HIV testing technologies

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Increasing the uptake of HIV testing in order to reduce rates of undiagnosed infection and late diagnosis is a key goal of the HIV Prevention England programme. Many non-clinical services are involved in providing or promoting HIV testing services. In order to accurately explain the benefits of HIV testing to clients, workers need to know what kind of test is being offered and how accurate it is, especially in the context of recent infection.

This briefing paper, produced by NAM for HIV Prevention England, provides an overview of HIV diagnostic tests for people planning, commissioning or providing HIV prevention activities in England.

What would an ideal test be like?

A set of criteria are commonly used to evaluate the appropriateness of using a medical test, particularly when it is offered to people who feel well. Firstly, the test must detect a medical condition that is not trivial and which can be treated. This is clearly the case for HIV.

Moreover, the test should ideally be:

- Very accurate in identifying people who have the infection ("sensitive").
- Very accurate in identifying people who do not have the infection ("specific").
- Very accurate in identifying people who have recently been infected.
- Reliable, giving the same result each time a sample is tested.
- Non-invasive (a needle or tube does not enter the body).
- Safe.
- Inexpensive.
- Simple to carry out, without complex equipment or training.
- Suitable for testing lots of people.
- Quick to give a result.

A test for HIV should also detect the full range of HIV-1 subtypes and identify people who are infected with HIV-2.

Unfortunately, no medical test is perfect. Choices must be made based on the available tests, the needs of the people being tested and our priorities.

- Which is more important – that people who have an infection are identified, or that people who do not have the infection are not falsely alarmed by the suggestion that they might be?
- If a rapid, convenient and non-invasive test encourages more people to test, should it be used even though it is less accurate than another test?

As no test is 100% accurate, it is important to stress that no one test is used in isolation, especially to give an HIV-positive diagnosis. If a test appears to give a positive result, the validity of this must always be verified with a series of confirmatory tests.

This briefing summarises some key issues with HIV testing technologies commonly used in laboratories, community settings, self-sampling and self-testing services.

Common questions about testing

How soon after taking a risk can I test?

Most clinics advise people who have recently taken a risk to test immediately, and believe that it is unhelpful to ask people to put off testing until later. If people are concerned about a very recent risk they have taken, they may be motivated to test now. If they are asked to wait, the issue may slip from their mind.

Antibody/antigen tests can sometimes detect infection just 10 days after infection, and most infections will be detected within a month. So, the clinic should take an initial test straightaway. If the result is negative, the person will usually be asked to return a few weeks later in order to be re-tested.

How long after taking a risk can I be sure that I am HIV-negative?

Although the majority of infections are detected within a month, there are occasional cases when it takes longer. All cases of infection should be detected by antibody/antigen tests within eight weeks. Following an event that would carry a high risk of HIV infection, BASHH recommends a test after eight weeks to be sure.

How often should I test?

BHIVA and NICE recommend annual HIV testing for people in groups or communities with high rates of HIV. People with continued risk behaviour (such as gay and bisexual men having condomless anal sex with new or casual partners, or drug users who share injecting equipment) as well as people taking PrEP should test every three months.

HIV testing technologies

HIV PREVENTION ENGLAND

Antibody/antigen laboratory tests

The most accurate and reliable routinely used diagnostic HIV test is a laboratory test that can detect both HIV antibodies and p24 antigen.

HIV antibodies are not part of HIV itself, but are produced by the human body in response to HIV infection. In the weeks after exposure to HIV, the immune system recognises antigens that belong to HIV and begins to generate HIV antibodies (this period is known as 'seroconversion'). These antibodies persist for life.

An HIV antigen, known as p24, is a structural protein that makes up most of the HIV viral core. High levels of p24 are present in the blood during the short period between HIV infection and seroconversion, before fading away. Since p24 antigen is usually detectable a few days before HIV antibodies, a test that can detect p24 has a slightly shorter window period than a test that only detects antibodies.

A blood sample is taken through a needle from a vein in the arm. Samples from many individuals are analysed at the same time, in a machine at a laboratory. These tests may also be referred to as "fourth-generation" tests or as an ELISA (enzyme linked immunosorbent assay). The first- and second-generation tests are no longer in use. The third-generation test only detects antibodies and is no longer recommended for routine use in the UK.

It is hard to say exactly how long the window period for a test lasts, as there are variations between individuals and it is a difficult topic to research. Nonetheless, it is estimated that the median window period for antibody/antigen tests is 18 days, with half of all infections being detected between 13 and 24 days after exposure. While occasionally this period will be a little longer, 99% of HIV-infected individuals would be detectable within 44 days of exposure.

