Over 100 different kinds of HIV test are currently available worldwide. More are likely to become available in the future. The choice of test depends on factors including laboratory requirements, how easy the test is to use, how accurate it is, and how much it costs.

The main types of HIV tests are tests that detect HIV antibodies and tests that detect the virus itself.

This section looks at HIV tests available, which tests are most appropriate for which purpose and how to choose the most appropriate tests.

The human immunodeficiency virus (HIV) is a virus that damages the body’s immune system (the body’s defence system against attack from viruses, bacteria and other harmful organisms).

Two major types of HIV affect people: HIV-1 and HIV-2. HIV-1 is most prevalent worldwide. HIV-2 is most prevalent in some countries in West Africa. There are also other sub-types (sometimes called strains or variants) of HIV.

Antibody testing is the most common, efficient and widely-used method for determining whether an individual is infected with HIV and for screening blood.

When someone is infected with a virus or bacteria, their body produces antibodies to fight off the infection. Antibodies are produced by the immune system and are specific to the infecting organism. Antibodies are much easier to detect than the virus itself.

Most HIV antibody tests detect antibodies to HIV in a sample of a person’s blood. If no antibodies are detected, the result is seronegative or HIV negative. If HIV antibodies are present, the result is seropositive or HIV positive.

However, a test may have a negative result if a person has only recently been infected. This is because it can take a few weeks for the body to start producing antibodies after an infection. Antibodies can usually be detected 3-8 weeks after infection with HIV. The time between infection and the production of enough antibodies for a test to detect is called the window period. During the window period, levels of HIV antibody are too low to detect and an antibody test result will be negative even if the person is infected with the virus (see diagram).

Tests that detect HIV antibodies in blood include ELISA tests, simple/rapid tests and confirmatory tests. Tests are also now available that can detect antibodies in saliva and urine. Additional information about HIV tests is provided in Appendix 1.

2.1 HIV antibody tests

Antibody testing is the most common, efficient and widely-used method for determining whether an individual is infected with HIV and for screening blood.

When someone is infected with a virus or bacteria, their body produces antibodies to fight off the infection. Antibodies are produced by the immune system and are specific to the infecting organism. Antibodies are much easier to detect than the virus itself.

Most HIV antibody tests detect antibodies to HIV in a sample of a person’s blood. If no antibodies are detected, the result is seronegative or HIV negative. If HIV antibodies are present, the result is seropositive or HIV positive.

ELISA tests

Enzyme linked immunosorbent assay (ELISA) tests detect HIV antibodies in blood. They were the first HIV tests available in the 1980s. Early ELISA tests were not very accurate (see Section 2.3). Most ELISA tests are now very accurate. The likelihood that an infection will not be detected with an ELISA test during the window period has been considerably reduced.

ELISA tests are:
- cheap
- efficient
- suitable for testing large numbers of samples (more than 100 in one day)
- able to detect HIV-1, HIV-2 and HIV variants
- suitable for use in surveillance and centralised blood transfusion services.

However, ELISA tests require:
- skilled and well-trained technical and laboratory staff
• sophisticated, well-maintained equipment, such as automated pipettes, washing systems, incubators and readers
• a reliable and constant power supply
• a minimum number of specimens to be efficient
• adequate time.
There is also usually a delay in receiving the results of an ELISA test. People may need to come back after several days, which means that some people do not return for their results.

Simple/rapid tests
Simple/rapid tests (assays) are now available to test for HIV antibodies in blood. Some can be performed in less than 10 minutes. These are called rapid tests. Some require 30 minutes to two hours. These are called simple tests. There are four types of simple/rapid tests: agglutination assays, comb/dipstick assays, flow-through membrane assays, and chromatographic membrane assays.

Simple/rapid tests give results that are as accurate as ELISA tests. In addition, they:
• can be done using a whole blood sample or a filter paper (see below) with blood from a finger prick
• can be done quickly, enabling people to obtain the results the same day
• usually come in a simple kit form and require no special equipment, such as microscopes or electricity
• are simple, involving between two and eight steps, reducing the chance of error
• can be carried out by staff with limited laboratory training
• do not require electricity
• are portable and flexible
• are easy to read – for most simple/rapid tests, a positive result is indicated by the appearance of a clearly visible dot or line
• sometimes have an internal control that ensures that the test result is accurate
• are designed either as single tests or in a multiple format for a limited number of specimens, giving greater flexibility than ELISAs in the number of tests that can be performed at one time. This also makes simple and rapid tests more cost-effective if only a few tests are carried out at one time or during a day. Simple/rapid tests could increase access to HIV testing in areas that lack laboratory services and highly trained technicians. However, simple/rapid tests also have disadvantages. They:
• are more expensive than ELISA tests
• may require refrigeration (although some can be stored at temperatures of between 2°C and 30°C)
• could increase the potential for mandatory testing on the spot
• could lead to results being given to people who have not had the chance to think through the implications. Some voluntary counselling and testing services that use simple/rapid tests advise people to go away and think for

Testing blood for HIV in Kenya.

a few hours after pre-test counselling, to decide whether they really want to go ahead with the test.

