HIV TESTING TECHNOLOGIES

The knowledge, the will and the power (KWP) is the National African HIV Prevention (NAHIP) programme's strategic plan to prevent sexual HIV transmissions among African people in England. One of the aims of KWP is that African people in England have accurate clinical knowledge of their own and their partner's HIV status. This briefing provides an overview of HIV diagnostic tests commonly used in laboratories and in community settings (often referred to as the point-of-care) for HIV health promoters involved in promoting or providing 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 should be read alongside our previous KWP briefing on HIV testing among African people 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.
- 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 detect the full range of HIV-1 subtypes and identify infection with HIV-2. Worldwide, the predominant virus is HIV-1, and generally when people refer to HIV without specifying the type they will be referring to HIV-1. The relatively uncommon HIV-2 type is concentrated in West Africa and is rarely found in the UK.

Unfortunately, no medical test is perfect. Choices must be made based on the available tests 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 infected?
- If a rapid, non-invasive test encourages more people to test, should it be used even though it is less accurate than another test?

Although 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 will always be verified with a series of confirmatory tests.

ANTIBODY LABORATORY TESTS

The HIV tests that most people are most familiar with test for HIV antibodies only. 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 "third-generation" tests or as an ELISA (enzyme linked immunosorbent assay). The first and second generation tests are no longer in use.

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. Production of these antibodies persists for life.

The typical time before a third-generation test can detect HIV antibodies is thought to be between 20 and 25 days, although it can be longer in some cases. This is called the "window period" which is the period after exposure to HIV during which tests are not able to detect any HIV antibodies (either because none have been produced yet, or because they are too few in number for the test to pick up).

The period during which antibodies are first produced is called "seroconversion". It is often, but not always, accompanied by a set of symptoms commonly called a seroconversion illness, which may be misdiagnosed as flu or glandular fever (or ignored). The most common symptoms are fever, rash, sore throat, swollen lymph nodes, muscle aches and joint pains. When these symptoms appear, they normally do so within six weeks of the HIV exposure.

Except in the case of recent infection, third-generation tests are extremely accurate. For example, a Health Protection Agency evaluation[1] of 16 tests found that all except one had a sensitivity of 100% – in other words, all HIV-positive people tested were correctly diagnosed. Moreover, all had a specificity of 99.8% or over – in other words, if 1000 HIV-negative people were tested, 998 would be correctly diagnosed as such, while two samples would test positive. However in practice, confirmatory tests would be used and individuals would not receive an incorrect positive diagnosis.

ANTIBODY / ANTIGEN LABORATORY TESTS

Antibody only laboratory tests are no longer recommended for routine use in the UK. UK national guidelines for HIV testing[2] recommend the use of tests which detect both HIV antibodies and p24 antigen, otherwise known as "fourth-generation" tests.

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. A fourth-generation HIV test adds a technique for detecting p24 antigen to the traditional antibody test. Otherwise, the test is carried out in the same way, with blood samples at a laboratory.

Since p24 antigen is usually detectable a few days before HIV antibodies, the window period is somewhat reduced. Some people who have been HIV infected but have not yet seroconverted will have their infection diagnosed with this test.

It is hard to say exactly how long the window period for these tests lasts, as there are variations between individuals and it is a difficult topic to research (recently infected people would need to know exactly when they were exposed to HIV and then give multiple blood samples over the following days and weeks). Nonetheless, some experts believe that combined tests usually detect infection approximately 11 to 16 days after exposure, but occasionally this period will be a little longer. The UK HIV testing guidelines[2] say that, when this test is used, the majority of infections will be detected within one month.

Antibody / antigen laboratory tests are extremely accurate. In terms of sensitivity (correct identification of people with HIV), a Health Protection Agency evaluation[1] found that nine out of the ten tests they evaluated had a sensitivity of 100%, while a French evaluation[3] 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. All tests checked by the Health Protection Agency evaluation[1] had a specificity of 99.7% or above, and the French study[3] found that all tests produced after the year 2000 had a specificity of 99.8% or above.

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 may 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?

To be certain, BASHH (2010)[4] recommends re-testing three months after the last possible exposure to HIV, and many clinicians still describe the window period as being up to 3 months. Although the majority of infections can be detected within a month, and almost all within six weeks, there are occasional cases when it takes longer. This period will be longer if someone has taken post-exposure prophylaxis (PEP).

How often should I test?

NICE[5] recommend a routine offer of a HIV test by all health professionals to (among others): those from a country of high HIV prevalence and those living in areas where HIV prevalence is greater than 2 in 1000 people. However, NICE[5] does not make does not make an explicit recommendation on the ideal frequency of HIV testing for African people in England, instead outlining various 'trigger points' where testing should be considered (for example, the start of a new relationship). However, NAT (2012)[6] recently argued a new recommendation is justified for sexually active African people to test at least annually.

