).

The cost of an EIA ranges from USD1-2 per test. When considering both direct and indirect costs (technology, human resources, and equipment requirements), the actual cost may exceed USD15-20 per test (Brown and Burke 1995). However, if the direct and indirect costs are shared across all programs (blood screening, diagnosis, and surveillance), a more realistic cost for surveillance activities would be USD4-6.

**recommendation**

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

### 4.1.2.3 Performing an EIA

EIAs can be performed with serum, plasma, urine, oral fluids, or dried blood spots (once eluted). They can take from 2 to 4 hours to perform (including specimen preparation and dilution) and an additional 3 to 4 hours if a screening result has to be confirmed. Manufacturer's instructions provided with the specific EIA used should be followed.

General steps for performing an EIA include

1. Dilute the specimen in the specimen buffer and put it in a microwell plate containing HIV antigen already bound to the plate.
2. Incubate the plate as per protocol and then wash as indicated.
3. Add antihuman immunoglobulin-enzyme conjugate, which will react with the HIV-specific antibody, if present.
4. Incubate.
5. Wash the plate, add the enzyme substrate, and incubate as prescribed.
6. Add a stopping solution to terminate the enzyme reaction, and read the absorbance of the solution in a spectrophotometer.

A positive reaction has occurred if the specimen in the specimen well changes color or becomes colored, which indicates the presence of HIV-specific antibody in the specimen. The reaction is best read quantitatively with an EIA plate spectrophotometer.

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 incubation and dilution times described in the manufacturer's instructions
- Use of deionized water
- Use of a spectrophotometer to read results accurately and objectively
- Training with the technology being used
- Consistent source of power without outages that would affect the storage of reagents or the functioning of equipment

## 4.1.3 Rapid Tests

### 4.1.3.1 General Description

Interest in the development of HIV antibody tests that provide same-day results and do not require reagents or equipment not contained in the kit led to the currently available HIV rapid tests. Current rapid tests are based on four immunologic principles: particle agglutination, immunodot (dipstick), immunofiltration (flow-through device), and immunochromatography (lateral flow) (UNAIDS/WHO 1998). Most HIV rapid tests contain antigens to HIV-1 and HIV-2 and detect antibodies to both (Tam 1999; UNAIDS/WHO 1999). A positive 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 developing 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 fewer than 100 HIV tests per day, for laboratories without electricity or equipment, and for geographic areas with limited laboratory infrastructure. In some instances, even if a laboratory performs more than 100 tests per day but only during a limited time in a year, rapid tests may be more appropriate than EIAs. A result can usually be obtained in less than 45 minutes, and it is easy to interpret. However, some training is required to correctly perform the test and interpret the results. The test kits generally contain all reagents needed to run the assay: no additional reagents or equipment are required. Many rapid tests do not require electricity, special equipment, refrigeration, or highly skilled staff, although a few require refrigeration for heat-sensitive reagents.

Rapid tests may be appropriate for linked confidential testing among hard-to-reach populations (e.g., injection drug users, female sex workers) 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 (Respess, Rayfield, and Dondero 2001). 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 between USD1-3, 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 EIAs. In addition, most rapid tests include an internal quality control. However, when using rapid tests, quality assurance and external quality control measures need to be developed and implemented at all sites that are using them (Table).

**recommendation**

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

## Table. A Comparison of HIV Testing Technologies: Enzyme Immunoassays and Rapid Tests

| HIV Testing Technology | Specimens | Advantages | Limitations | Cost[a] (USD) | Complexity[b] |
|---|---|---|---|---|---|
| EIA | Serum<br>Plasma<br>Dried blood spots<br>Oral fluids<br>Urine | • Can be batched: good for ≥100 specimens at a time<br>• Can be automated<br>• QA/QC done at national and regional laboratories: easier to control<br>• Cost per test less than cost per rapid test<br>• Identifies seroconverters earlier: highly sensitive, which reduces non-reactive period | • Not flexible in testing (need minimum numbers filled for maximum efficiency)<br>• Requires skilled, trained technicians to perform and read results<br>• Requires >2 hours for results (if need to run two EIAs, >5 hours)<br>• Requires special equipment<br>• Requires maintenance of equipment<br>• Reagents must be refrigerated | 1-2 | 4 |
| Rapid test | Serum<br>Plasma<br>Whole blood<br>Oral fluids[c] | • Good for testing 1 to 100 specimens at a time<br>• Requires minimal equipment and reagents<br>• Can be performed in a clinic (on-site testing)<br>• Highly skilled staff not required<br>• Very easy to interpret test results<br>• Results in < 45 minutes<br>• Test kits can be stored at room temperature (increased stability) | • Not good for testing >100 specimens at a time<br>• The QA/QC is performed at multiple sites: requires more control<br>• May cost more per individual test than EIA<br>• Choice of testing strategy may require multiple specimens<br>• Interreader variability may provide inconsistent results with some assay formats (e.g., particle agglutination) | 1-3 | For tests based on<br><br>Immuno-chromato-graphy — 1<br><br>Dipstick and membrane-flow-through technology — 2<br><br>Agglutination — 3 |