Filter paper method Tests can be carried out either directly on a blood sample, or via the ‘filter paper method’ – where blood is collected on specially prepared filter paper. The filter paper can then be transported over a long distance, for example from a rural area to the main regional testing laboratory. The dried blood spot is eluated (brought into solution) which is then tested.

Saliva and urine tests
Saliva and urine tests detect HIV antibodies in saliva and urine. Advantages of these types of tests include:
• simpler procedures for collecting samples than taking blood
• appropriate for people who object to giving blood for religious or other reasons
• reduced occupational risk from needlestick injury, disposal of needles and cuts from glass tubes
• safer to handle than blood, because urine and saliva contain less virus (an amount that is insufficient for HIV transmission).
However, saliva and urine tests have potential disadvantages, including:
• the need to follow specific testing procedures very carefully because the level of antibodies is lower than in blood
• potential for mandatory testing, because they are easier to carry out without obtaining informed consent
• may encourage myths about transmission of HIV through kissing
• have not been widely evaluated in the field, especially in Africa.
Confirmatory tests

Western blot tests are used to confirm a positive result from the first test. They are used rarely now because they are expensive, require special equipment and laboratory facilities, need highly trained specialist staff to interpret the results and can produce indeterminate (unclear) results.

Line immunoassays are also used as confirmatory tests. They produce fewer indeterminate results than Western blot but are equally expensive.

Home testing kits

Home testing kits are simple/rapid blood or saliva tests that can be used at home, either to obtain a result immediately or to send a sample, collected at home with a home collection kit, to a testing facility. Home collection kits were on trial for a time in the USA, but demand was low and some companies failed to meet the specifications required by the drug regulatory body.

No home testing or collection kits are currently approved by any of the regulatory authorities. No home testing kits have been approved by the World Health Organization, although some have falsely claimed WHO approval.

Because there is no pre-test or post-test counselling and no confirmatory testing system following a positive test result, the possibility of false positives or a test being taken during the window period is very high.

There are currently no controls to ensure that test kits are of a high quality and that the result is confirmed. Effective confidential record-keeping cannot be ensured. Even if home testing is approved, it will only be feasible where mail services are reliable and people have access to a telephone to obtain their results and post-test counselling.

2.2 Tests to detect the virus

The first tests that detected the HIV virus detected antigen, particles of the HIV virus. Their usefulness is limited because levels of virus particles vary at different stages of infection and are not always detectable.

The two main types of test are viral culture and nucleic acid amplification technologies (NAT), such as polymerase chain reaction (PCR) tests...

- Viral culture grows the virus from a sample of blood, in a laboratory. If HIV can be cultured, this means that there was some virus in the blood to start with and that the person is infected. However, viral culture is expensive and difficult and requires sophisticated technology and expertise, including a special safety laboratory (P3), to grow infectious material, so it is not feasible for use where resources are limited.

- Nucleic acid amplification technologies (NAT), such as polymerase chain reaction (PCR) tests, work by detecting the genetic material of the virus. Like viral culture, PCR testing is expensive, requires sophisticated facilities and highly trained technicians, and is not feasible in most developing countries. These new tests can detect very low levels of the virus and can be used to monitor anti-viral therapy.

2.3 Accuracy of the test

No HIV test is 100 per cent accurate. Some tests are likely to produce more false positive results than others, while some are more likely to produce more false negatives. Before deciding which test to use, it is important to know how accurate a test is likely to be in any given situation. The accuracy of a test result depends on the characteristics of the test itself (in terms of sensitivity and specificity) and the prevalence of HIV in the population (see diagram on page 12).

- Sensitivity is the likelihood (expressed as a percentage) that a test result will be positive when antibodies to HIV are present.

  A test with high sensitivity can detect very small amounts of antibodies and will minimise the likelihood of a false negative result. This is particularly important when testing for blood for transfusion, so that people are not given infected blood.

- Specificity is the likelihood (expressed as a percentage) that test results will be negative if HIV antibodies are not present.

  A test with a high specificity will identify HIV-negative samples correctly and will minimise the likelihood of a false positive result. This is particularly important when testing individuals, so they are not informed that they are HIV positive when, in fact, they are not.

