HIV Diagnostic Technology and Advances

What is HIV Diagnostic Technology?

HIV diagnostic technology refers to both HIV testing technology and a broader set of diagnostic tools to measure the impact of HIV on the health of people living with HIV (PLHIV).

HIV testing refers to tools and techniques used to determine the presence or absence of HIV in a person's body. Different testing methods seek to identify and/or measure different HIV-related molecules in the body, such as HIV antibodies, antigens from the virus, or viral RNA. The body fluids most commonly used to detect the presence of the virus are blood and oral fluids.

The broader field of HIV diagnostics also includes tests to monitor the health of PLHIV, including immune system function and the effectiveness of antiretroviral treatment. Of these diagnostic tests, the most commonly used are tests to measure CD4 count and viral load. These technologies are crucial for determining when to initiate treatment and when to adjust antiretroviral regimens. They are also useful in monitoring of community viral load (see *Viral Load*).

HIV Testing: Sensitivity and Specificity

The ability of different tests to accurately detect HIV depends on a combination of sensitivity and specificity. The sensitivity of a test is defined as the percentage of positive results in individuals who are actually infected. The higher the percentage the more sensitive the test; a test that is 100% sensitive will produce no false negatives. The specificity of a test refers to the percentage of negative results among HIV-negative individuals. The higher the percentage the more specific the test; a test that is 100% specific will produce no false positives.

Both false positives and false negatives can have harmful consequences. False positives can be traumatic, but they are often identified quickly through confirmation testing. False negatives, on the other hand, require no confirmation testing and may go unnoticed until the next time an individual gets tested, creating opportunities for the virus to be unknowingly passed on.[1]

Types of HIV Tests

A number of different HIV tests have been developed over the years. They each have their own particularities and are used in different situations. They can be grouped in 2 ways: according to the molecules they search for in body fluids (HIV antigens, HIV nucleic acids, or HIV antibodies), or according to the body fluid used for the procedure (blood tests, oral tests).

Antibody Tests

HIV antibody tests are designed to detect the antibodies produced by the immune system in reaction to HIV infection. Examples of antibody tests include the Enzyme Linked Immuno Sorbent Assay (ELISA) and Western Blot tests. The ELISA test has a high sensitivity, and therefore a negative result with ELISA is usually sufficient to rule out HIV infection. When an ELISA test produces a positive result, it is often confirmed with a Western Blot, which has a high degree of specificity.

HIV antibody tests are highly reliable at a relatively low cost. For these reasons, they are a popular option for HIV testing in large populations of adult individuals.

Nucleic Acid-based Tests (NAT)

Nucleic acid-based tests (NAT), also known as NAAT (nucleic acid amplification tests), capture portions of HIV's RNA and amplify them in order to facilitate viral detection. Because of the extremely high sensitivity of NAT tests, window periods are short but false positives are common. Positive results must be confirmed with standard testing for HIV antibodies as soon as possible.

Given their high costs, NAT tests are not routinely used nor are they cost-effective for testing among large populations. NAT testing is most commonly used for detection of HIV in newborns and blood banks. NAT tests are also occasionally used to diagnose primary or recent infection.

Antigen Tests

Antigen tests detect the presence of a protein called p24, which helps make up HIV's protein shell. Antigen tests are 100% specific but have a low sensitivity of 89%, meaning that they produce no false positives but some false negatives. This low sensitivity is related to the fact that p24 antigen is detectable for only a short period of time, between 2 weeks after infection and the development of HIV antibodies by the immune system 2–3 months after infection,[2] at which point the interaction between p24 antigen and HIV antibodies renders the test no longer reactive.[3] A negative result does not rule out infection and a positive result requires confirmation with Western Blot.

Antigen tests can be used to identify cases of acute infection in blood bank screening and to diagnose infection in newborns.[4,5] However, since the development of more effective techniques such as NAT tests, the antigen test has fallen into disuse.

Fourth Generation HIV Tests

Fourth generation tests combine technologies in order to identify both p24 antigens and HIV antibodies. This combination, also known as an HIV Combo Assay, has a sensitivity of 73.7% and a specificity of 99.8%.[6] Fourth generation tests have the potential to reduce the window period and detect the presence of HIV during primary infection. These tests are not widely used due to their low cost-effectiveness in settings where community HIV incidence is low.[7] While cost-effectiveness increases in high-incidence settings, the initial investment required to test large numbers of people is still prohibitive in most high-incidence countries.