[a]The cost of a testing technology will be affected by the direct and indirect costs.
[b]UNAIDS/WHO's four categories of complexity for HIV antibody tests: (1) No additional equipment or laboratory experience is required; (2) Reagent preparation or a multistep process is required; (3) Specific skills such as diluting are required; and (4) Equipment and trained laboratory technician are required (UNAIDS/WHO 1998).
[c]Rapid tests using oral fluids are under evaluation in field settings.
EIA, enzyme immunoassay; QA/QC, quality assurance/quality control.

#### 4.1.3.3 Performing a Rapid Test

Rapid tests can be performed with serum, plasma, whole blood, and oral fluids. Oral fluid-based rapid tests are under evaluation in field settings. No rapid test is currently available for use with urine. For some rapid tests, specimen preparation may take as long as 10-20 minutes, while the simplest rapid tests require no specimen preparation. Thus, confirmed test results can be obtained in less than 45 minutes after the specimen is applied to the device or strip. 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 with the technology being used
- Adherence to manufacturer's instructions
- Correct interpretation of results by person reading results

## 4.2 HIV Testing Strategies for Surveillance

UNAIDS and WHO recommend three criteria for choosing an HIV testing strategy (i.e., selecting appropriate HIV testing technologies or a combination of tests) (UNAIDS/WHO 1998):

1. Objective of the test (surveillance, blood screening, or diagnosis),
2. Sensitivity and specificity of the test(s) being used, and
3. HIV prevalence in the population being tested

After these three criteria are defined, an HIV testing strategy can be selected to maximize sensitivity and specificity while minimizing cost. The three HIV testing strategies recommended by UNAIDS and WHO are described below (Figure 4).

### Strategy I:

- Requires one test.
- For use in diagnostic testing in populations with an HIV prevalence >30% among persons with clinical signs or symptoms of HIV infection.
- For use in blood screening, for all prevalence rates.
- For use in surveillance testing in populations with an HIV prevalence >10% (e.g., unlinked anonymous testing for surveillance among pregnant women at antenatal clinics). No results are provided.