Minimum standards for HIV tests are a sensitivity of greater than 99 per cent and a specificity of greater than 98 per cent.

- HIV prevalence is the percentage of a population that has HIV at a particular time. Knowing local HIV prevalence is necessary before deciding on a testing strategy for individuals, because the prevalence will indicate how accurate test results are likely to be. For example, in a population with low HIV prevalence, most of the positive results from a test that is more likely to produce a false positive result (high sensitivity) would be from uninfected people. However, if the same test was used in a population with high HIV prevalence, most of the positive results would be from people with HIV.

Calculating test accuracy

Positive predictive value (PPV) describes the accuracy and reliability of a test result in a given situation. It is the likelihood (expressed as a percentage) that a unit of blood is truly positive when the test result is positive. It is calculated by dividing the number of true positives detected by the test by the total number of positive test results (both true and false positive results). First, the sensitivity and specificity of the test needs to be known. The HIV prevalence should also ideally be known.
Calculating the PPV

Example 1: In a population with an HIV prevalence of 0.1 per cent, one person in 1,000 people has HIV. So out of 1,000 people there would be one true positive. A test with a 99 per cent specificity, carried out on 1,000 people, would give true positive results for one person and false positive results for 1 per cent of the 999 uninfected people, that is, 10 people. Most of the positive results would in fact be from uninfected people.

True positives = 1
False positives = 10 (1 per cent of 999)
PPV is \( \frac{1}{1+10} = 0.091 \) expressed as a percentage = 9.1%

In a low HIV prevalence population, a test with high specificity will lead to a substantial number of false positives.

Example 2: In a population with an HIV prevalence of 10 per cent, 100 in 1,000 people have HIV. The same test with 99 per cent specificity, if carried out on 1,000 people, would give true positive results for 100 people and false positive results for nine people (1 per cent of the 900 uninfected people). So, out of a population of 1,000 people there would be 100 true positives and 9 false positives (1 per cent of 900). Most positive results would be from people who have HIV.

True positives = 100
False positives = 9 (1 per cent of 900)
PPV is \( \frac{100}{100+9} = 0.9174 \) expressed as a percentage = 91.74%

As these examples show, the higher the prevalence, the greater the probability that a person testing positive is really infected (the greater the positive predictive value) and the more likely it is that positive results will accurately indicate that infection is present.

If the prevalence is low, false positive results may outweigh true ones and most people with positive results will not actually be infected.

**2.4 Different tests for different purposes**

The purpose of the test affects the choice of test. Testing may be carried out to screen blood or organs, to diagnose HIV in an individual, or for surveillance.

**Screening blood or organs**

One test is adequate for screening donated blood or organs, if the donor is not to be notified of their result.

Tests with the highest possible sensitivity should be used for screening blood, to avoid false negative results and to detect infections as early as possible. Tests also need to be as specific as possible, to avoid unnecessary wastage of blood due to false positive results. Depending on local circumstances, tests for screening blood should be able to detect at least HIV-1 and HIV-2.

Where large numbers of blood samples are tested on a daily basis in one centre, ELISA tests are the most appropriate option. However, in hospitals and blood banks in rural areas, the number of samples screened at one time may be limited, laboratory facilities may be basic, equipment may be poorly maintained and technicians may be inadequately trained. In these circumstances, simple/rapid tests would be more appropriate.

**Surveillance**

One test is adequate for surveillance and research purposes, if the participants are not told their results. In most studies large numbers of samples are collected anonymously over a period of time. Collecting blood samples from a finger prick, using the filter paper method, may be the most practical in such studies (see page 10). As results are not needed immediately,
unclear results. The most appropriate combination of tests will need to be decided locally, on the basis of local HIV prevalence and conditions.

Tests with the highest possible specificity should be used for diagnosing HIV infection in individuals, so that people are not wrongly informed that they are infected. This is especially important in areas where HIV prevalence is low, because a high proportion of positive results from one test are likely to be false positives. Test combinations also need to be selected carefully. As HIV tests have become more sensitive, the probability of a false positive result from two separate tests has increased.

2.5 Choice of test
Choice of test depends on a number of factors including:

- whether testing will be used for screening blood, for surveillance or for diagnosis
- how accurate the test is (sensitivity and specificity)
- which testing kits are approved for use locally
- how easy it is to perform the test and to read the results
- how long it takes to do the test and obtain results
- whether the test is able to detect the local types or strains of HIV, for example HIV-1 or HIV-2
- what storage conditions are required, for example, refrigeration, and the shelf life of the test and of reagents
- what laboratory facilities, equipment and staff are available
- how much the test costs
- how many samples will be tested each day.

The suitability of different tests for different circumstances is shown in Appendix 2 on pages 36-40.