Therefore, UK guidelines state that an antibody/antigen laboratory test will detect the great majority of individuals who have been infected with HIV four weeks after specific exposure.

Antibody/antigen laboratory tests are extremely accurate. In terms of sensitivity (correct identification of people with HIV), a Centers for Disease Control and Prevention (CDC) review identified four studies of two different assays, with sensitivity always above 99.7% for established infection. A Health Protection Agency evaluation found that nine out of the ten tests they evaluated had a sensitivity of 100%, while a French evaluation found that ten of twelve tests had a sensitivity of 100%. The lowest sensitivity was 99.8%.

Similar results were found for specificity, in other words, the ability of a test to correctly give an HIV-negative result. Both tests checked by the CDC had a specificity of 99.5% or above; all tests in the Health Protection Agency evaluation had a specificity of 99.7% or above; and the French study found that all tests produced after the year 2000 had a specificity of 99.8% or above.

Rapid, point-of-care tests

From the point of view of a hospital doctor, the laboratory tests previously described have considerable advantages. They give exceptionally accurate results, processes are automated and quality control can be assured in a laboratory environment. Also, if a test appears to give either a positive result or one that is difficult to interpret, there is plenty of time to re-test the blood sample to clarify the diagnosis.

But laboratory tests have some disadvantages, especially from the point of view of people testing. Some people dislike having blood taken with a needle. Getting the results usually requires coming back on another day, something that a lot of people fail to do. Laboratory tests tend to be offered in hospital settings.

"Point-of-care" tests (PoCT) do not require specialised laboratory equipment, so they can be administered and interpreted in any setting. Most point-of-care tests require a tiny sample of blood (the fingertip is pricked with a lancet). Other tests require oral fluid (an absorbent pad is swabbed around the outer gums, adjacent to the teeth). They are called "rapid" tests because the result can usually be given within 30 minutes. These tests are often used in community settings on the assumption that more people will be willing to test for HIV if they can do so at venues they go to anyway.

However, the accuracy of point-of-care tests is not always equal to those of laboratory tests, especially in relation to recent infection. This is for two main reasons:

- What the test looks for. While one antibody/antigen test is available, the other tests look for antibodies only. Moreover, some can only detect immunoglobulin G (IgG) antibodies, but not immunoglobulin M (IgM) antibodies, which appear sooner.
- The sample taken. Point-of-care tests are usually performed on whole blood taken from a fingerprick. This has a lower concentration of antibodies and p24 than the blood plasma that is separated from whole blood in a laboratory and then tested. Samples of oral fluid have a concentration of antibodies that is lower still.

HIV testing technologies

HIV PREVENTION ENGLAND

As a result, the window period of commonly used rapid tests such as the **Alere HIV Combo** and the **INSTI HIV-1/HIV-2 Antibody Test** may be one to two weeks longer than for antibody/antigen tests. Other rapid tests, based on older technology, may have longer window periods than this.

Rapid tests can be performed by staff with limited laboratory training. However, reading the test result relies on subjective interpretation, and when the result is borderline, experienced staff give more consistently accurate results. It is good practice for test results to be reread by a second member of staff, within the time frame specified on the test packaging. Organisations using point-of-care tests must maintain strong links with a pathology laboratory that provides support with clinical governance and quality assurance.

Point-of-care testing is supported in specific scenarios by the British HIV Association (BHIVA) and the National Institute for Health and Care Excellence (NICE). These include: testing at community sites; when it is important to avoid a delay in receiving results; in situations where it would be difficult to give people their results; or if a person does not want to give a venous blood sample.

When used in a population with a low prevalence of HIV, false-positive results can be a problem. The tests always produce a small number of false positive results, but in a setting where very few people have HIV, the majority of apparent positive results will in fact be incorrect. However, as the proportion of people with HIV being tested increases, the true positives start to outnumber false positives. This means it is more appropriate to use point-of-care-tests in high-prevalence populations, such as with gay and bisexual men, than in the general population.

As noted above, all HIV tests need to have reactive ("positive") results confirmed with further tests. Most providers tell people who are testing that a negative result is definitive, but that a reactive result simply indicates the need for further laboratory testing.

The accuracy of different rapid tests

A wide range of point-of-care tests have been manufactured in many countries, but only a few of them have been subject to rigorous, independent evaluations, and even fewer are marketed in the UK. Research on HIV tests is only occasionally published in medical journals. Informally, laboratory professionals may have insights into which tests perform best.