### Strategy II:

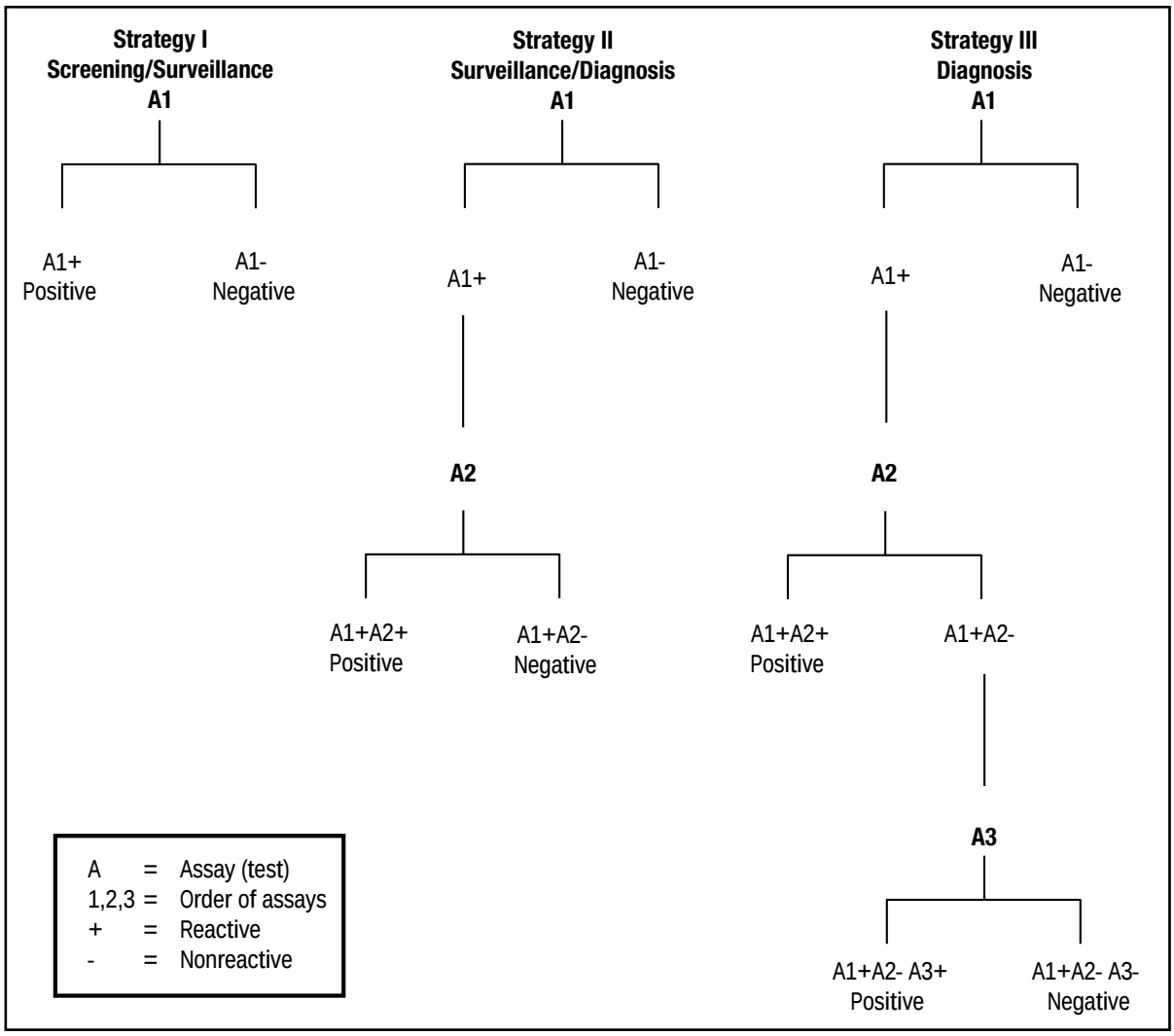
- Requires up to two tests.
- For use in diagnostic testing in populations with an HIV prevalence ≤30% among persons with clinical signs or symptoms of HIV infection or >10% among asymptomatic persons.

- For use in surveillance testing in populations with an HIV prevalence ≤10% (e.g., unlinked anonymous testing for surveillance among patients at antenatal clinics or sexually transmitted infection clinics). No results are provided.

Strategy III:

- Requires up to three tests.
- For use in diagnostic testing in populations with an HIV prevalence ≤10% among asymptomatic persons.

Figure 4. UNAIDS and WHO HIV Testing Strategies



For HIV testing strategies where more than one test may be required (Strategies II and III), the selection of testing technologies and the order in which they are used (testing algorithm) are important for obtaining accurate test results. First, the tests should contain different antigens. Second, the first test should have as high a sensitivity as possible, and the second test should have as high a specificity as possible. The first test is the screening test, so it is ideal to have a more sensitive test to detect all positives. Because a few false positives will occur, the second test (confirmation test) needs to be highly specific to ensure that all truly negative test results are identified as negative. In addition, when Strategy II or III is used, tests that can use serum, plasma, or dried blood spots are recommended so that multiple tests can be performed from a single specimen, avoiding collection of additional specimens.

For rapid tests that use oral fluid or whole blood from a finger stick, the specimen is placed directly on the rapid test apparatus; thus, a second specimen must be collected for confirmation if the first test is reactive. Therefore, for testing strategies II and III, it is suggested that, if feasible, two rapid tests be performed simultaneously on a single oral swab or finger stick and then analyzed as if performed sequentially.