Limitations of HIV Testing

The most important limitations of HIV diagnostic tests relate to the window period and cross-reaction with other molecules present in body fluids.

Window Period

The term "window period" refers to the time between initial HIV infection and the ability of diagnostic tests to detect infection.[8] Each type of test has a different window period. For the most widely used antibody tests, the window period may range between one and six months. However, it is generally accepted that 97% of those infected would produce antibodies three months after infection, ending the window period. For nucleic acids tests and antigen tests, the window period can be as short as 2 weeks. Even when it is not possible to detect HIV infection during the window period, people living with HIV can still transmit the virus to others.

Cross-Reactions

A cross-reaction is a false positive result produced by interactions with other molecules in a person's body fluids. Cross-reactions can happen in people with hypergammaglobulinemia, people recently vaccinated against Hepatitis B or the influenza virus, and/or people with antibodies that have similar characteristics to HIV antibodies (eg, antibodies directed at other infectious agents). Pregnancy can also produce false positives due to cross-reactions with molecules similar to HIV antigens that are present in a normal placenta.

Point of Care Tests (Rapid Tests)

Point of care tests, or rapid tests, allow for testing to be conducted outside of the traditional laboratory setting. Point of care tests can be performed in a consultation room by a counsellor, at a patient's bedside in the hospital, or even at the patient's home.

Rapid tests have a low rate of false positives, but positive results still require confirmation by a Western Blot. In addition, rapid tests are not highly sensitive, so a negative result does not definitively rule out HIV infection. Rapid tests aim to reduce barriers to HIV testing by reducing the amount of time it takes to complete a test and eliminating the need to return for a test result at a later date. Rapid tests also reduce the level of technology required to perform the test, making HIV testing more accessible for large populations, as well as for populations in resource-limited settings, rural areas, and other challenging locations.

Over-the-Counter Tests (OTC)

Over-the-counter tests (OTC) are HIV test kits that can be purchased at a pharmacy and then used in the privacy of one's own home.

The OraQuick HIV test by OraSure Technologies is the first and currently the only OTC test to be approved for distribution. The test uses an oral swab to detect HIV antibodies in oral fluids within a 30-minute time period. The test is simple to use and the results are easy to read, with one red line indicating a negative result and two red lines indicating a positive result. The absence of any red lines indicates that the test was not conducted correctly and must be taken again using a new test kit. The test currently costs $40 when purchased online.[9]

As a testing approach, OTC tests have a number of strengths and weaknesses. The ability to take the test in the privacy of one's own home helps ensure confidentiality and anonymity, potentially increasing rates of testing among individuals who had avoided testing due to stigma, discrimination, or breaches of confidentiality in health care settings.[10] OraSure's clinical trials indicate that the test has a sensitivity of 93%, which is below the US Food and Drug Administration (FDA) recommendation of 95%. This lower rate of accuracy is believed to stem from human error due to the fact that the test is self-administered by untrained individuals at home, as opposed to trained heath care professionals.

Testing outside of health care settings also raises questions about the counselling and referral components of the testing process, which are important for reducing risk behavior, mediating the psychological impact of a positive test result, and connecting those who test positive with essential follow-up services. Finally, the cost may be prohibitive for use by many of the people at highest risk for HIV infection.

Despite the lower rate of accuracy and questions about counseling, referral, and cost, the US FDA approved the OraQuick test in July 2012 because of the expected public health benefits of an OTC testing option.[11] By increasing testing and diagnosis, early initiation into treatment is expected to increase. This would boost health outcomes for people living with HIV and prevent new infections by reducing community viral load. The US FDA has estimated that OraQuick could identify 45 000 new cases of HIV per year in the United States and help to prevent 4000 new infections annually.

Given that MSM are disproportionately impacted by HIV and frequently experience homophobia in health care settings, OTC tests may increase individual and community-level testing among gay men. Gay men and other MSM may feel more comfortable testing in the privacy of their own homes rather than face harassment or unprofessional conduct at the hands of health care workers.

Despite its potential advantages, the frequency of false negative results obtained with OraQuick OTC represents a challenge for gay men and other MSM. A false negative could result in behavioral disinhibition and the potential exposure of future partners.[12] Education is needed to avail MSM of the strengths and limits of this testing approach, along with increased advocacy and resources to help ensure that gay men and other MSM have access to HIV treatment and care.