It is important to verify that any test used is CE marked. This should mean that the test conforms to European health and safety legislation, although it does not necessarily mean that test performance has been independently evaluated.

There are variations in accuracy from one test to another, with some older tests that are generally not marketed in the UK having a sub-optimal sensitivity and specificity. However, the evaluation data that are available for the tests more commonly used in the UK are more encouraging. World Health Organization evaluation of the **Alere Determine HIV-1/2**, **Uni-Gold HIV**, **INSTI HIV-1/HIV-2 Antibody Test** and **SD BIOLINE HIV-1/2 3.0** found that all had a sensitivity (the ability to detect all true positive results) and a specificity (the number of negative samples correctly identified as negative) in the range of 99-100%.

The sensitivity of the **OraQuick** test with fingerstick blood samples was slightly less (sensitivity 98.1%, specificity 100%). Furthermore, performance is poorer when testing samples of oral fluid – some people with HIV may receive a false negative result. This is because quantities of antibodies are lower in oral fluid than in blood.

There is one rapid, point-of-care test that looks for both antibodies and p24 antigen, in a similar way to antibody/antigen laboratory tests. The **Alere HIV Combo** is an improved version of the **Alere Determine HIV-1/2,** which was originally introduced in 2009. Although the older version performed well in respect of established HIV infection, the p24 part of the test was quite insensitive, meaning that many cases of acute infection were missed. The handful of studies published so far on the newer version suggests it has better performance, although it still does not match that of antibody/antigen laboratory tests. The **Alere HIV Combo**'s sensitivity during acute infection has been variously estimated to be 28% (in three African countries), 54% (France), 65% (the Netherlands) and 88% (UK). The test may perform better with HIV-1 subtype B (common in Europe) than with other subtypes (more common in Africa, for example).

One analysis pooled the results of five separate studies in which a point-of-care test (including the **Alere Determine HIV-1/2** and **OraQuick)** was compared with an antibody/antigen laboratory test. The estimated sensitivity of the point-of-care tests was 94.5% and specificity was 99.6%. This means that of 1000 people who were diagnosed with a laboratory test, 945 would also be correctly diagnosed with a point-of-care test, whereas 55 people would be given a false negative result. On the other hand, there would be only four cases of false positives for each 1000 negative results.

HIV testing technologies

HIV PREVENTION ENGLAND

Antiretrovirals and test accuracy

Antiretroviral drugs suppress or slow the replication of HIV. As a result, the immune response and production of antibodies is sometimes modified. This can have an impact on test performance in people taking antiretrovirals.

- Infrequently, rapid test and self-tests give false negative results to individuals taking HIV treatment. These tests should not be used to confirm HIV infection after starting treatment.
- This occurs more frequently in people who began HIV treatment in the first few weeks after infection. It appears that the evolution of HIV antibodies over time is profoundly altered by prompt initiation of antiretroviral therapy.
- A similar phenomenon affects people taking pre-exposure prophylaxis (PrEP), for example an individually who acquires HIV just before starting PrEP and who initially tested during the window period. He or she will be taking antiretrovirals during the early stages of infection, which may delay their antibody response.

Self-testing

Self-testing is sometimes referred to as "home-testing" and involves the end-user carrying out all the test procedures themselves, including reading and interpreting the test result.

Self-testing can increase rates of HIV testing by removing social and structural barriers, including the time required and geographical distance from testing facilities (particularly for people living in rural areas). Self-testing is attractive to people who particularly value privacy, confidentiality and autonomy. It often reaches people who have not tested before or who have not done so for some time.

Self-tests are usually modified versions of rapid, point-of-care test kits that were originally designed for healthcare professionals. Their processes, packaging and instructions have been simplified so as to guide the user through the steps of taking a test. Their sensitivity, specificity and window periods should be the same as that of the professional product.

The two self-tests which are most commonly used in the UK are the **BioSURE HIV Self Test** and the **INSTI HIV Self Test.** Both require a tiny sample of blood (the fingertip is pricked with a lancet). These tests are extremely sensitive and specific in relation to chronic (long standing) HIV infection. The **BioSURE** test is a second-generation antibody test (sensitive only to IgG), with a longer window period. **INSTI** is a third-generation antibody test (sensitive to both IgG and IgM) that is likely to perform relatively well in cases of recent infection.

While self-testing can facilitate regular HIV testing, a longer window period could mean that a recent HIV infection is missed and this could be a particular problem if self-tests are used by high-risk populations – and are relied upon in sexual decision making.