This testing situation may occur when conducting linked HIV testing among hard-to-reach populations. In this situation, there is only one opportunity during which to obtain informed consent, collect the specimen, conduct the test, and provide the results and adequate counseling to the person tested. Only rapid tests allow for on-site same-day screening and confirmation. If blood is the specimen collected, dried blood spots can be prepared for quality assurance purposes (Respess, Rayfield, and Dondero 2001).

**recommendation**

When Strategy II or III is used, tests that can use serum, plasma, or dried blood spots are recommended so that multiple tests can be performed from a single specimen, avoiding 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 Factors
  - UNAIDS/WHO Recommendations Regarding Selection of EIAs or Rapid Tests
  - UNAIDS/WHO Annual Report of Operational Characteristics of Available Tests in Laboratory Settings
  - Operational Characteristics of Tests in the Field
  - Country Conditions
- Country Evaluation of Selected Tests
  - Evaluation of Tests at the National Reference Laboratory, Regional Laboratory, and Settings Where They Will Most Likely Be Used

Many countries use a variety of different HIV testing technologies and testing algorithms. For example, the national reference laboratory may screen with a rapid test and confirm with an EIA while the regional laboratory may use different rapid tests than the national laboratory for both screening and confirmation. Because of these differences, it is important for the national surveillance coordinator to document all of the testing technologies and testing algorithms that are being used in the country. In addition, in order to ensure reliable and comparable surveillance data over time, the number of testing technologies that are in use in a country should be minimized.

recommendation

It is important for the surveillance coordinator to document all of the testing technologies and testing algorithms that are being used in the country.