Diagnostic Technology in HIV Treatment and Care

In addition to HIV testing, diagnostic technology is also used in the treatment and care of PLHIV. Diagnostics relevant to clinical management of HIV infection include tests to monitor CD4 count and viral load. These diagnostics are used to make decisions regarding initiation of treatment, to determine prognosis, and to evaluate the efficacy of antiretroviral medicines. This is valuable not only for optimizing the health of individuals on treatment, but also for monitoring community viral load.

CD4 Count

CD4 tests are used to measure the number of CD4 T-cells in the blood. Determining a person's CD4 T-cell count helps physicians to assess damage in the immune system and decide when to start someone on treatment. CD4 tests are normally performed shortly after initial diagnosis, then regularly every 3 to 6 months until initiation of treatment, and then every 6 months once treatment has started.

The World Health Organization (WHO)'s 2010 guidelines on *Antiretroviral Therapy for HIV Infection in Adults and Adolescents* recommend that treatment be initiated when someone's CD4 count drops below 350.[13] The decision to increase the threshold for treatment initiation to 350 (older versions of the guidelines used a threshold of 200) was based on clinical trials showing better prognosis and less progression to AIDS in patients who started treatment earlier.[14] Recent research confirms that earlier initiation of treatment corresponds to lower mortality and better prognosis.[15] While

most low- and middle-income countries try to follow the WHO's 2010 guidelines, some countries continue to wait for CD4 counts to drop below 200 due to cost constraints. Conversely, doctors in other countries may decide to initiate treatment much earlier, at CD4 counts as high as 500.

CD4 counts are also used to determine prophylactic measures taken to prevent opportunistic infections. For example, a CD4 count lower than 200 indicates the need to initiate cotrimoxazol prophylaxis in order to prevent pneumocistosis.[16] CD4 tests are also used to evaluate the effectiveness of treatment: an increase in CD4 count is a sign that a patient's current treatment regimen is effective; conversely, when CD4 counts remain low or continue to decrease, physicians can identify treatment failure and switch a patient to a different drug regimen.[17]

Viral Load (VL)

The viral load quantitative test determines the number of copies of HIV per milliliter of blood. Standardized categorical measures for viral load have been defined as follows:

- High VL (>100,000 copies/mL)
- Suppressed VL (where ≤200 copies/mL) / not suppressed (>200 copies/mL)
- Undetectable VL (≤50 copies/mL)[18]

The VL test is used to monitor HIV infection and potential progression to AIDS. This monitoring process can help physicians decide when to start someone on treatment, though this decision relies primarily on the CD4 test. A VL test can also help evaluate the effectiveness of antiretroviral treatment and identify resistance to drugs by detecting increases in viral load while a patient is on treatment.

After initiation of treatment, the number of HIV copies may drop below 50/mL, the test's threshold for detection of the virus. This *"undetectable viral load"* does not indicate eradication of the virus. However, an undetectable VL is associated with better prognosis, slower progression to AIDS, lower risk of transmitting the virus, and a prolonged life.[19] VL tests should be requested shortly after diagnosis, every 6 months before treatment, and a month after initiation of treatment to evaluate efficacy of the chosen treatment regimen.

At the community level, VL testing has a much broader use. *Community viral load* (CVL) refers to the amount of virus in a community. By totalling the VL of a community's members, CVL indicates the progression of the disease within a group of people.[20] The CVL of a given cohort can be helpful in assessing the effectiveness of interventions, like prevention campaigns or testing initiatives, as well as for assessing "per act transmission risk" (a single act of unprotected sex with an unknown partner carries a higher risk of infection in a community with a higher CVL).[21,22] An understanding of CVL can be particularly helpful in efforts to expand community-level awareness and to develop community-level HIV intervention strategies and programs.

Conclusion

HIV diagnostics have benefited from the simplification of the technology required for their implementation, leading to an increase in the number of patients tested and the number of HIV cases identified. Advances like home testing represent new and important opportunities for addressing HIV among MSM. However, as technology development continues to push forward, we must ensure that new technologies are matched with well thought-out implementation strategies that take into account both the latest scientific advances and the needs of the communities that need them most.

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