Several studies have assessed whether people able to understand test instructions and use self-tests reliably. In each one, users' test results were compared with those of a healthcare worker who performed the test on the user at the same time. Overall, most people can reliably and accurately use the test kits. Nonetheless, invalid results occur more frequently in people using blood-based tests than in people using oral fluid-based tests (not currently available in the UK). Common errors included incomplete sample collection, spilling the buffer solution, problems transferring blood samples, and difficulties with the interpretation of results. Simpler test procedures and clearer instructions can mitigate these problems.

One concern with self-testing is whether people getting a reactive result approach health services for confirmatory testing. As noted above, all tests can give false-positive results and in populations where very few people have HIV, the majority of apparent positive results will in fact be incorrect. It's therefore vital that the packaging and instructions that come with a test make it clear that a 'reactive' result is not the same as a diagnosis of HIV. It simply means that the individual needs to take more tests to confirm the result.

Self-sampling

Self-sampling is different from self-testing in that the user does not perform the test or interpret their own result. The user collects their own fingerprick blood sample and sends this to a laboratory for analysis. The laboratory makes the results available by phone or text a few days later.

When a blood sample is sent to a laboratory it will be tested with the same kind of assay that is used to test venous blood samples. Typically this is a fourth-generation antibody/antigen test. In theory, the test will be as accurate with a self-collected sample of fingerprick blood as with venous blood, both in relation to chronic (long-standing) and acute (recent) infection.

HIV testing technologies

HIV PREVENTION ENGLAND

Nonetheless, there is little published research to confirm that self-sampling works as well as can be expected. Most studies deal with acceptability and feasibility, rather than accuracy.

England's National HIV self-sampling service (www.test.hiv) has been successful at engaging first time testers (26% of users) and people who have not tested for more than a year (32%) of users. The majority of users are gay and bisexual men (68%), with fewer black African users (8%). Just under 1% of samples returned are reactive, with higher proportions in first-time testers and black African people.

Sixty per cent of people who request a kit return a sample. The vast majority of samples can be processed in the laboratory. However, 2.4% of kits are returned with an insufficient sample and 2.7% of kits are returned with a degraded sample that could not be analysed. This may be due to delayed delivery of the sample, extreme weather or not allowing alcohol from the swab to fully dry before taking the sample.

Recent infection testing algorithm (RITA)

Individuals who are newly diagnosed with HIV may also have their blood tested by the RITA method. This is a laboratory technique which aims to distinguish between recent and more established HIV infection.

RITA depends on looking for specific antibody markers, which give different results in the months following infection. If a test gives a result below a pre-determined cut-off point, it is deemed to be an infection that probably occurred in the last six months.

RITA was designed to help public health officials monitor the number of new HIV infections in a population, in order to better inform HIV prevention work. Because of person-to-person variability in the development of immune response, the tests are seen as being unable to give a definitive date for an individual's infection. They are only able to suggest rough timings, and have a considerable margin of error.

RITA is more reliable with the HIV subtype (B) that is most commonly found in Europe. With subtypes that are more commonly found in Africa and elsewhere, its results may sometimes be inaccurate. Moreover, some people may be misclassified as having recent infection when they have a low CD4 cell count or when they have taken antiretroviral drugs, either as treatment, post-exposure prophylaxis (PEP) or pre-exposure prophylaxis (PrEP).

Blood for RITA may be taken alongside samples needed for viral load testing, CD4 counts and other tests. The test is done at a Public Health England laboratory. The results are returned to the HIV clinician, who decides whether to discuss them with the patient. Clinicians are encouraged to explain the limitations of the test and to present the results in the context of the patient's clinical history and recent sexual behaviour.

Key points

- UK guidelines recommend the use of combined antibody/antigen laboratory tests. Antibody-only tests are no longer recommended.
- Combined antibody/antigen laboratory tests are exceptionally accurate and usually able to detect infection within a month after exposure.
- Point-of-care (rapid) tests, self-testing and self-sampling services are more convenient for some users. Improving the availability of these services may lead to more people receiving HIV test results.
- There are limitations to the performance of some of the more convenient testing methods – they do not always reach the standards of combined laboratory tests.
- An HIV-positive diagnosis should never be given on the basis of a single test result – confirmatory tests are always required.

Further reading

Centers for Disease Control and Prevention. **Laboratory testing for the diagnosis of HIV infection: updated recommendations**, 2014.

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Delaney KP et al. **Time Until Emergence of HIV Test Reactivity Following Infection With HIV-1: Implications for Interpreting Test Results and Retesting After Exposure.** Clinical Infectious Diseases 64: 53-59, 2017.

HIVST (HIV self-testing research and policy hub). www.hivst.org

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