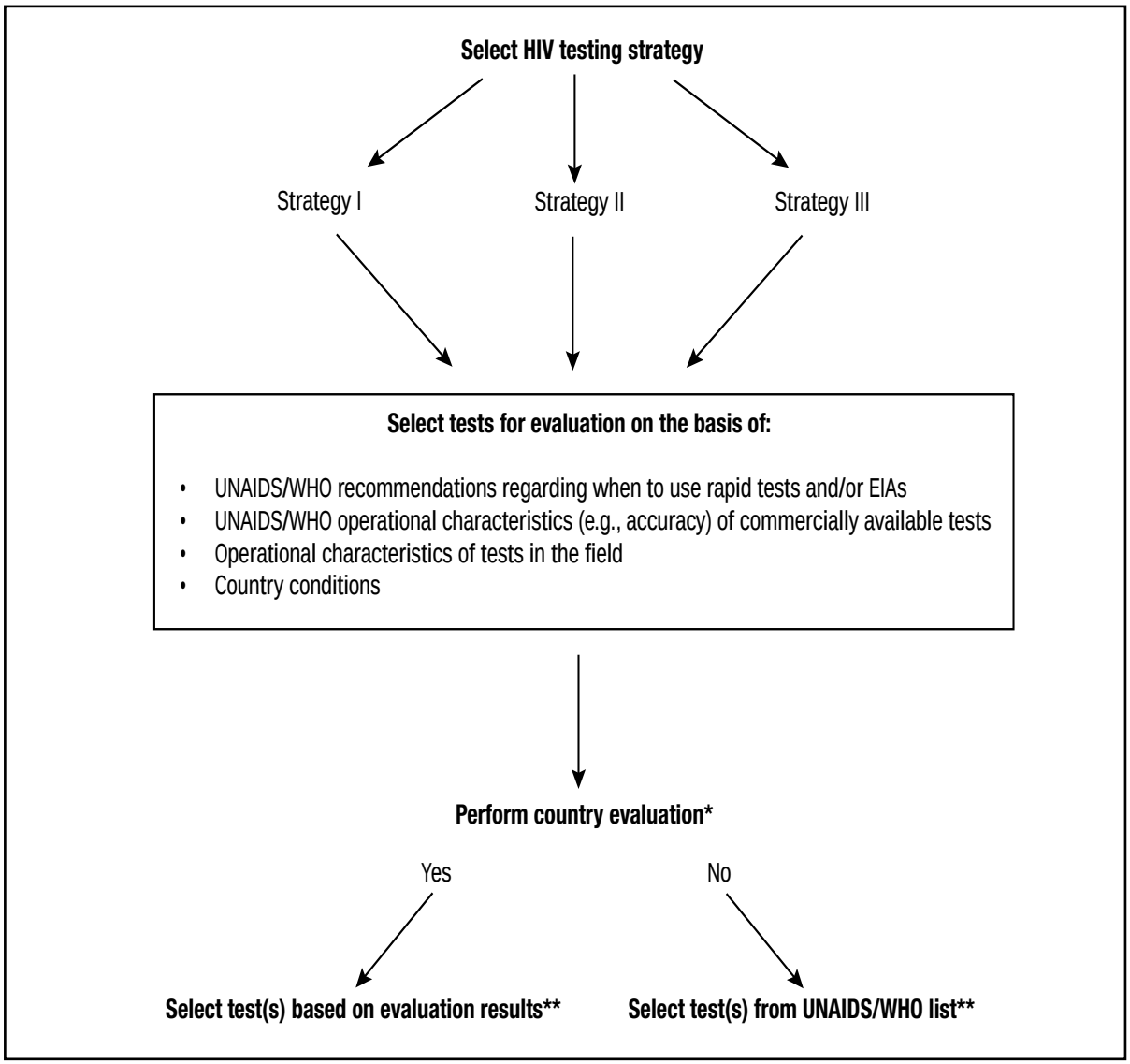
## 5.1 How to Select Tests

Selecting appropriate HIV testing technologies for use in developing countries may be a challenge since there may not be a national technical advisory group to review and evaluate available HIV tests. Therefore, the decision about which test(s) to select may often be made by a donor agency, may be based on price, or may be made as a result of a manufacturer's influence.

Instead, if the laboratory infrastructure at the national level is sufficient, countries should evaluate the performance of HIV testing technologies in their laboratory settings. However, the evaluation of testing technologies in a country can be limited by the high cost of equipment and lack of technical expertise, as well as insufficient capabilities to process specimens and then transport them to the national laboratory.

If it is not feasible to conduct a country evaluation, a country should review the selection factors below and then either select a test(s) from the UNAIDS/WHO list of currently available HIV tests or select a test based on evaluations, preferably in the region, by another independent, noncommercial source (Figure 5).

Figure 5. How to Select an HIV Test




* Determine the sensitivity and specificity of selected HIV tests in clinic and laboratory settings.
**Select the best test(s) and, for Strategies II and III, order of tests.

**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, select tests on the basis of evaluation by an independent, noncommercial source.

## Selection Factors

The first decision in selecting HIV testing technologies should be which testing format will be used (EIA, rapid test, or a combination of the two). Then, the operational characteristics of the testing technologies should be reviewed in relation to country conditions: the laboratory infrastructure, the availability of skilled laboratory personnel, and the existence of quality assurance and control measures.

- UNAIDS/WHO Recommendations Regarding Selection of EIAs or Rapid Tests. UNAIDS and WHO recommend rapid tests instead of EIAs in geographically remote areas, areas with little or no laboratory infrastructure, and small hospitals that perform fewer than 100 tests per day. In these situations, rapid tests are preferred because they are fast and easy to perform, and most have a longer shelf life than reagents for EIAs (UNAIDS/WHO 1998). Rapid tests are ideal for use at the local level because of their simplicity and low indirect cost (especially for equipment and maintenance of equipment). UNAIDS and WHO recommend EIAs for areas with good laboratory infrastructure and trained staff and for hospitals that perform 100 or more tests per day, as EIAs are very efficient for batched testing and easily implemented in laboratories at the national or regional level (UNAIDS/WHO 1998).
- UNAIDS/WHO Annual Report of Operational Characteristics of Commercially Available HIV Tests. UNAIDS and WHO publish an annual report of operational characteristics of commercially available HIV tests, which includes evaluations of the tests' sensitivity and specificity, ease of performance, suitability for use in small laboratories, and cost (UNAIDS/WHO 1999). Copies of this report may be obtained by contacting UNAIDS or WHO at unaids@unaids.org or publications@who.int. As described in Section 4.2, a highly accurate test is recommended so that the percentage of false negatives and false positives is reduced. The revised recommendations of UNAIDS and WHO state that HIV antibody tests used for surveillance need to have a specificity of at least 95% (UNAIDS/WHO 1998). Currently, most tests have a specificity of 98% or higher, but the actual sensitivity and specificity of the tests when conducted in the field may vary by the geographic origin of specimens (UNAIDS 1997).
- Operational Characteristics of the Tests in the Field. It is important to consider the selected tests' operational characteristics in the country in which they will be used. Important characteristics include ease of performance, cost per test, storage requirements, normal shelf life of the product, equipment and maintenance required, training of personnel, and regular availability of the tests from the manufacturers (UNAIDS 1997).