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 results may be inaccurate when a person does not have the most common HIV-1 subtype (B). 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 or pre-exposure prophylaxis.

Blood for RITA may be taken alongside samples needed for viral load testing, CD4 counts and other tests. The test is done at a Health Protection Agency 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.

RAPID, POINT-OF-CARE TESTS

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

But laboratory tests have some disadvantages, especially from the point-of-view of people testing. Some people dislike having a blood sample 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 many of which are only open during "working hours" or are inaccessible to those with immigration difficulties.

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. Point-of-care test kits are cheap and do not require specialised laboratory equipment, so they can be administered and interpreted in any setting. Alongside promoting access to more traditional testing, many HIV charitable organisations offer point-of-care tests in their offices or from other community settings including sporting and cultural events. This allows organisations to deliver rapid HIV testing services to people who might not attend an NHS setting to test.

Rapid tests can be performed by staff with limited laboratory training. Given that the test result relies on subjective interpretation (particularly with a borderline result), it is essential that those delivering point-of-care test results in community settings are well-trained and experienced. It is entirely possible that comparative studies of these tests have been adversely affected by problems with staff training or quality control, rather than intrinsic limitations of the tests. Nonetheless, this does highlight real-world difficulties in delivering consistently reliable results.

While several studies have shown point-of-care tests to be almost as accurate as antibody laboratory tests, performance has not always reached these standards. The UK HIV testing guidelines[2] are cautious about the use of point-of-care tests, recommending that they are only used:

- At community testing sites.
- In clinical settings where a rapid turnaround of test results is desirable.
- For urgent source testing (for example, following a needle-stick injury).
- If a person refuses to give a venous blood sample.

When used in a population with a low prevalence of HIV, false-positive results can be a problem. In a setting where very few people have HIV, the majority of apparent positive results may 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 African migrants to England, 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.

ANTIBODY POINT-OF-CARE 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.

With one exception, all point-of-care tests look for antibodies only. This explains, in part, the scepticism of some health professionals. Moreover, the window periods of these tests are usually a few days longer than of antibody laboratory tests.

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 wide variations in accuracy from one test to another. Some tests, mostly ones which are not marketed in the UK, have a sensitivity (the ability to detect all true positive results) or specificity (the number of negative samples correctly identified as negative) of 95% to 97%, rather than 99-100%[7,8]. However the evaluation data that is available for the tests more commonly used in the UK has generally been more encouraging.

In most but not all studies of the *Determine HIV 1/2*, *INSTI* and the *Vikia HIV 1/2* tests, they performed well, usually with sensitivities and specificities in the range of 99-100%.

Performance of the *OraQuick* test with finger-prick blood samples has generally been good, with sensitivity and specificity in the range of 99-100%. However performance is slightly poorer when testing samples of oral fluid – some people with infection may receive a false negative result. This may be because quantities of antibodies are lower in oral fluid than in blood[9].

ANTIBODY / ANTIGEN POINT-OF-CARE TESTS

Introduced in 2009, the *Determine HIV-1/2 Ag/Ab Combo* test looks for both antibodies and p24 antigen, in a similar way to antibody / antigen laboratory tests. At the time of writing, it is the only point-of-care test to do so.

Because it detects p24 antigen as well as antibodies, the window period should be reduced. The manufacturer says the window period is an average of five days shorter than for the previous *Determine* test, but this varies from individual to individual (range: 2 to 20 days). The manufacturer also reports that on tests with 1179 positive and 2343 negative samples, sensitivity was 100% and specificity was 99.2%.

However, other research suggests that while the test performs well in respect to established HIV infection, its ability to detect very recent HIV infection does not match that of laboratory antibody / antigen tests. Research in Malawi found high sensitivity with people who had been infected for several months or more, but the test identified only two of eight people with very recent infection[10]. UK researchers tried the test on stored samples from 36 HIV-positive people who had detectable p24 according to laboratory tests. The rapid test detected p24 antigen for only half those tested (sensitivity 50%). The test failed to detect HIV infection (either via p24 antigen or antibodies) in ten of 36 cases, nine of whom had very recent infection[11].

FIVE KEY POINTS

- UK HIV testing guidelines[2] 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.
- Many users find point-of-care (rapid) tests more convenient and their use may lead to more people receiving HIV test results.
- There are limitations to the performance of point-of-care tests – they do not reach the standards of combined laboratory tests – but they bring other advantages.
- An HIV-positive diagnosis should never be given on the basis of a single test result – confirmatory tests are always required. Community organisations offering point-of-care testing will need to establish a fast referral pathway to the nearest NHS service where a confirmatory test can be undertaken.

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