- Country Conditions. Country conditions that will influence test selection include number of tests performed daily, staff expertise, technological skills of laboratory personnel, test costs, laboratory infrastructure, existence of a reference laboratory, quality control and quality assurance measures in the laboratory, storage conditions (temperature and refrigeration controls), and laboratory logistics (e.g., availability of supplies, continued source of electricity).

## 5.2 Country Evaluation of Selected Tests

Ideally, 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 performance of different testing algorithms based on the results of the sensitivity and specificity of the tests evaluated (Branson 2000). A three-phase approach may be used for a country evaluation (Box 3). The first phase is conducted at the national reference laboratory to determine the sensitivity and specificity of the new test(s) under ideal laboratory conditions. In Phases II and III, specimens are collected prospectively. In the second phase, the specimens are collected at a few regional clinics and evaluated at regional laboratories. In the third phase, the specimens are collected and evaluated under the field conditions in which they will be used.

This type of three-phase evaluation has been conducted in Honduras and in Uganda (Stetler et al. 1997; Downing et al. 1998). In Honduras, seven rapid tests were evaluated in Phase I by testing 600 specimens stored at the national reference laboratory. Five tests were selected, based on their sensitivity and specificity and ease of performance, to be evaluated prospectively in Phase II (n=900). Three of the five tests were then further evaluated prospectively in Phase III at rural hospitals and clinics. The positive and negative predictive values were calculated for the different testing algorithms using the three tests to determine which testing algorithm was most appropriate. A general outline of three phases for a country evaluation is provided in Box 3; however, the outline would need to be adapted and expanded upon for specific country evaluations.

## Box 3. General outline for a country evaluation of HIV testing technologies

Phase I: Retrospectively evaluate the sensitivity and specificity of selected tests at the national reference laboratory.

- Select approximately 500 serum or plasma specimens (approximately 250 HIV antibody-positive and 250 HIV antibody-negative) from specimens already collected and stored frozen (e.g., specimens collected from surveillance activities) or from blood stored at blood banks.
- Prior to the evaluation of the tests, create a proficiency panel of approximately 25 HIV antibody-positive and 25 HIV antibody-negative specimens to evaluate the proficiency of the technicians performing the tests. Have the technicians who pass proficiency testing test the selected specimens, masked to the serostatus, using the current testing algorithm and the selected tests.* The current testing algorithm should be considered the "gold" standard against which the selected tests are evaluated. Determine the sensitivity and specificity of the selected tests.

Phase II: Prospectively evaluate the selected tests at the regional laboratories.

- Assuming adequate performance (e.g., sensitivity, specificity, ease of performance) of the selected tests in Phase I, prospectively evaluate the selected tests and current tests at a few representative regional laboratories and hospitals in the country. Approximately 100 HIV antibody-positive specimens and 200 HIV antibody-negative specimens per site (in total, 600-900 specimens) should be collected.
- Each specimen should be given a unique code so that it can later be matched with corresponding demographic information collected at the particular site. One possible method of specimen selection is the following. Those specimens found to be antibody-positive using current tests should be tested with the selected tests. For each antibody-positive specimen, the next two antibody-negative specimens should be tested until 100 positive and 200 negative test results are obtained.
- Blood should be tested using the selected tests, following manufacturers' instructions, and should also be tested using the current tests and testing algorithm. Only results from tests currently being used in the country's testing algorithm should be returned to the person; results from tests under evaluation should not be returned.
- The HIV test results obtained from the current testing algorithm should be considered the "gold" standard against which the selected tests and the selected testing algorithm(s) should be evaluated to determine the sensitivity and specificity of the selected tests. Based on the sensitivity and specificity of the selected tests in this phase, select two to three tests for evaluation in Phase III.

Phase III: Prospectively evaluate the selected tests at settings where they will most likely be used.

- Prospectively collect blood specimens from a total of 500-900 persons at four to six settings where testing with the selected tests is most likely to be performed. The goal is to have at least 50 HIV-positive specimens per site. This is best done in areas with high HIV prevalence.
- Follow the same selection procedures at the settings as described in Phase II.
- Determine the specificity and sensitivity of the selected tests and selected testing algorithms and compare them to those obtained using the country's current testing algorithm. On the basis of results of the evaluation of the selected tests and of the selected testing algorithms, recommend HIV testing technology(ies) and a testing algorithm for the country.

*Testing technologies that use only whole blood or oral fluid can be evaluated only in Phases II and III.

Adapted from Stetler et al. 1997

# 6.0 Laboratory Quality Assurance and Safety

summary

## Laboratory Quality Assurance and Safety

- National Quality Assurance
  - Proficiency Testing
- Laboratory Quality Assurance
  - Preanalytical Phase
  - Analytical Phase
  - Postanalytical Phase
- Safety

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 (UNAIDS/WHO 1996). Therefore, the ongoing process of monitoring the laboratory system, both internally and externally, is essential. Quality assurance is the dynamic and ongoing process of monitoring a system for reliability and reproducibility of results that permits corrective action when established criteria are not met.

## 6.1 National Quality Assurance

Countries should require that laboratories at all levels (e.g., HIV laboratories in hospitals, blood transfusion services, and private HIV laboratories) participate in an external quality assurance of their performance. The national reference laboratory, in collaboration with the national AIDS program, should monitor the effectiveness of the participating laboratories' quality assurance systems to identify any laboratory that might require further training or other required action.

The most common method for external quality assurance of a laboratory's performance is proficiency testing (UNAIDS/WHO 1996). The national reference laboratory should send to all participating laboratories a proficiency panel of approximately six specimens to identify as HIV-positive or HIV-negative. This panel should contain HIV-negative and HIV-positive (weak to strong) specimens representative of the HIV strains circulating in a country and of the different stages of HIV infection (UNAIDS/WHO 1996). Proficiency testing should be done once or twice each year. In geographic areas with limited laboratory infrastructure, laboratories can prepare a

dried blood spot on filter paper to be tested at the national reference laboratory for quality control purposes. External quality assurance for the national reference laboratory may be provided by an independent laboratory (e.g., a university) or by one of WHO's regional quality assurance programs.

A quality assurance system can help create good national laboratory practices using standard procedures and will also allow for exchange of information between laboratories (UNAIDS/WHO 1996). It is also important to provide feedback to the participating laboratories regarding their evaluations. Elements of the quality assurance system should be included in procedure manuals used by laboratory technicians and other laboratory staff. Compliance with such programs will maximize the reliability and accuracy of test results.

**recommendation**

Countries should require that all laboratories at all levels (e.g., national reference laboratory, HIV laboratories in hospitals, blood transfusion services, and private HIV laboratories) participate in an external quality assurance of a laboratory's performance.

## 6.2 Laboratory Quality Assurance

Laboratories at all levels (national, regional, and local) that conduct HIV testing should have a functioning internal quality assurance program. Each laboratory conducting HIV testing should routinely monitor and assess the quality in the preanalytical, analytical, and postanalytical phases of the testing process.

### 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, labeling, and transport conditions
- Treatment of specimens before testing
- Sources and types of specimens tested
- Number of specimens tested
- Selection of test kits
- Expiration dates of test kits. Kits need to be used before expiration 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 expiration dates and before newer kits) will result in a more efficient use of test kit supplies.

- 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.

### 6.2.2 Analytical Phase

The analytical phase encompasses the testing process itself. Some of the components to be reviewed by the quality assurance program include

- Specimen processing and storage
- Written procedure manual
- Reagent preparation
- Testing performance
- Performance and maintenance of equipment (e.g., spectrophotometers, washers)
- Correct use of reagents
- Inclusion of internal quality controls in the test kits
- Quality control 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
- Entering data into the tracking system (computer or hard copy)
- Maintaining records
- Reviewing quality control

## 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. See WHO, 1991, and CDC, 1988, for further recommendations on safety precautions. Precautions for the prevention of transmission of HIV and other blood-borne pathogens are summarized in the Appendix. For information about postexposure prophylaxis, consult the country's national AIDS control program.

Proper disposal of all contaminated laboratory waste is essential (WHO 1991). 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 (Richmond and McKinney 1993). Methods to decontaminate all contaminated waste (e.g., autoclaving, chemical disinfecting, 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. Universal Precautions for Prevention of Transmission of HIV, Hepatitis B Virus, and Other Bloodborne Pathogens in Health-Care Settings

The risk of nosocomial transmission of HIV, HBV, and other bloodborne pathogens can be minimized if health-care workers use the following general guidelines:

1. 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. Do not recap used needles by hand; do not remove used needles from disposable syringes by hand; and do not bend, break, or otherwise manipulate used needles by hand. Place used disposable syringes and needles, scalpel blades, and other sharp items in puncture-resistant containers for disposal.
2. Use protective barriers (e.g., rubber gloves) to prevent exposure to blood, body fluids containing visible blood, and other fluids to which universal precautions apply. The type of protective barrier(s) should be appropriate for the procedure being performed and the type of exposure anticipated.
3. Immediately and thoroughly wash hands and other skin surfaces that are contaminated with blood, body fluids containing visible blood, or other body fluids to which universal precautions apply.

## Glove Use for Phlebotomy

Gloves should reduce the incidence of blood contamination of hands during phlebotomy (drawing blood samples), but they cannot prevent penetrating injuries caused by needles or other sharp instruments. In universal precautions, all blood is assumed to be potentially infective for bloodborne pathogens, but in certain settings (e.g., volunteer blood donation centers), the prevalence of infection with some bloodborne pathogens (e.g., HIV, HBV) is known to be very low.

Institutions that judge that routine gloving for all phlebotomies is not necessary should periodically reevaluate their policy. Gloves should always be available to health-care workers who wish to use them for phlebotomy. In addition, the following general guidelines apply:

1. Use gloves for performing phlebotomy when the health-care worker has cuts, scratches, or other breaks in his/her skin.
2. Use gloves in situations where the health-care worker judges that hand contamination with blood may occur, for example, when performing phlebotomy on an uncooperative patient.
3. Use gloves for performing finger and/or heel sticks on infants and children.

4. Use gloves when persons are receiving training in phlebotomy.
5. Use sterile gloves for procedures involving contact with normally sterile areas of the body. Use examination gloves for procedures involving contact with mucous membranes, unless otherwise indicated, and for other patient care or diagnostic procedures that do not require the use of sterile gloves.
6. Change gloves between patient contacts.
7. Do not wash or disinfect surgical or examination gloves for reuse. Washing with surfactants may cause "wicking," i.e., the enhanced penetration of liquids through undetected holes in the glove. Disinfecting agents may cause deterioration.
8. Use general-purpose utility gloves (e.g., rubber household gloves) for housekeeping chores involving potential blood contact and for instrument cleaning and decontamination procedures. Utility gloves may be decontaminated and reused but should be discarded if they are peeling, cracked, or discolored, or if they have punctures, tears, or other evidence of deterioration.

Source: Centers for Disease Control. Perspectives in disease prevention and health promotion update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other pathogens in health-care settings. MMWR Morb Mortal Wkly Rep 1988; 37(24): 377-88.

# Glossary

Code: A code is used to identify a specimen. It is unique to a specimen and may or may not be linked to any personal identifying information.

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.)

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 that occurs in a defined population within a specified time period (i.e., the rate of incidence).

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 counseling) 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-behavior 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.)

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

Positive predictive value: In HIV testing, 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 and of the different stages of HIV infection. The panel, approximately six specimens, should be sent to participating laboratories once or twice each year for quality assurance testing.

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.

Second generation HIV surveillance: Developed by the World Health Organization and the Joint United Nations Programme on HIV/AIDS, second generation HIV surveillance is a conceptual framework to improve HIV surveillance. Guidelines for second generation HIV surveillance suggest approaches to make better use of data to increase and improve the response to the HIV epidemic.

Sensitivity of a test: A measure of the probability of correctly identifying an HIV-infected person.

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 presence or absence of HIV antibody. Latent, subclinical infections and carrier states can thus be detected, in addition to clinically overt cases.

Specificity of a test: A measure of the probability of correctly identifying an HIV-uninfected person.

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 information that could identify the source of the blood is eliminated from the sample.

Western blot: A type of HIV test, Western blot uses an electroblotting method in which proteins are transferred from a gel to a thin, rigid support and detected by binding of labeled antibody to HIV.

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